PROCEEDINGS

The 7th ASIAN CROP SCIENCE ASSOCIATION CONFERENCE

Improving food, energy and environment with better crops

IPB International Convention Center
Bogor, Indonesia, 27-30 September 2011

Research Center for Bioresources and Biotechnology
Bogor Agricultural University

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Foreword from the Chairman of Organizing Committee

We are currently facing an increasing population, diminishing resources and also escalating environmental problems. These inconvenient conditions could be solved by intensifying our attention to develop efficient, stable, and sustainable crop production systems. To achieve this goal, the cooperation of crop scientists working on different aspects from basic to applied sciences, and different commodities from food crops as considered the basic need of human being to ornamental crops considered as secondary need, transcending the boundaries of region and nations is necessary.

With the intention to accommodate the communication among researchers on crop science, the Asian Crop Science Association (ACSA) conducts regular international conference every three years. During four days on September 27-30th, 2011, we had successfully organized the 7th Conference of ACSA with the theme on **Improving Food, Energy, and Environment with Better Crops**, in Bogor, Indonesia. This event had been attended by 240 participants coming from 17 different countries. During three days of the conference, 13 papers were presented by invited speakers in the plenary sessions, 81 papers were in the parallel sessions, and 71 posters were presented in the poster sessions. This conference was in conjunction to the 48th anniversary of Bogor Agricultural University, annual meeting of Crop Science Society of Japan (CSSJ), Japanese Society of Breeding (JSB) and Japanese Society of Tropical Agriculture (JSTA), and the kick off meeting and workshop of Japan International Research Center for Agricultural Science (JIRCAS).

The scientific activities were composed of three seminars: (1) **ACSA Conference** with the theme on “Improving food, energy, and environment with better crops”, (2) **seminar of annual meeting of CSSJ, JSB and JSTA** with the theme on “Improvement of crop performance for sustainable agricultural development in wetlands”, and (3) **workshop of JIRCAS** with the theme on "Rice innovation for environmentally sustainable production systems”.

On behalf of the editor and also the organizing committee, I would like to express our happiness that the scientific papers presented in the Conference can be finally compiled and published in the form of Proceedings of the 7th Asian Crop Science Association Conference.

We apologize for the delay to publish these proceedings. We sincerely hope that these Proceedings will be useful and beneficial for all parties.

Chairman of OC and Chief Editors,

Prof. Dr. SONY SUHARSONO

Foreword from the Rector of Bogor Agricultural University

I would like to express my happiness that The Proceedings of The 7th Conference of Asian Crop Science Association (ACSA) can finally be published. Looking on the number of scientific papers coming from different countries and aspects, it is certainly that many tasks had been done to publish these proceedings. My expression of appreciation and gratitude are addressed to the editors who had been working very hard to finish the proceedings.

Crop is very important to human and there is no life without crop. From crop we obtain food and feed. With the increasing of the world population, the requirement for crop is increasing substantially, therefore, serious efforts must be undertaken to improve crop production to supply sufficient food for human. In addition, the climate change forces us to adapt to this environmental changes. From this point of view, the development of crop science is highly important, from basic to applied sciences, and collaboration amongst scientists is necessary to accelerate the progress of crop development. Crop does not only provide food, but also provides a future energy sources which is more friendly to the environment, therefore, the proceedings consisted of the scientific papers of the 7th Conference of ACSA with the theme of "*Improving food, energy, and environment with better crops"* is highly relevant to the current situation.

ACSA is expected to play major role in crop science development and in coordinating the collaboration amongst scientists, not only in Asia but also throughout the world. Therefore I personally and as a Rector of Bogor Agricultural University strongly support ACSA as a medium for collaboration to share knowledge and experiences in recent advances of crop science development.

Finally, I extend my gratitude to contributors, and sponsors for participating in the compiling all scientific papers in the proceedings. I believe that these proceedings will enrich our knowledge, especially in crop sciences, and beneficial to mankind.

Rector of Bogor Agricultural University,

Prof. HERRY SUHARDIYANTO, PhD.

MINISTRY OF AGRICULTURE
REPUBLIC OF INDONESIA

KEYNOTE ADDRESS
MINISTER OF AGRICULTURE REPUBLIC OF INDONESIA
AT THE OPENING CEREMONY OF THE 7th ACSA CONFERENCE
Bogor, 27-30 September 2011

Distinguished guests,

- Rector of Bogor Agricultural University,
- Chairman of Asian Crop Science Association (ACSA),
- Chairman of Crop Science Society of Japan,
- Director of Japan International Research Center for Agricultural Sciences (JIRCAS),

Ladies and Gentlemen,

Assalamu'alaikum warahmatullaahi wabarakaatuh,

It is a great pleasure for me to be here with you and warmly welcome you all in this important gathering of "The 7th ACSA conference". On behalf of the Government of the Republic of Indonesia, it is also a great honor and privilege for me to extend our warm welcome to all participants.

It is a great pleasure also for me to congratulate for 48th anniversary of IPB and warm welcome and success for kick off meeting of Crop Science Society and Japan International Research Center for Agricultural Sciences (JIRCAS).

Ladies and Gentlemen,

The on going changes in the economy, resources competition from other sectors, environmental changes, increasing commercialization of agricultural activities, and the importance of international trade mean that the way of agricultural product will be produced in the future will be substantially different.

Some traditional agriculture-growing areas may lose their comparative advantage while others may become new growth centers for agriculture product. Clearly, there is a need to develop a new vision for future agriculture activities given these global trends and likely scenarios. This vision is needed to strategically position investment in agricultural research, technology delivery, and the design of policy reforms.

Agriculture in the next future will not only need to produce enough food and energy for population, but will also be the key to conserve environment for a sustainable food production. FAO's latest forecast for world cereal production in 2011 stands at 2,302 million tonnes, 2.9 percent up from the 2010 harvest. World cereal utilization in 2011/12 is forecast to increase by 1.2 percent from 2010/11. Given the latest production forecast and the expectation for utilization in 2011/12,

world cereal stocks at the close of crop season ending in 2012 are now estimated at about 486 million tonnes.

Except for rice, the inventories of which are forecast to increase, stocks of coarse grains and wheat anticipated to decline. With total cereal production in 2011 below the anticipated utilization, international price are likely to remain high, especially in the wheat and coarse grain market. In Asian countries, high food prices and the generally good rainfall situation have been conducive to bumper food production. In the 2011, annual harvest for rice are expected to increase in China, India, Vietnam, Indonesia, Thailand and Philippines.

Up to 2050 the world's population will rise from 6 bn to 8 bn (33%) and about 50% of the total population are lives in Asia. Simultaneously, demand of food will increase by 50%, demand of water will increase 30%, and demand for energy will increase by 50%.

Considering those features, the approach need for adaptation are (1) public awareness to enhance participation, (2) improving institutional capacity building, (3) policy reform, (4) strong enhancing research and study.

Ladies and Gentlemen,

In terms of energy, more than 40% of our populations engage in producing energy from fossil fuel and wood to run their daily activities including agriculture. So there is a need for using a sustainable source of fuel in the rural area in the future.

The Central Bureau of Statistics (BPS) reported that population growth in Indonesia during the period of 2000 to 2010 was 1.49 percent per year, compared to 1.45 percent during the period of 1990 to 2000. The 0.04 percent increase for the current population (237 million people) resulted in a very big increase in food demand. If we do not make a serious effort to control birth rate, the country's population may reach an alarming 400 million in the year 2060. In the case of rice, with consumption rate of 134 kg/cap/year, the demand for hulled rice in 2015 will be 44,4 million tons. In addition to the pressure on the demand, large population also represent a serious threat to environment that cause unsanitary living conditions, the depletion of resources, environmental pollution, and poverty, which are the root of environmental problems.

The second problem is diminishing natural resources, particularly land and water resources. Irrigation water is an important production factor in rice production systems but it is no longer available in unlimited quantities in rice-growing areas due the increase of competition from domestic use. Meanwhile, conversion of rice field to non-agriculture use is estimated about 100.000 ha annually. These two will significantly limit food production capacity in the country.

The third problem is the impact of global warming. Indonesia is already a significant emitter of greenhouse gases due to deforestation and land-use change, estimated at 2 million hectares per year and accounts for 85 percent of the country's annual greenhouse gas emissions. The effects will be felt more acutely by the poorest people, who are living in the most marginal areas that are vulnerable to drought, floods and landslides. International Food Policy Institute estimates that rice production in developing countries, will be reduced as much as fifteen percent (15%), and the price of rice will increase to twelve percent (12%) in 2050.

Ladies and Gentlemen,

Opportunities to cope with fore mentioned problems are by using environmentally friendly technologies. Luckily, our researchers have generated innovative technology for better crops that increase productivity with environmentally friendly manners. Among of them are more aerobic cultural practices, include intermittent irrigation and aerobic rice varieties, specific site nutrient management, integrated pest management, and higher yield rice varieties.

Ladies and Gentlemen,

I believe that through the 7th Asian Crop Science Association Conference, we are all will share knowledge and experience; therefore, I hope this conference will come up with recommendation to cope all those problems.

Finally, with **Bismillahirrahmanirrohim, I declare The 7th ACSA Conference officially open.** Have a pleasant meeting and please enjoy your stay in Indonesia, especially in Bogor.

Wassalaamu'alaikum warahmatullaahi wabarakaatuh.

Minister of Agriculture,

SUSWONO

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Estate and Forest species

Improving Photosynthesis to Increase the Productivity of Crops

Akiho Yokota

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan
yokota@bs.naist.jp

Abstract

Environmentally friendly energy-producing systems that utilize natural sources of energy, such as solar, wind, land heat, and sea waves, have been developed around the world. These new energy systems are expected to replace fossil fuels for energy production. However, they do not assimilate CO_2 liberated into the atmosphere artificially, nor do they produce food or feed for heterotrophs. Thus, it would be prudent to utilize plant systems to synthesize organic matter from inorganic CO_2 with solar energy. However, there are intrinsic restrictions on plant production, one of which is the ability of the key photosynthetic enzyme, ribulose 1,5-bisphosphate (RuBP) carboxylase/oxygenase (RuBisCO), to fix CO_2 via photosynthesis. Photosynthesis converts CO_2 into sugars and lipids. This process relies on biochemical energy in the forms of reduced nicotinamide adenine dinucleotide phosphate and adenosine triphosphate, which are produced from solar energy. As a catalyst, RuBisCO has disadvantages. It has a low reaction rate, a low affinity for its substrate, CO_2, and it catalyzes the oxidation and evolution of carbons in sugars as CO_2. In addition to RuBisCO, activities of two phosphatases have been reported to be too low to give other enzymes full play. Here, I would like to address our successes in improving photosynthetic performance of tobacco and potato.

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Impacts of Climate Change and Climate Variability on Productivity of Food Grain Crops

P V Vara Prasad

Department of Agronomy, Kansas State University, Manhattan, Kansas 66506, USA
2004 Throckmorton Plant Sciences Center, Department of Agronomy,
Kansas State University, Kansas 66506, USA
Tel.: +1 785 532 3746; Fax: +1 785 532 6094
vara@ksu.edu

Abstract

Crop production is highly sensitive to changing environmental conditions. In recent years long-term climate change and year-to-year climate variability has become a major challenge to crop productivity. Current knowledge on effects of season-long high temperatures and elevated carbon dioxide concentrations; and effects of short periods of high temperature stress on various physiological, growth and yield processes will be presented and discussed. Main focus will be on major grain crops (dry bean, groundnut, soybean, rice, wheat and sorghum). Results indicate that above optimum temperatures will have negative impacts on reproductive processes (such as pollen production, pollen germination, fertilization, seed numbers and individual seed weight) resulting in lower seed yield. The beneficial effects of elevated carbon dioxide mediated through increased photosynthesis will be negated by rising temperatures resulting in lower seed yields. Grain crops are most sensitive to high temperature stress during micro-sporogenesis and flowering. High temperature stress during these stages leads to loss of pollen fertility, poor pollination and decreased fertilization, resulting in fewer seed numbers. Development high temperature tolerant cultivars will be of prime importance for adaptation to climate change and climate variability. However, there are several challenges to develop stress tolerance genotypes, some of these include (a) availability of genetic variability in trait of interest; (b) development and use of efficient and reliable screening method; (c) identification of stress tolerant genotypes; (d) understanding physiological and biochemical mechanism(s) associated with tolerance or susceptibility; and (e) heritability of identified trait(s) and incorporation of tolerant traits into existing high yielding genotypes. Genetic variability exists in high temperature tolerance in few grain crops. Some physiological traits that may contribute to high temperature tolerance include increased membrane thermostability, increased green leaf duration, higher reproductive fertility, canopy temperature depression, increased thermal tolerance and high temperature avoidance during flowering by time of the day of flowering. New emerging biochemical and molecular tools provides some opportunities for screening and phenotyping. Continued collaboration between physiologists, breeders, molecular biologists and agronomists is essential for developing strategies to combat effects of climate change and climate variability on crop production.

Keywords: temperature stress, carbon dioxide, pollen viability, seed yield, gen

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The Plant Improvement of Tolerance to Oxidative Stresses

Shigeru Shigeoka

Dept. Advanced Bioscience, Faculty of Agriculture, Kinki University
Nakamachi, Nara 631-8505, Japan
Tel: +81 742 43 8083
shigeoka@nara.kindai.ac.jp

Summary

The explosive increase in world population and in environmental deterioration poses a serious threat to agricultural production and food supplies. To improve food production efficiency, crops that mature earlier with enhanced environmental stress tolerance and higher yield must be developed. When plants are exposed to a variety of environmental stresses, excess amount of reactive oxygen species (ROS) is generated, resulting in oxidative damage at the cellular level (*J. Exp. Bot.* 53,1305-1319,2002;*Plant Cell Physiol.* 45,1586-1594,2004). Despite their potential for causing harmful oxidations, ROS are also powerful signaling molecules that are involved in the control of plant growth and development as well as priming acclimatory responses to stress stimuli (*Plant Cell Physiol.*51,190-200,2010; *Plant Physiol*.155,93-100, 2011). A wide range of strategies, including molecular genetics and genetic engineering approaches, have been used to enhance the tolerance of plants to abiotic stress. Many attempts have been made over the last two decades to protect photosynthesis against stress-induced inhibition by manipulation of component antioxidant enzymes, and an extensive literature exists showing that enhancing the capacity of the water-water cycle through genetic engineering, including the overexpression of superoxide dismutase, glutathione reductase, and dehydroascorbate reductase, can improve the tolerance of plants to abiotic stress. Overall, the enhancement of chloroplast antioxidant defenses has proved to be one of the most effective ways of protecting plant cells from abiotic stress (*Biosci. Biotechnol. Biochem.* 72,1143-1154, 2008, *Plant Physiol*. 155,93-100,2011). Transgenic tobacco plants overexpressing thylakoid membrane-bound ascorbate peroxidase (APX) in the chloroplasts exhibited enhanced tolerance to high-light (HL) and paraquat (PQ) treatment, a combination that is designed to maximize photooxidative stress (*Plant J.* 32, 915-925, 2002). Similarly, transgenic tobacco and tomato plants overexpressing KatE, a gene encoding an *Escherichia coli* catalase (CAT) in the stroma, resulted in a greatly enhanced tolerance to photooxidative stress (*Plant Cell Physiol*.41,311-320,2000; *Plant Cell Environ*. 26,2037-2046,2003; *Plant Physiol.*145,258-265,2007). In this case, the enhanced tolerance was explained in terms of compensation for the inactivation of chloroplastic APXs by KatE. Ectopic expression of useful genes from cyanobacteria or Chlamydomonas (*Plant J* 37,12-33, 2004) into the chloroplasts of higher plants has proved to be effective at enhancing tolerance to abiotic stress. The GPX-like proteins of *Synechocystis* PCC 6803 are able to reduce unsaturated fatty acid hydroperoxides using NADPH as an electron donor, but not GSH and TRX (*Plant Physiol*., 136, 2855-2861, 2004). The expression of *Synechocystis* GPX in Arabidopsis chloroplasts resulted in a suppression of photoinhibition and an increased tolerance to photooxidative stress (*Physiol. Plant* 128, 251-262, 2006). Recently we have isolated various HL and heat-shock (HS) stress-inducible genes, including a heat shock transcription factor A2 (HsfA2), by suppression-subtractive hybridization from Arabidopsis plants. HsfA2-overexpressing Arabidopsis (Pro35S:HsfA2) plants showed increased tolerance to combined stress conditions, HL+ HS plus PQ treatments due to the increased expression of 46 genes, including a large number of heat-shock proteins, APX 2 and galactinol synthase 1 and 2 (GolS1 and GolS2)

(*Plant J.* 48, 535–547, 2006). Our findings indicated that HsfA2 is a key regulator in the induction of the defense system under several types of environmental stress (*Plant Cell Physiol.* 51,486–496, 2010; 52,933-945, 2011). Furthermore, mutant pqr-216 from an Arabidopsis activation-tagged line showed a phenotype of increased tolerance to oxidative stress after treatment with 3 μM PQ. Based on the phenotype of transgenic plants overexpressing the genes flanking the T-DNA insert, it was clear that enhanced expression of a Nudix (nucleoside diphosphates linked to some moiety X) hydrolase gene, AtNUDX2 encoding ADP-ribose pyrophosphatase, was responsible for the tolerance, resulting from maintenance of NAD+ and ATP levels by nucleotide recycling from free ADP-ribose molecules under stressful conditions (*J. Biol. Chem*. 280, 25277-25283, 2005; *Plant Cell Physiol* 48,1438-1449,2007; *Plant Physiol.*148,1412-1424,2008; 151,741-754, 2009; 152, 2000-2012, 2010; *Plant J.* 57,289–301,2009; *Plant Signaling Behavior* 5,839-841, 2010). The evidence described above confirms the importance of the antioxidative enzymes and the value of genetically enhanced antioxidants and other defense compounds as a mechanism for stabilizing photosynthesis in stress situations. Other metabolites can serve protective functions in chloroplasts. Oligosaccharides such as galactinol and raffinose, which often accumulate to high concentrations, can act as antioxidants as well as osmoprotectants in plant cells. Transgenic Arabidopsis plants overexpressing GolS1 and GolS2, key enzymes in the synthesis of galactinol and raffinose, had increased levels of these oligosaccharides and enhanced tolerance to enhanced oxidation caused by exposure to paraquat, chilling, and osmotic stress (*Plant Physiol*. 147, 1251-1263, 2008). These findings suggest that carbohydrates including raffinose family oligosaccharides and sugar alcohols are present at high levels under normal and/or stressfull conditions and act as antioxidants to protect plant cells from oxidative damage and maintain redox homeostasis (*Plant Signaling & Behavior* 3, 1016-1018, 2008).

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Salt Resistance Mechanism of *Metroxylon sagu*, Starch-producing Palm

Hiroshi Ehara [a,*], Wikanya Prathumyot [a,b], Hitoshi Naito [c]

[a] *Graduate School of Bioresources, Mie University, Tsu, Mie, Japan*
[b] *Faculty of Agricultural Technology, Rambhai Barni Rajabhat University, Muang, Chantaburi, Thailand*
[c] *College of Life Sciences, Kurachiki University of Science and the Arts, Kurashiki, Okayama, Japan*
** Corresponding author: Graduate School of Bioresources, Mie University, Kurimamachiya-cho 1577, Tsu, Mie 514-8507, Japan.*
Tel.: +81 59 231 9495; fax: +81 59 231 9495.
ehara@bio.mie-u.ac.jp

Abstract

Sago palm (*Metroxylon sagu* Rottb.) distributed in Southeast Asia and Melanesia grows in swampy, peaty, and alluvial soils in areas with both fresh and brackish water where almost no other crops grow without drainage and/or soil improvement. This palm species stores a large amount of starch in the trunk. The starch of sago palm is processed into various basic raw materials and is receiving attention as a sustainable energy resource as well as for use in the production of bioethanol due to the current situation, in which competition between biofuel and food production is taking place. However, sago palm is harvested primarily from natural forests and is considered to be an unexploited plant. Thus, the Na^+ and some other ion concentrations in different plant parts as well as the physiological and morphological features under NaCl treatments (86 to 342mM NaCl for 1 or 4 months in a hydroponic system) were investigated to study the salt resistance mechanism of sago palm to develop a sustainable method of cultivation that is essential for the improvement of sago palm as an economic plant. (1) Sago palm maintained a low Na^+ concentration in the leaflets, which may be attributed to Na^+ storage mainly in the roots. K^+ absorption and distribution to the leaflets may not be affected by changes in the Na^+ concentration in the roots and petioles in sago palm. (2) Based on X-ray micro-analysis, a dense distribution of Na was observed around the endodermis of the adventitious roots. Salt resistance of sago palm might be due to salt avoidance to mechanically restrict an excess of Na distribution in plant tissues as well as maintain the water status in the leaves by restricting the transpiration. (3) The development of Casparian strips in the endodermis can be considered as an important mechanical factor relating to the avoidance mechanism for preventing the excess influx of Na^+ into the stele and its translocation from root to shoot in sago palm. (4) The factor limiting the photosynthetic rate under NaCl stress was the reduction in stomatal conductance that resulted from a trade-off with the decrease in the transpiration rate to maintain the water status in the leaves. (5) Although chlorophyll production was depressed, the absorption of macronutrients was not inhibited by salt stress and there was no lack of materials, such as N and Mg for chlorophyll production. The chlorophyll concentration could increase up to high levels over a comparatively long time. These factors may account for the resistance of sago palm to salt stress and its ability to grow even with a reduction of the growth rate.

Keywords: Casparian strip, Na^+ absorption, sago palm, salt avoidance, transpiration rate

Introduction

Sago palm (*Metroxylon sagu* Rottb.) that is distributed in Southeast Asia and South Pacific areas grows in swampy, alluvial and peaty soils where almost no other crops can grow without drainage or soil improvement (Flach, 1977). It supplied carbohydrates and, like banana and taro, has long been cultivated (Barrau, 1959). This rare palm species stores a large amount of starch in the trunk, approximately 300kg (dry wt.) per tree (Ehara 2006). The importance of sago palm as a staple food is well recognized and the palm is still being in some areas of Southeast Asia and South Pacific (Ehara et al., 2000). Its carbohydrate can be further processed into various basic raw materials for food, animal feed and for industrial uses. Sago palm is one of the most important crops for sustainable agriculture and for rural development in swampy areas of the tropics.

Since, sago palm is distributed not only in fresh water areas but also in brackish water areas near the coast, it is considered to be salt-resistant. Flach (1977) reported that saline water treatment up to EC values of 6 to 7 mmho/cm did not affect leaf emergence in sago palm. However, there are few studies on the mechanisms of salt tolerance in sago palm. Thus, we investigated the Na^+ and the other ions concentrations of different plant parts with some other physiological features under NaCl treatment to study the absorption and distribution of Na^+ in sago palm.

Materials and Methods

Expt 1. Seedlings at the 8th leaf stage (mean plant length: 50cm) were treated with the culture solution lacking or containing NaCl concentrations of the rates of 86, 171 and 342mM NaCl (0.5, 1.0 and 2.0%) in a pot filled with vermiculite for 30 days (n=3) under a 25klx at 30ºC and RH75%. Kimura B culture solutions (Baba and Takahashi, 1958) containing the different NaCl concentrations (pH5.5) were supplied every day according to the amount of solution consumed and then they were renewed once a week. The Na^+ and K^+ concentrations in the leaflets, petioles and roots determined using an ion chromatograph with a conductivity detector (Shimadzu CDD-6A, IC-C2, Japan).

Expt 2. The seedlings at the 8th or 9th leaf stage were used for the NaCl treatments. The culture solution containing 342mM NaCl was used from July 9 to August 9, 2004 in the phytotron under natural sunlight. Ion concentration in the plant tissue was measured. A portion in the region above the extension zone of the large roots of the treated plants was soaked in liquid nitrogen after sampling and kept at -70°C. The frozen root samples were freeze-dried and prepared as transverse sections. The sections were coated with gold ion and used for SEM observation and X-ray micro-analysis (Horiba EMAX-5770W, Japan).

Expt 3. At ten months after germination, the longest adventitious root was taken as sample and preserved in 70% ethanol solution. The transverse sections of the root were prepared as 1st position: root tip, 2nd position: 10 mm from root tip, 3rd position: 20 mm from root tip, 4th position: 30 mm from root tip, 5th position: 40 mm from the root tip, 6th position: between the first and second lateral roots, and 7th position: above the second lateral root. The sections were stained according to Brundrett et al. (1988) and observed with a fluorescence microscope (Axio Imager A1; Carl Zeiss, Germany).

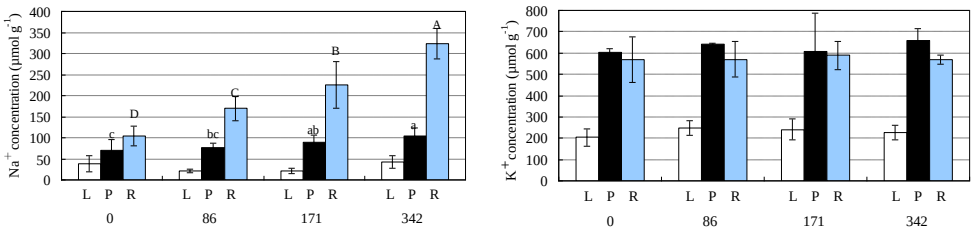
Expt 4. Four spiny seedlings were selected and transferred individually to a 7.3 L plastic pot filled with vermiculite. The mean value of plant length of all the plant materials was 79.3 cm. Two treated pots were connected individually to the first peristaltic pump that was used to supply Kimura B culture solution containing NaCl 224 mM (1.3% NaCl) (pH 5.5) to the treated pots for 6h and the 2nd pump that was used to supply the culture solution without NaCl for another 6h by turns twice a day. The 4th and 5th leaves from the top were used for the measurement of the photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Cs) by a portable

photosynthetic meter (Analytical Development Company limited LCA-4, England) under 900 µmol m^{-2} s^{-1} PAR.

Results and Discussion

Na^+ and K^+ concentrations in different plant parts

There were no significant differences in the Na^+ concentrations in the leaflets between the three NaCl concentrations in the culture solution, the values of which ranged from 22 to 44 µmol/g (Figure 1). In the petioles, Na^+ concentrations under the 342mM NaCl treatment were significantly higher than those in the absence of NaCl treatment (one and half times), and the differences between the absence of NaCl treatment and the 86mM NaCl treatment, 86mM and 171mM NaCl treatments, and 171mM and 342mM NaCl treatments were not significant. The Na^+ concentrations in the petioles tended to be higher at higher NaCl concentrations, but with a maximum increase of 1.5 times. The Na^+ concentrations in the roots were significantly higher at higher NaCl concentrations, increasing 1.5, 2.2 and 3.1 times under the 86, 171 and 342mM NaCl treatments, respectively. Two types of salt tolerance include an avoidance mechanism and a resistance mechanism (Yeo and Flowers, 1983). Through the avoidance mechanism, a low Na^+ concentrations maintained in the leaf blade. Sago palm can be considered to display an avoidance mechanism to maintain a low Na^+ concentration in the leaflets by storing Na^+ in the roots and petioles. The K^+ concentrations in the leaflets, petioles and roots did not show any significant differences at three NaCl concentrations in the culture solution and in the absence of NaCl treatment, which seems very important to understand the mechanism of salt tolerance in sago palm (Figure 1). K^+ accumulation may be associated with osmotic adjustment in sago palm.

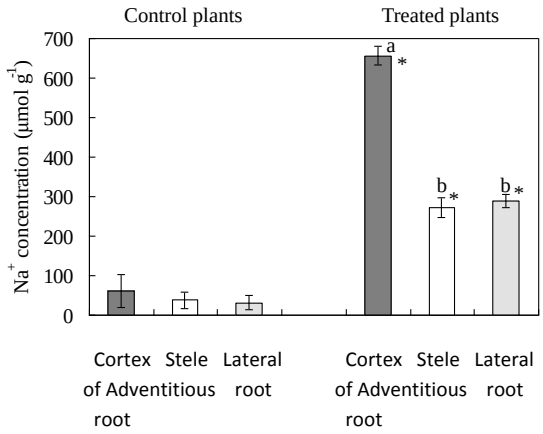


Different letters in the figure indicate significant differences (0.05 probability level, Tukey-Kramer test).

Figure 1. Na^+ and K^+ concentrations in leaflets (L), petioles (P) and roots (R) in different NaCl treatments (0, 86, 171 and 342mM).

Na^+ concentration in different parts of the roots

The Na^+ concentration in the roots increased with the NaCl treatment (Figure 1). In the adventitious roots, the Na^+ concentration was lower in the stele than in the cortex (Figure 2). Figure 3 shows the Na distribution from the cortex to the stele in the adventitious roots of the treated plants based on X-ray micro-analysis. The amount of Na was larger in the cortex than in the stele. A dense distribution of Na was observed in the inner region of the cortex near the endodermis. Based on our present findings, it appeared that the region with the endodermis was able to trap some of the excess influx of Na into the large roots. This mechanism must be very important to restrict the translocation of Na^+ from root to shoot under salt stress.



Different letters in the figure indicate significant differences in different parts (0.05 probability level, Tukey-Kramer test). Asterisks indicate significant difference in each part (0.05 probability level, T-test).

Figure 2. Na^+ concentration in different parts of the roots.

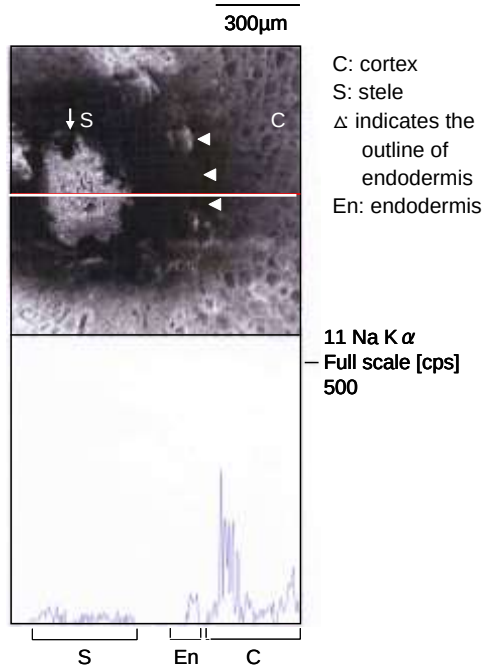


Figure 3. SEM of adventitious root and Na distribution based on X-ray micro-analysis.

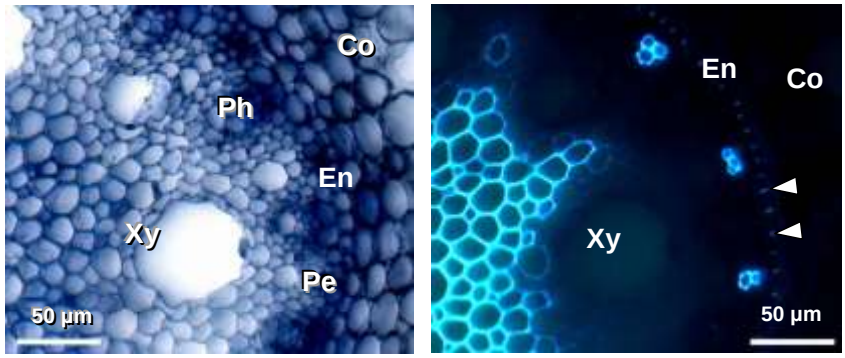


Figure 4. The structure and component of adventitious root at 10 mm from the root tip under visible light (left) and UV (right) microscope. Arrowheads indicate Casparian strips in the radial cell walls of endodermis. Co: cortex, En: endodermis, Pe: pericycle, Ph: phloem, Xy: xylem.

Casparian strip in the root

We found that the Casparian strip was located in the endodermal cell wall of both adventitious roots of sago palm (Figure 4). Taking into account the previous findings of Ehara et al. (2008) that a dense distribution of Na was observed around the endodermis in the extension zone of the adventitious root, therefore, the Casparian strip develops in the endodermis of which can be considered as one of the important mechanical factors relating to functional role of the avoidance mechanism for preventing the excess influx of Na^+ through an apoplastic pathway into the stele and its translocation from root to shoot in sago palm.

Photosynthetic rate and related characteristics

After the 4th week of the treatment, the SPAD value of the control plants started to increase and became stable after the 6th week (Figure 5). Due to the slow rate of increase in the SPAD value of the treated plants, the difference in the SPAD value between the control and treated plants became more appreciable in the 5th to 7th week and gradually decreased after the 7th week. Finally, the SPAD value of the treated plants reached nearly the same level as that of the control plants. The SPAD value is a parameter positively related to the chlorophyll concentration in plant leaf or chlorophyll content per unit leaf area. Therefore, it can be suggested that the rate of increase in the chlorophyll concentration of the treated plants also may be low. Based on this result, it can be suggested that sago palm is able to produce chlorophyll even under NaCl stress, although the rate of chlorophyll production was low. The important structural components of chlorophyll are magnesium and nitrogen that are located in the centre of chlorophyll (Lack and Evans, 2001). We found that the total N and Mg^{2+} concentrations of the leaflets did not display any distinct differences between the control and treated plants, and it was therefore clear that the deficiency of N and Mg was not the cause of the low chlorophyll production.

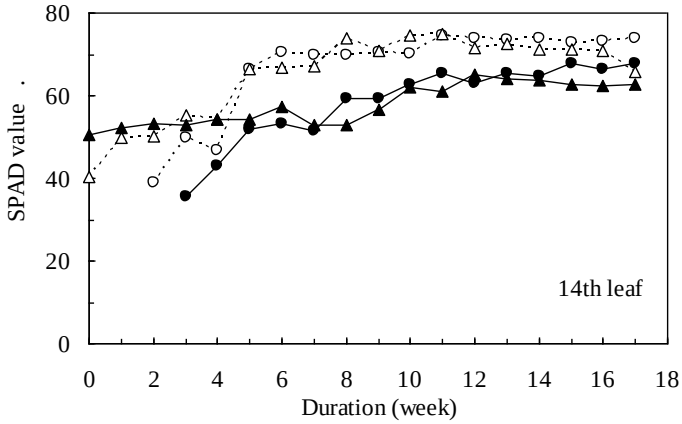


Figure 5. Changes in SPAD value of the leaflets at the 14th leaf of the control plants (C1, C2) and treated plants (T1, T2) during the NaCl treatment. Open and close symbols indicate the control and treated plants, respectively. Circle and triangle symbols indicate plant replication No. 1 and 2 of the control and treatment.

The photosynthetic rate, transpiration rate and stomatal conductance in both leaves of the treated plants decreased by 40%, compared to the value of the control plants (Figure 6). Based on the water content, which was not appreciably different between the control and NaCl treatment, it is suggested that a decrease in the stomatal conductance, which leads to the decrease in the transpiration rate and also to the decrease in the photosynthetic rate is the mechanism that sago palm used to avoid water loss and maintain the water status in the plant body under saline conditions. The decrease in the photosynthetic rate can be interpreted as a trade off with the decrease in the transpiration rate. The decrease of the photosynthetic rate might be caused by 2 main factors, i.e. reduction in the stomatal conductance and low chlorophyll production in the leaves.

In conclusion, sago palm growing under NaCl treatment can maintain a low Na^+ concentration in the leaflets by storing Na^+ mainly in the roots and petioles at lower leaf positions with a fair reduction of the growth rate. This accounts for the slow morphogenesis and the decrease in the stomatal conductance and results from the maintenance of the water status in the plant body.

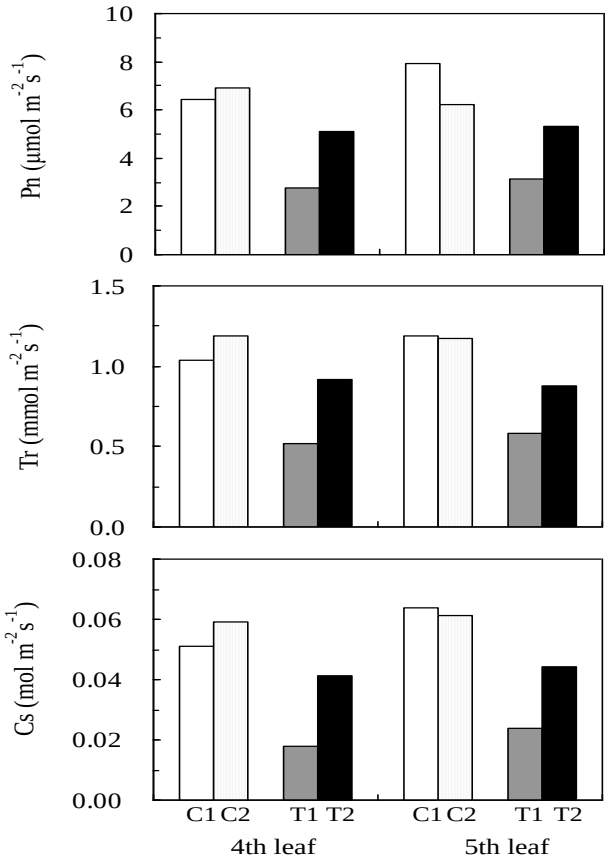


Figure 6. Photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Cs) of the 4th and 5th leaves from the top of the control plants (C1, C2) and treated plants (T1, T2) at 900 PAR in October 2007.

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How to Increase Food Crop Productivity in Asia

Narasim Upadyayula

Monsanto Singapore Co (Pte) Ltd
Singapore. Tel.: +65 97220310; fax: +65 64885610
narasimham.upadyayula@monsanto.com

Abstract

At Monsanto we believe that by improving agriculture, we improve people's lives. In the hands of farmers, better seeds along with good agronomic practices can help meet the needs of our rapidly growing population. We believe that we need to increase productivity while protecting the Earth's natural resources. Monsanto's technologies are striving to make agriculture truly sustainable by getting more from each acre, each raindrop and each seed. In this presentation I will talk about the 3 main pillars that we think we can help us increase the productivity by using less resources: (1) Breeding, (2) Agronomic Practices, and (3) Biotechnology.

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Biosynthesis of Nicotine as an Anti-insect Defense in Plants

Takashi Hashimoto

Graduate School of Biological Sciences, Nara Institute of Science and Technology,
630-0192 Nara, Japan
hasimoto@bs.naist.jp

Abstract

Nicotine and related pyridine alkaloids are synthesized in the root and then transported to the aerial parts of tobacco plants. Expression of biosynthetic genes for these alkaloids is enhanced 3-4-fold upon insect herbivory to the leaf. Current model suggests that jasmonate acts as a transmissible signal from the damaged leaf to the underground part, where it activates structural genes of nicotine biosynthesis via the conserved COI1-JAZ-MYC2 pathway. In Arabidopsis, the MYC2-family basic-helix-loop-helix transcription factors mediate transcriptional regulation of jasmonate-responsive genes, and their transcriptional activities are suppressed by physical interactions with Jasmonate-ZIM domain (JAZ) suppressors. Regulatory *NIC* loci that positively regulate nicotine biosynthesis have been genetically identified and their mutant alleles have been used to breed low-nicotine tobacco varieties. The *NIC2* locus comprises tandemly arrayed transcription factor genes of an Ethylene Response Factor (ERF) subfamily; in the *nic2* mutant, at least seven *ERF* genes are deleted altogether. Overexpression, suppression, and dominant-repression experiments using transgenic tobacco roots showed functional redundancy and divergence among the *NIC2*-locus *ERF* genes. These transcription factors recognized a GCC-box element in the promoters of nicotine pathway genes, and specifically activated all known structural genes in the pathway. We also demonstrate that tobacco*MYC2* controls nicotine biosynthesis genes in two combinatorial ways, by directly binding G-box in the target promoters, as well as by up-regulating the *NIC2*-locus *ERF* genes.

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Food Security and Agriculture Biotechnology Progress in Indonesia

Tantono Subagyo, Fadlilla Dewi Rakhmawaty

Syngenta Indonesia, Perkantoran Hijau Arkadia, Tower C, 8FL Jakarta

Abstract

Food security in Indonesia become serious problems because land conversion from agriculture is high and almost uncontrollable and the population growth rate is still high, the conditions worsened by climate uncertainty. One of the answer to this problems is to increase yield and it can be achieved by increasing seed quality using available technology. Although it is not panacea, biotechnology can provide tools to increase seed quality. Biotechnology research in Indonesia is done since 1980 but so far little products come to the market due to the regulations. Recent improvements in the system and regulations make the product pipeline moving, and it is hoped that the first launch of transgenic plants happen in 2012. However some improvements is still needed in order to make regulations in Indonesia facilitating biotechnology development.

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14-3-3 Proteins Act as Intracellular Receptors for Rice Hd3a Florigen

Hiroyuki Tsuji[1], Ken-ichiro Taoka[1], Izuru Ohki[2], , Kyoko Furuita[2] , Kokoro Hayashi[2], Tomoko Yanase[1], Midori Yamaguchi[1], Chika Nakashima[1], Yekti Asih Purwestri[1, †], Shojiro Tamaki[1], Yuka Ogaki[1], Chihiro Shimada[1], Atsushi Nakagawa[3], Chojiro Kojima[2,3] and Ko Shimamoto[1]

[1]*Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan*
[2]*Laboratory of Biophysics, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan*
[3]*Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
†Present address: Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

Abstract

'Florigen' was proposed 75 years ago to be synthesized in the leaf and transported to the shoot apex, where it induces flowering. Only recently have genetic and biochemical studies established that florigen is encoded by *FLOWERING LOCUS T* (*FT)*, a gene that is universally conserved in higher plants. Nonetheless, the exact function of florigen during floral induction remains poorly understood and receptors for florigen have not been identified. We show here that the rice FT homologue Hd3a interacts with 14-3-3 proteins in shoot apical cells, yielding a complex that translocates to the nucleus and binds to OsFD1, a rice homologue of *Arabidopsis* FD. The resultant ternary 'florigen activation complex' (FAC) induces transcription of *OsMADS15*, a homologue of *Arabidopsis APETALA1 (AP1)*, which leads to flowering. We determined the 2.4-Å crystal structure of a rice FAC. The modelled FAC structure provides a mechanistic basis for florigen function in flowering. Our results suggest that 14-3-3 proteins act as intracellular receptors for florigen in shoot apical cells and offer new approaches to manipulate flowering in various crops and trees.

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Food Security and Sustainable Agriculture

Paul P.S. Teng

S Rajaratnam School of International Studies
Centre for non-Traditional Security Studies (RSIS/CNTS)
and
National Institute of Education, Nanyang Technological University, Singapore 637616
paul.teng@nie.edu.sg

Abstract

Food security is a complex interplay of forces and is more than just an issue of supply and demand. Various definitions have been proposed but in general most consider access to safe food which is nutritious at all times to all people. Four dimensions of food security have been proposed in a conceptual model by RSIS/CNTS, namely food availability, physical access, economic access and utilization. While each dimension is necessary for overall food security, they likely have different weightings in a rural setting as compared with an urban setting and also across countries with different incomes and net food trade balances. Food availability, the first dimension of food security, addresses the 'supply side' and is determined by the level of food production, stock levels, food aid and net trade. Raising farm productivity is a core issue; whether by accessing or increasing inputs, improving seed varieties, or employing better farm management practices. Herein also lays the interplay with sustainable agriculture, as raising farm productivity has the potential to clash with sustainable agriculture goals. Food security implies surplus production over demand so that the excess may be sold or traded with food deficit households. If Sustainable Agriculture is taken to mean agriculture which is "environmentally friendly, economically sound and socially just", then ensuring food availability means ensuring that there is sustainable agriculture. A key debate is the use of appropriate technology and the modality in which it is practiced, whether in a conventional manner or through organic and subsistence farming. The debate is further complicated by the fast uptake of biotechnology crops. Food availability is often the focus of much of the debate on food security but raising farm productivity alone is not sufficient to ensure household food security. The second dimension is the physical access to food. This means an adequate amount of food must be within the physical reach of vulnerable households, whether through their own production or through the marketplace, to assure food security. Common threats to physical access to food are war, civil strife, poor infrastructure, inadequate logistics for food distribution and market imperfections. The third dimension is economic access to food or the ability of the household to purchase the food it requires. As the most recent food crisis demonstrated, urban households were among the hardest hit as they saw their purchasing power decline drastically and they had very limited capacity to produce their own food. Additional factors that will influence economic access include employment and income security, macroeconomic policies and social security programmes. The fourth dimension in food security is food utilization, typically reflected in the nutritional status of an individual and is determined by the quantity and quality of dietary intake, food safety and human health factors. Strategies to combat food insecurity arising from this dimension include bio-fortification of common staples through conventional or bio-technology. In the longer term, food security considered as a four-dimensional complex, is inextricably linked to sustainable agriculture,

which in an Asian context, must take into account the millions of smallholder farmers and the millions of urban poor.

Keywords: food security, sustainable agriculture

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Improvement of Oil Palm Productivity

Tony Liwang

Plant Production & Biotechnology Division,
PT SMART Tbk, BII Plaza, Tower 2, 10th Fl., Jalan M.H. Thamrin 51, Jakarta, Indonesia.
tony-liwang@smart-tbk.com

Abstract

Since 2009 Indonesia is the largest palm oil producer in the world. At the same time, Indonesia became the largest oil palm seed producer as well as consumer in the world. However, some major challenges facing in oil palm upstream industries in Indonesia are a narrow basic of genetic material, a long period needed for breeding cycle, limited human resources and research findings, and a limited genetic source for abiotic as well as biotic stress in some specific areas, and low absorption efficiency of nutrition. This paper explains the main research activities to improve the oil palm productivity. To enlarge and enrich the oil palm germplasm in Indonesia, a consortium of oil palm companies has carried out some explorations, such as to Cameron, Angola, and others countries. Moreover, the application of new gene resources has been studied for the phenotypic characters, genetic diversities and heterosities by using molecular markers. Elite varieties of oil palm could be obtained faster by the application of micro satellite and Single Nucleotide Polymorphism (SNP) markers from DNA genome sequences of selected genotype in early phase. The application and commercialization of oil palm tissue culture were the big challenge for the industry to improve the oil palm productivity at large scale, it might be included the clone of elite oil palm and DxP bi clonal seedlings with 20-30% higher of productivity compared to conventional oil palm. The originality of ramets could be done by using DNA fingerprinting. Genetic engineering technology focused on water stress tolerant, resistance to *Ganoderma sp.* and oil synthesis. Subsequently, the techniques of anther culture to produce dihaploid homozygous plant as a pure line will be used as a parental for F1 Tenera hybrid in order to produce oil palm with two to three times higher productivity compared to conventional oil palm.

Key words: oil palm, Elaeis guineensis *Jacq, biotechnology, tissue culture, productivity*

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Characterization of Water Availability, Management Practices and Grain Yield for Deepwater Rice in Northwest Cambodia

Nguyen T.B. Yen[a], Akihiko Kamoshita[a,*], Yuji Araki[a,b], Makara Ouk[c]

[a]*Asian Natural Environmental Science Center, The University of Tokyo, Japan*
[b]*Faculty of Education, Saitama University, Saitama, Japan*
[c]*Cambodian Agricultural and Development Institute, Phnom Penh, Cambodia*
**Corresponding author: Asian Natural Environmental Science Center,*
The University of Tokyo, 1-1-1 Midoricho, Nishitokyo, 188-0002 Japan.
Tel/fax.: +81-42-463-1696.
akamoshita@anesc.u-tokyo.ac.jp

Abstract

This study aimed to characterize rice area from the shallower (lower toposequence of) rainfed lowlands side to the deeper floating rice side in Northwest Cambodia during wet season rice (WSR) production in 2009 and 2010 for the yearly and spatial differences in field water conditions, management practices and grain yield. This area considered as deepwater rice (DWR) area was divided into three groups with (1) upper fields located near the National Road Number 5 (referred as ULR); (2) middle fields (referred as MLR and MFR where lowland rice (LR) and floating rice (FR) were planted, respectively); and (3) lower fields located near to the Lake (referred as LFR). Water came to the paddy fields from both the inundation from Tonle Sap Lake and rainfall in WSR 2009, but only from rainfall in WSR 2010. Water depths increased from upper to lower fields in 2009, while they were shallower and relatively similar between the field locations in 2010. Broadcasting time started earlier and harvesting time finished later in FR fields than in LR fields. The overall average grain yield for both years of 2009 and 2010 was low. The lowest yields were observed in MLR and MFR in 2009 as well as they were in MFR and LFR in 2010. Lower yield in WSR 2009 was mainly due to the water shortage at heading stage for both FR and LR, improper application of N fertilizer and insufficient weed management for LR, and late sowing for FR. Lower yield in WSR 2010 was mainly due to low water level for FR, low N fertilizer rate and insufficient weed management. This study identified important spatial and yearly variation in rice management and grain yield for farmers to cope with unpredictable flooding environments in DWR area.

Keywords: Cambodia, deepwater rice, flooding pattern, rice type

Introduction

In Cambodia, deepwater rice (DWR) areas are located in the provinces near to the Tonle Sap Lake, the Mekong River, and Tonle Bassac River. In 1960s, the DWR area occupied up to 16% of Cambodia's rice land (about 400,000 ha) (Javier, 1997; Seng *et al,* 1988). However, as the discouragement of growing DWR during Pol Pot regime, DWR area decreased sharply and it was only 120,000 ha in 1988 (Seng *et al.,* 1988). DWR presented 3.9% of the cultivated area in 2006 (MAFF, 2006). Cambodian's Tonle Sap Lake (TSL) floodplain is well known for its unique dynamic flooding pattern between dry and rainy season. Volume of the Lake ranges from about 1.3 to 75 km^3, its surface area varies from 2,500 to about 15,000 km^2, and its water level increases from 1.4 to 10.3 m above sea level, between dry and rainy season (MRC, 2010a). DWR is important source of livelihood to many poor villages in the TSL floodplain that do not have access to better agricultural land higher up. There is a need to further increase the productivity of DWR in order to improve the local farmers' livelihood and to conserve the DWR area. However, necessary information for yield improvement in DWR area such as flooding pattern, land use pattern, management practices in Cambodia are not sufficient. We conducted a study to quantify the yearly and spatially difference in field water condition in DWR area in Northwest Cambodia, and to assess rice management practices and grain yield in the area.

Materials and Methods

The study was carried out at a DWR area inside a flood plain area of TSL located in Kampong Preah village, Kampong Preah commune, Sangke district, Battambang province, Northwest Cambodia in 2009 and 2010. Eighty five fields (91.2 ha in planting area) were selected which were located continuously along a transect line from a toposequentially upper zone nearby National Road Number 5 toward TSL. These 85 fields were divided into three groups according to their field locations : (1) upper fields located closer to the National Road Number 5 where only LR was grown (19 fields; 11.4 ha) and referred as ULR; (2) middle fields where both LR and FR were grown (19 fields; 31.6 ha) and referred as either MLR or MFR; and (3) lower fields located near to the Lake where only FR was grown (37 fields; 48.2 ha) and referred as LFR. Variety, field size and type of rice were determined for the 85 fields in WSR 2009 and 2010. Thirty fields within the 85 fields were selected from each field group; sowing time, mid-season tillage practice, fertilizer input, pest control and grain yield were determined by the interviews to the owners in ending time of each WSR.

Results and Discussion

Field water environment

Field water regime of the studied area in WSR 2009 was completely different from that in WSR 2010 (Figure 1). In WSR 2009, water came to the fields from both the inundation from the Tonle Sap Lake and rainfall. Flood started earliest in lower fields in September, then middle and upper fields afterward. Flood started receding to the Lake in late October and rice fields became non-flooded conditions in middle fields and upper fields in late November and in lower fields in early to mid December. There was large difference in water depth between the 3 field locations in 2009. Rice ecosystems of the three locations could be classified as DWR for middle and lower fields and medium-deep rainfed lowland rice for upper fields (Mackill *et al*., 1996). In WSR 2010, water came to the fields only from the rainfall; field water regime was relatively similar to all the 3 field locations with the average maximum water depths less than 30 cm in mid October. These water conditions in 2010 were more favorable for rainfed lowland rice than floating rice. Low water level in Tonle Sap Lake leading to shrink floodplain area in 2010 was probably due to extremely low water level in Mekong River (IRIN news, 2010) which was caused likely by a combination of an early end to the 2009 wet season, low monsoon rainfall and very low rainfall in the dry season in upper Mekong Basin (MRC, 2010b).

Management practices

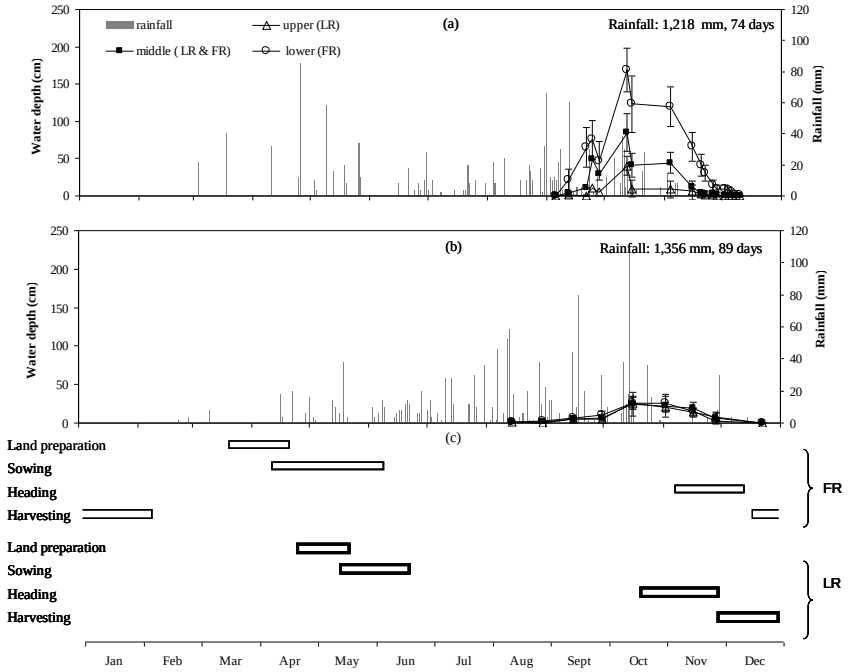
Crop calendar

Planting season for FR fields is earlier than that for LR fields. For FR fields, farmers start land preparation from early March to late April (Figure 1). Dry seeds with rate of about 100 kg ha^{-1} are broadcasted mainly from early April to early May. Farmers sometimes have to broadcast seeds for the second time in late May or early June if rice establishment failed due to drought. For LR fields, land preparation is often from mid April to mid May. Dry seeds with higher rate in comparison with FR (150 kg ha^{-1}) are broadcasted from mid May to mid June. Rice is harvested from early December to early February in FR fields and from late November to early January in LR fields, both depending on maturity type of rice varieties.

Distribution of rice type and varieties

LR and FR were both grown in the studied area in the both WSR 2009 and 2010. In general, early medium varieties of LR were only planted in upper fields while medium varieties of LR were planted in both upper and middle fields. Early varieties of FR were only planted in middle

fields while medium and late varieties of FR were planted in both middle and lower fields. In comparison between WSR 2009 and 2010, there was a small change in the area ratio of LR and FR in middle area. Area of LR decreased from 38% in WSR 2009 to 30% in WSR 2010 but area of FR increased from 62% in WSR 2009 to 70% in WSR 2010. Among the varieties planted in the studied area, early varieties of FR in the middle area had the largest increase in area percentage, from 22% in WSR 2009 to 47% in WSR 2010.



(a) and WSR 2010 (b), and cropping calendar for FR and LR (c) in the studied area. The error bars indicate standard deviations.

Figure 1. Rainfall distribution and water depth in the 3 field locations (upper, middle and lower) in WSR 2009.

Relationship between grain yield, environmental and management factors

In WSR 2009, grain yields of ULR and LFR (120 and 164 g m^{-2}, respectively) were significantly higher than those of MLR and MFR (50 and 56 g m^{-2}, respectively). However, grain yields of ULR and MLR (both 262 g m^{-2}) were significantly higher than those of MFR and LFR (87 and 111 g m^{-2}, respectively) in WSR 2010.

Average grain yield of both WSR 2009 and 2010 was low with only 180 g m^{-2} for LR and 110 g m^{-2} for FR. The lowest yields were observed in MLR and MFR in WSR 2009 which was due to risky water environments in middle fields. With maximum water depth of 85 cm and absence of standing water after late November (Figure 1), the water condition in this area was too deep for LR for a possible submergence damages and insufficient for medium and late maturing FR to maintain good grain filling. This was also supported by the positive correlation between maximum water depth and grain yield for FR and negative correlation between those for LR (Table 1). Farmers responded to this risky water condition by changing rice type/varieties from year to year based on yield obtained from previous year. The increase in area of early variety of FR in WSR 2010 was due to that farmers observed that yields of early variety of FR in middle fields were higher than other varieties of LR and FR in this area (data not shown).

Low yield in WSR 2009 was mainly due to (1) the water shortage at heading stage for both FR and LR, (2) non- or late application of N fertilizer for LR, (3) insufficient weed management for

LR and (4) late sowing for FR (Table 1). (1) Due to low rainfall occurring at pre-flood period (June to August; Figure 1a) in WSR 2009, rice plants suffered drought stress leading to delayed heading, mainly from late November to mid December while there was also low rainfall in the late season. This caused the shortage of water at heading stage of 10 out of total 29 fields. Those fields where rice plants did not suffer drought at late stage were mainly located in lower part, or bunded with high levees, or pumped water from the water source nearby. This indicated that well-water management, especially at heading stage, is crucial to improve yield in DWR area, particularly in middle and upper fields. (2) In WSR 2009, LR plants were likely submerged during high flood (40 cm in upper fields and 85 cm in middle fields), growing situation of rice plants before flood commence was very crucial for gaining high grain yield. Taller and more vigorous rice plants, which could be improved by basal application of N fertilizer (before flood commence), were able to withstand submergence and hence gave higher yield and this was also indicated in Puckridge (1991). It was suggested that rice plant should be able to uptake more than 20 kg N ha^{-1} before onset of flooding in order to reduce yield loss due to submergence (Puckridge, 1991) while Sharma and Gosh (1998) reported that optimum basal fertilizer rate for semi-deep water environment was 30 kg N ha^{-1}. The average N fertilizer applied in LR field in WSR 2009 was less than half of this recommendation rate (4-15 kg N ha^{-1}; data not shown). (3) Our study showed that conducting midseason tillage practice, a weed control method, helped to improve yield of LR in WSR 2009. All of LR fields were conducted the practice in WSR 2010 while about half of LR field number were not conducted in WSR 2009, which was perhaps due to water constraint (drought or flood at the time farmers wanted to do the practice). (4) Late sowing significantly decreased yield of LFR. High rainfall occurred mainly from late April to mid May while only few rains with small amount occurred in June and July (Figure 1a). This might be the reason leading the poor establishment of crop with late sowing. When the flood arrived, this late sowing rice was likely more susceptible to the rapidly rising water in September. The importance of sowing in time was also mentioned by Catling (1983), Javier (1997) and Sing *et al*. (2004).

Table 1. Correlation between rice yield and water condition, management factors of LR and FR in WSR 2009 and WSR 2010

	WSR 2009		WSR 2010	
Items	LR (n=13)	FR (n=16)	LR (n=13)	FR (n=17)
Water condition				
max WD (cm)	-0.784***	0.679***	0.374	0.326
flooded at heading stage (n=29) [a]	0.715***			
Sowing time (DOY)	0.145	-0.345*	-0.269	0.157
Fertilizer management				
fertilizing before flood commence [b]	0.581**		0.386	
N fertilizer (kg ha^{-1})	0.331		0.504*	
Weed management				
conducting mid-season tillage [c]	0.492*			
plowing for mid-season tillage [d]			0.653**	
herbicide (g a.i. ha^{-1})	0.253	-0.400*	-0.193	0.628***

*P<0.1; **P<0.05; ***P<0.01

[a] values for flooded at heading stage and those for other water conditions at heading were 1 and 0, respectively, as a dummy variable

[b] values for inorganic fertilizer applied before flood commence and those for not applied or after flood commence were 1 and 0, respectively, as a dummy variable

[c] values for conducting mid-season tillage and those without the practice were 1 and 0, respectively, as a dummy variable

[d] values for conducting mid-season tillage by plowing and those for conducting the practice by harrowing were 1 and 0, respectively, as a dummy variable

Low yield of FR in WSR 2010 was mainly due to low water level as the discussion at beginning of this section. Beside that insufficient weed control was also another reason. Higher application rate of herbicide was applied in WSR 2010 in comparison with that in WSR 2009 and low application rate of herbicide significantly reduced yield of FR in WSR 2010 (Table 1). This was because that weed infestation was more severe throughout the crop season due to the low water level in WSR 2010 while FR fields were not conducted midseason tillage for controlling weed like LR. Different from WSR 2009, water condition in WSR 2010 was more favorable for growing LR like in rainfed lowland environment. Therefore, yield of LR in WSR 2010 could be further improved by conducting midseason tillage with plowing for controlling weed (Table 1) and increase application rate of N fertilizer. The N fertilizer rate applied this WSR was only about one third of the recommendation rate for rainfed lowland rice in drought or submerged prone area with rate of 60 kg ha^{-1} (Balasubramnian and Hill, 2002). However, it should be noticed that field water environment in WSR 2010 was not representative for most years in the studied area.

In short, it is risky for farmers to attempt to increase planting higher yielding lowland rice in the middle part of the floodplain of TSL, due to the occurrence of deep flood incidence, but farmers could miss chances of higher yield if planting traditional floating rice varieties just to escape from submergence damage. It is desirable if information on water situation in the area is informed to farmers by a long-term weather forecast before the cropping season (February or March). So that farmers will be able to make a right decision of selecting rice type/variety to grow in the area. Beside that early maturity varieties with higher yield potential and tolerance to submergence (suitable for medium-deep water area) can be introduced in to the area in order to improve grain yield in a sustainable manner.

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Submergence Escape in *Oryza glaberrima* Steud.

Jun-Ichi Sakagami[a,*], Chiharu Sone[a], Naoyoshi Kawano[b]

[a]*Crop, Livestock and Environment Division, Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan*
[b]*Rural Development Division, Japan International Research Center for Agricultural Sciences, Tsukuba, Ibaraki, Japan*
**Corresponding author: Crop, Livestock and Environment Division, Japan International Research Center for Agricultural Sciences, 1-1, Ohwashi, Tsukuba, Ibaraki 305-8686, Japan*
Tel.:+81 29 838 6368; Fax: +81 29 838 6355.
sakajun@affrc.go.jp

Abstract

Oryza glaberrima, an African monocarpic annual rice derived from *Oryza barthii*, is grown in traditional rice producing wetland areas of West Africa. *Oryza sativa*, an Asian rice that varies from annual to perennial, is derived from *Oryza rufipogon*. Genotypes of *O. glaberrima* are inherently lower yielding than those of *O. sativa* and are, therefore, cultivated in fewer areas. However, because they grow adequately in unstable environments such as those with water stress, they appear to tolerate severe environmental stress. Cultivars of *O. glaberrima* are roughly divisible into two ecotypes: upland and lowland. However, it might be that *O. glaberrima* is a valuable rice species for flooding conditions in all cases. To elucidate the physiological responses of young rice plants to short-term submergence stress, so-called flash flooding, under rainfed conditions for *O. glaberrima* by comparison with several genotypes for lowland adapted, deepwater adapted shoot elongated escape and *Sub1* of *O. sativa*, 30-day-old seedlings were submerged completely for 10 d at 45 cm water depth at 13 d after transplantation in a lowland field. In fact, *O. glaberrima* showed higher shoot elongation ability during submergence than any genotype of *O. sativa* that we tested. However, *O. glaberrima* lodged easily after the end of submergence because of longer and more rapid shoot elongation during submergence. Therefore, it triggered a decrease in its survival rate. On the other hand, various lines of 35 *O. sativa* and 27 *O. glaberrima*, including some classified as short-term submergence tolerant, were compared for submergence tolerance in field and pot experiments to long-term submergence tolerant varieties in other words, deepwater varieties. Submergence-tolerant cultivars of *O. sativa* were unable to survive prolonged complete submergence for 31–37 d, which indicated that the mechanism of suppressed leaf elongation that confered increased survival of short-term submergence was inadequate for surviving long periods underwater. The superior tolerance of deepwater *O. sativa* and *O. glaberrima* genotypes to prolonged complete submergence appeared to be attributable to their greater photosynthetic capacity developed by leaves that had newly emerged above the floodwater. Cultivars of *O. glaberrima* adapt to long-term complete submergence. Cultivar “Saligbeli” adapted to short to long term submergence.

Keywords: glaberrima, lodging, photosynthesis, shoot elongation, submergence escape

Introduction

Rice farming in Africa began approximately 3500 years ago (Porter, 1970). The rice variety cultivated at the time was African rice (*Oryza glaberrima* Steud.), which is native to the continent (Chu and Oka, 1972). African rice is well adapted to the severe local climate, but it has had little opportunity to be improved or selected by humans. It is, therefore, regarded as wild and of low productivity. For that reason, Asian rice (*O. sativa* L.) accounts for a high proportion of the rice consumed in Africa today. However, the development and promotion of a NEw RIce for AfriCA (NERICA) is attracting attention in Africa. Moreover, the properties of African rice, which was employed as a parent strain in the production of NERICA, are now being reassessed. In this manner, rice farming in Africa has been developed differently from that in Asia, and cultivation and cooking methods also differ in many ways. Farmers cultivate rice in irrigated lowlands in Asia,

although rainfed uplands in Africa account for most rice fields. African farmers attach great importance to the quantity immediately after cooking in many cases (Sakagami *et al*., 2008). Although rice consumption in African countries has shown an increasing trend in recent years, the production rate is not sufficient to meet the demand. The volume of imported rice is increasing annually, which is having a detrimental effect on African countries' economies.

In general, rice cultivation is vulnerable to natural disasters in West Africa. One reason is the shortage of suitable irrigation systems. The area of irrigation is less than 20% of the total area of rice cultivation, and most rice is planted in rainfed regions (Balasubramanian *et al*., 2007). Therefore, rice cultivation in West Africa is strongly influenced by precipitation or overflow from rivers. However, the status of damage to rice plants by flooding in West Africa has not been understood well until now. Upland rice is severely influenced by rainfall because of the lack of standing water. Yields of upland rice are very low (around 1 t ha^{-1}) compared with those of lowland rice cultivation (around 2 t ha^{-1}) (Norman and Otoo, 2003). Lowlands, therefore, offer greater potential for raising rice production, and represent about 20–50 million hectares, depending on the definition used. At present, only about 10–20% of this area is under cultivation (Africa Rice Center, 2004), thereby offering great potential for rice farming expansion. However, rice plants in lowland areas are often damaged by floods caused by heavy rain. It is, therefore, important to study the effects of submergence on rice plants to develop sustainable rice production in West Africa.

Genotypes of *O. glaberrima* are inherently lower yielding than those of *O. sativa* and are, therefore, cultivated in fewer areas (Linares, 2002). However, because they grow adequately in unstable environments such as those with water stress, they appear to tolerate severe environmental stress. Flooding imposes severe selection pressure on plants, principally because excess water in the plant surroundings can deprive them of certain basic needs, notably of oxygen and of carbon dioxide and light for photosynthesis. It is a major abiotic influence on species' distribution and agricultural productivity world-wide. Based on our analysis, most *O. glaberrima* varieties adapt well when floods are deeper and when they entail long-term submergence in Africa because of their greater photosynthetic capacity developed by leaves that have newly emerged above floodwaters through rapid shoot elongation.

Materials and Methods

Experiment 1: Responses to short-term submergence "flash flood"

To elucidate the physiological responses of young rice plants to short-term submergence stress, so-called flash flooding, under rainfed conditions for *O. glaberrima* by comparison with several genotypes for lowland adapted, deepwater adapted shoot elongated escape and *Sub1* of *O. sativa*, 30-day-old seedlings were submerged completely for 10 d at 45 cm water depth at 13 d after transplantation in a lowland field of Guinea. Dry matter production, plant height, lodging and surviving rate were compared.

Experiment 2: Responses to long-term submergence "deep water"

Various lines of 35 *O. sativa* and 27 *O. glaberrima*, including some classified as short-term submergence tolerant, were compared for submergence tolerance in field and pot experiments to long-term submergence tolerant varieties in other words, deep-water varieties. Plants were submerged completely for 31 d in a field experiment, and partially or completely for 37 d in a pot experiment in a growth chamber. Dry matter production, plant height, leaf area, and photosynthetic rate were compared.

Experiment 3: Flash flood tolerance for cultivated species

Lodging, plant height, and dry matter accumulation for 99 cultivars in *O. sativa*, *O. glaberrima*, and interspecific hybridization progenies (IHP) were measured when 12-day-old

seedlings were submerged completely for 7 d in pots and in fields to make an evaluation of flash flood tolerance.

Results and Discussion

There was a high positive correlation ($P < 0.01$, $r = 0.86$) between shoot length elongation during short term submergence and lodging score at 15 DAS (Day After Submergence) in experiment 1. DRL (Deep-water to Rainfed Lowland genotype in *O. glaberrima*) showed higher shoot elongation during submergence and a higher lodging score after desubmergence than other genotypic groups. On the other hand, ST (Submergence Tolerance genotype) showed the opposite features to DRL with lower shoot elongation and lodging score. RL (Rainfed Lowland genotype), DW (Deep-Water genotype) and SE (Shoot Elongation genotype) showed intermediate traits in shoot length elongation and lodging score between DRL and ST. Figure 1 shows the relationship between shoot length elongation during submergence and survival rate at 19 DAS. There was a negative correlation between shoot length elongation and survival rate ($P < 0.05$, $r = -0.66$).

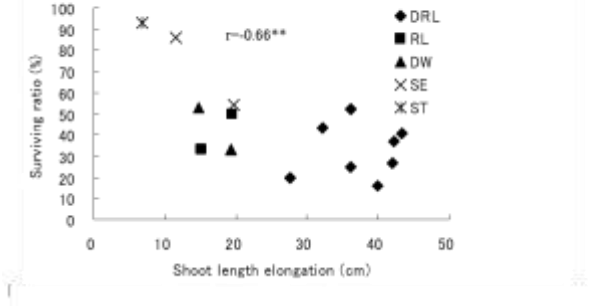
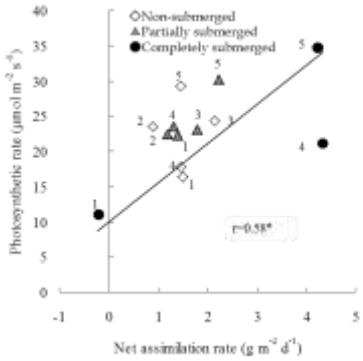


Figure 1. Effect of shoot length elongation on survival rate. Survival rate was calculated for number of plants before submergence divided by number of surviving plants at 19 day after desubmegence

ST of *Sub1* showed the highest survival rate (93%) and the shortest shoot length elongation (6.8 cm) as well as IR 62293- 2B-18-2-2-1-3-2-3 (86%, 11.5 cm) in the SE group. The survival rate of DRL was lower compared with ST. *O. glaberrima* showed higher shoot elongation ability during submergence than any genotype of *O. sativa* tested. However, *O. glaberrima* lodged easily after desubmergence due to longer and rapid shoot elongation during submergence, and thus triggered a decrease in its survival rate. We suggested that *O. glaberrima* was susceptible to short-term submergence while it may be adapted to prolonged flooding because of improved restoration of aerial photosynthesis and survival rate through shoot elongation ability

All cultivars of *O. sativa* with submergence tolerance based on the quiescence strategy failed to regain contact with the aerial environment and died during 31 d submergence in the field experiment 2. In contrast, all *O. glaberrima* genotypes resurfaced and survived submergence. The photosynthetic rates of the youngest fully expanded leaf of the main shoot of pot-grown plants were measured in pot experiment 2. The non-submerged rate for 'IR73020' (30.9 μmol m^{-2} s^{-1}) 1 DBS(Day Before Submergence) was significantly above those for other genotypes. The rate for 'Yele1A' (6.74 μmol m^{-2} s^{-1}) was the lowest. It was, however, significantly greater for Yele1A' than for other genotypes in non-submergence plots (29.2 μmol m^{-2} s^{-1}), in partial submergence plots (30.3 μmol m^{-2} s^{-1}) and in complete submergence plots (34.8 μmol m^{-2} s^{-1}) 37 DAS. The photosynthetic rate at 37 DAS in partial and complete submergence was closely related to the NAR (Net Assimilation Rate) during submergence in the pot experiment (Figure 2).

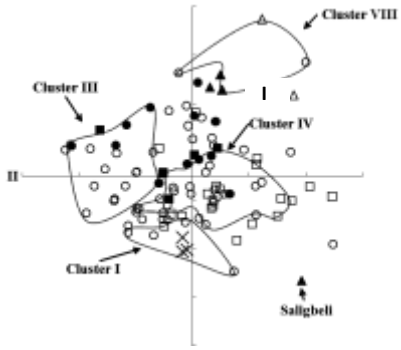


Symbols represent non-submerged plants, partially submerged plants and completely submerged plants as indicated. The number next to each symbol indicate the cultivars: 1, 'Banjoulou'; 2, 'IR71700'; 3, 'IR73020'; 4, 'Nylon'; and 5, Yele1A'

Figure 2. Relationship between net assimilation rate during submergence and photosynthetic rate after 37 d submergence in a pot experiment.

The superior tolerance of deepwater *O. sativa* and *O. glaberrima* genotypes to prolonged complete submergence appears to be due to their greater photosynthetic capacity developed by leaves newly emerged above the floodwater. Vigorous upward leaf elongation during prolonged submergence is, therefore, critical for ensuring shoot emergence from water, leaf area extension above the water surface and a subsequent strong increase in shoot biomass. Increase in shoot DMA after desubmergence was negatively correlated with shoot elongation during submergence, $r = -0.36$ ($P < 0.01$) in experiment 3. ST were plotted in the area of short shoot elongation and high increase in DMA (Dry Matter Accumulation), while *O. glaberrima*, except for Saligbeli, were plotted in the area opposite to that of ST. Some LS (Lowland Sativa genotype) and LI (Lowland Interspecific progenies genotype) were plotted close to the ST for both shoot elongation and shoot DMA. Increase in DMA after desubmergence of all US (Upland Sativa genotype) was less than that of ST.

The first principal component explained 46.8%, and for the classification of cultivars according to their physiological responses to flash floods. The results of the cluster analysis were compared to the principal component analysis results (Figure 3). Principal component analysis was performed with the increase in DMA from 1 DAD to 14 DAD, and lodging at 1 DAD, shoot elongation during submergence, and increased DMA during submergence. In Clusters I, III, and VIII, the main genotypes belonging to each cluster group were classified on the principal component analysis. Cluster I, including ST cultivars, and Cluster VIII, including *O. glaberrima*, were positioned in opposite regions except for Saligbeli. Saligbeli exhibited enhanced shoot elongation with the increase in DMA during submergence. These features seemed to be a unique way to cope with submergence.



Axis I is the first principal component (y =−0.403942 x1 + 0.434866 x2 + 0.329416 x3 −0.271996 x4). Axis II is the second principal component (y =−0.068947 x1 −0.080874 x2 + 0.618871 x3 +0.722613 x4), x1, x2, x3 and x4 represent increase in DMA after desubmergence, lodging score, shoot elongation and increase in DMA during submergence respectively. All data are standardized. (●) Upland Sativa, (○) Lowland Sativa, (▲) Upland Glaberrima (△) Lowland Glaberrima, (■) Upland IHP, (□) Lowland IHP, (×) Submergence tolerance.

Figure 3. Principal component analysis of the physiological traits linked to submergence (shoot elongation during submergence, increase in dry matter accumulation during submergence, lodging score and increase in dry matter accumulation after desubmergence.

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Toposequnential Variation in Soil Fertility and Limiting Nutrient for Rice Growth in the White Volta Floodplain of Northern Ghana

Yasuhiro Tsujimoto[a*], Yukiyo Yamamoto[a], Keiichi Hayashi[a], Alhassan I. Zakaria[b], Yahaya Inusah[b], Tamao Hatta[a], Mathias Fosu[b], Jun-Ichi Sakagami[a]

[a]*Japan International Research Center for Agricultural Sciences, Japan*
[b] *Savanna Agricultural Research Institute, Council for Scientific and Industrial Research, Ghana*
**Corresponding author: Japan International Research Center for Agricultural Sciences,*
1-1 Ohwashi Tsukuba, Ibaraki 305-8686, Japan
Tel.: +81 29 838 6368; fax: +81 29 838 6355.
tsjmt@affrc.go.jp

Abstract

Integrated floodplain resource management for rice cultivation is imperative to satisfy the growing demand of rice in West Africa. Irrigated pot experiments were conducted with different fertilizer treatments to identify toposequential variation in soil fertility and limiting nutrient for rice growth within the White Volta floodplains in Ghana. Eighteen experimental soils were examined across a riverside to upland gradient, ranging from 898 to 4200 m in distance from the main riverside, from 73 to 106 m in elevation, and from 0.7 to 2338.1 m in distance from water sources. The soil analysis revealed close correlation between N-mineralization rates and carbon contents of the soils, which were exponentially decayed with distance from water sources. In the non-fertilized treatments, plant N uptakes at maturity also decreased along the same transect from the water sources. However, the dry matter production was little relevant to this toposequential factor. Various fertilizer treatments identified remarkable effect of sulfur on rice growth, which was more significant on soils closer to waster sources. NPK application without S increased only N concentration and N: S ratio in plant tissues but not biomass production. The results indicated that sulfur is the primary limiting element for rice growth, and its supplementation would be more beneficial as closer to water sources so as to effectively utilize greater N-supplying capacity of this agro-environmental soils.

Keywords: rice, sulfur deficiency, floodplain, soil carbon, water source

Introduction

River floodplains, consisting of wide and flat plain of alluvium bordering rivers, are expected to support a large expansion in rice cultivation area and production in Africa, of which the major share is currently unexploited. Major constraints to expand agricultural activities into river floodplains include difficulty in water control, risk of complete submergence of plants, occurrence of water-borne diseases, and disadvantage in access to the road and market (Balasubramanian *et al*. 2007). On the other hand, this geographical environment can provide water resources as well as relatively fertile alluvial soils compared to uplands (Buri *et al*. 1999). Moreover, the use of river floodplains for rice cultivation should not cause spatial competition with the other crops due to the risk of periodic flooding.

In the traditional lowlands in Asia, large variance in cultivation conditions and rice productivity are commonly recognized within relatively small areas, and farmers' management practices are adapted to this variance according to the sequential changes in soil fertility and water availability (Fukai *et al*. 1998). However, either quantitative data or farmers' practical adoption on the toposeqential distribution of environmental resources are scarce for rice cultivation within river floodplains in West Africa.

Primary objectives of this study were, therefore (1) to quantify the rice productivity of soils in relation to small-scale toposequence and (2) to identify deficient nutrient for rice growth, which

results can help effective use of soil nutrient resources and development of toposequence-specific fertilizer management practices within a target environment. To achieve these objectives, phytometry experiments with rice were conducted under various fertilizer treatments by using multiple soils collected within a Whiter Volta floodplain in the northern Ghana. In addition to the major macronutrients of N, P and K, target nutrients included Zn, Si and S which deficiency have been previously reported in the region and in the similar hydromorphic soils in the other parts of the sub-Sahara Africa (Buri *et al*. 2000; Tsujimoto *et al.* 2010).

Materials and Methods

Sampling of experimental soils

Experimental soils were collected from the top 0-15 cm at 18 points across a riverside to upland gradient in the White Volta floodplains of northern Ghana at the beginning of rainy season in June, 2010. The sampling points ranged from 898.7 to 4200.0 m in distance from the main Volta riverside, from 73 to 106 m in elevation (ASTER-GDEM), and from 0.7 to 2338.1 m in distance from water sources. The average slope angle was 0.86 % between the closest and furthest sampling points from the riverside. Distance from water sources were calculated after extracting rivers and back-swamps by the image analysis of Quickbird imagery. Experimental soils were air-dried for a week in a screen house at the Savanna Agricultural Research Institute (9˚ 26' N, 0˚ 59' W, 183 m asl.). Thereafter, the soils at the air-dry equivalent weight (4.2 kg) were put into 7-l plastic pots followed by flooding and puddling with different fertilizer treatments.

The chemical and physical properties of the experimental soils were analyzed after air-dried and sieved. Total C and N were determined by automatic high sensitive NC analyzer, Sumigraph NC-220F (SCAS, Japan). Mineralizable nitrogen was determined by a 4-week anaerobic incubation at 30°C as the amount of NH_4^+-N extracted with 10% KCl solution. Available phosphorus content was measured by Bray No.2 method. Extractable sulfate was determined by extraction with KH_2PO_4 solution containing 500 mg l^{-1} P. CEC was measured by the ammonium acetate extract method at pH 7.0. Exchangeable bases were determined by plant atomic emission spectrometer, ICPE-9000 (Shimadzu, Japan). Soil texture was determined by sieving and pipetting method.

Experiment design and measurements

Two pot experiments were conducted. In the Experiment 1, rice was grown with no fertilizer inputs, using all the 18 experimental soils. In the Experiment 2, three of the 18 experimental soils were selected in a wide range of the distance from water sources (*Bottom*: 40 m; *Middle*: 501 m; *Top*: 1870 m), for which ten different fertilizer treatments were established including no fertilizer treatment in the Experiment 1: 1. Control (no fertilizer); 2. +N; 3. +P, 4. +K, 5. +NP; 6. +NK; 7. +NPK, 8. +NPKSi, 9. +NPKZn, 10. +NPKS. The chemical forms and application rates for each nutrient were 0.70 g N, 0.22 g P, 0.36 g K, 1.87 g Si, 0.05 g Zn, and 0.23 g S per pot as Urea, NaH_2PO_4, KCl, SiO_2, $ZnCl_2$, and Na_2SO_4, respectively. Both experiments had three replicates, followed by the same cultivation management and measurements. Two 21-day old seedlings of a local cultivar, *Jasmine85*, were transplanted at a rate of one hill per pot on June 26 in 2010, and were subsequently harvested on October 8 in 2010. The water level was kept above 2 cm throughout the rice growing period. Weeds were removed manually. No specific pest management was conducted.

The plants were harvested from each pot at maturity. The dry weights of the plant samples were determined after oven drying at 80˚C to a constant weight. The plant N concentration was determined by using automatic high sensitive NC analyzer, Sumigraph NC-220F (SCAS, Japan). The plant nutrient concentrations of P, K, S, Mg, Ca, Zn, Fe, Mn, Cu, and B were analyzed by plant atomic emission spectrometer, ICPE-9000 (Shimadzu, Japan), after digesting each sample with HNO_3 and H_2O_2 in microwave digestion system (MLS-1200 MEGA, Milestone Inc.). The plant

nutrient uptakes were calculated as the product of the dry weight and the nutrient concentration of each plant tissue. Statistic analysis was performed by using JMP 8 software (SAS Institute Inc.).

Results and Discussion

Toposequential variation in soil fertility and plant growth (Experiment 1)

The results of soil analysis revealed large variances in soil properties within the gently sloping floodplain area (Table 1). The total carbon (TC) and total nitrogen (TN) contents ranged from 3.48 to 30.15 g kg^{-1} and from 0.28 to 2.25 g kg^{-1}, respectively. Although differing among the locations, available P and extractable S were both below the critical deficiency levels shown by Dobermann and Fairhurst (2000). This was in accordance with the previous studies conducted in the same agro-ecological zones of West Africa (Buri *et al.* 1999, 2000).

Table 1. Soil properties and its correlation with the total carbon contents (n=18).

	Mean	Max	Min	coefficiency of variation (%)	correlation coefficient to Total C
pH 1:2.5 (H_2O)	6.29	7.56	5.42	10.8	-0.43
Clay (%)	13.65	30.96	5.60	54.2	0.80***
Total C (g kg^{-1})	11.91	30.15	3.48	64.4	-
Total N (g kg^{-1})	0.89	2.25	0.28	65.3	0.91***
Mineralizable N (mg kg^{-1})	43.53	141.10	1.42	104.5	0.96***
Available P (mg kg^{-1})	11.26	31.85	1.98	79.5	0.58*
Extractable S (mg kg^{-1})	8.72	14.48	6.09	24.4	0.50*
CEC (cmol kg^{-1})	6.97	15.10	3.07	56.8	0.80***
Exchangeable cation (cmol kg^{-1})					
Na	0.75	1.54	0.33	50.3	0.57*
K	0.35	0.47	0.24	23.3	0.64**
Mg	1.05	2.65	0.40	68.9	0.80***
Ca	1.89	4.21	0.80	59.6	0.78***

The total carbon (TC) contents of the soils correlated to most of the other soil properties, exponentially decreased as the distance from water sources (Figure 1a). This toposequential gradient in the TC, SOC or clay contents of the soils was also observed in the other rice-growing lowlands of Asia (Homma *et al.* 2003; Tsubo *et al.* 2006). The accumulated TC contents in the lower parts of the toposequence were most likely attributable to greater deposition of clay minerals, and to longer periods of submergence that alleviates the decomposition of organic substrates (Sahrawat 2004).

The total N uptakes of rice plants also showed exponential reduction along the same transect from the water sources (Figure 1b). This could be explained by large differences in soil mineralizable N, that were highly correlated with the TC contents (Table 2). Soil mineralizable N was regarded as a good index of soil N-supplying capacity, and sufficiently correlated with plant N uptakes under non-N fertilized conditions in previous studies (Russell *et al.* 2006; Tsujimoto *et al*. 2010). However, top dry matter (TDM) yield was less relevant to this toposequential factor (Figure 1b). The TDM production highly fluctuated within a 100 m radius of water sources, and one had a relatively high yield level at upper area of the floodplain. This discrepancy in responses with rice production to N-supplying capacity of the soils indicated that there existed other limiting elements apart from N.

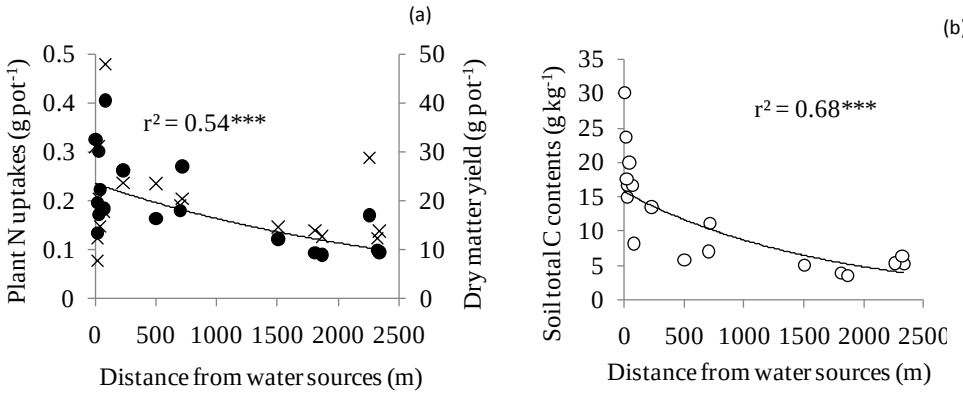

Figure1. Relationship between distance from water sources and (a) soil TC contents and (b) plant N uptakes (closed circles: ●) and top dry matter yield (cross marks: X).

Effects of fertilizer treatment and soil type on rice growth (Experiment 2)

The results of ANOVA showed significant F-values for the main and interactive effects of fertilizer treatment and soil type on the TDM production. The application of NPKS demonstrated stunningly higher TDM yields compared to the Control plots (Table 2). The differences in TDM yields between NPKS and Control plots were greater as closer to water sources (80.5; 40.7; and 29.7 g pot^{-1} on the *Bottom*, *Middle*, and *Top* soils, respectively). On the other hand, the application of N, P, and K or combinations of these elements without S showed no effects on the TDM yields for any of the experimental soils.

Table 2. Top dry matter yields and N: S concentration ratio at maturity as affected by different fertilizer treatments for the three experimental soils.

Fertilizer	Top dry matter yield (g pot^{-1})			N: S ratio		
	Bottom	*Middle*	*Top*	*Bottom*	*Middle*	*Top*
C	14.7 $^{fghijkl\#}$	23.5 ^{def}	12.7 ^{hijkl}	29.2 ^{f}	13.0 ^{hi}	12.9 ^{hi}
N	12.4 ^{ghijklm}	27.0 ^{d}	10.1 ^{jklm}	51.6 ^{ab}	25.4 ^{fgh}	47.9 ^{ab}
P	14.6 ^{fghijkl}	22.2 ^{defg}	12.8 ^{hijkl}	27.8 ^{fg}	11.3 ^{i}	14.7 ^{ghi}
K	18.6 ^{defghij}	17.1 ^{defghijk}	11.5 ^{hijklm}	26.9 ^{fgh}	12.5 ^{hi}	14.9 ^{ghi}
NP	10.7 ^{ijklm}	24.5 ^{def}	9.3 ^{ijklm}	45.0 ^{abcd}	31.6 ^{ef}	50.4 ^{ab}
NK	14.6 ^{fghijkl}	20.3 ^{defgh}	7.9 ^{klm}	57.1 ^{a}	34.0 ^{cdef}	50.8 ^{ab}
NPK	16.5 ^{fghijkl}	16.5 ^{efghijk}	5.9 ^{lm}	51.8 ^{ab}	43.8 ^{bcde}	47.1 ^{abc}
NPKSi	18.9 ^{defghij}	25.2 ^{de}	12.0 ^{hijklm}	49.6 ^{ab}	33.6 ^{def}	48.3 ^{ab}
NPKZn	19.5 ^{defghi}	12.0 ^{hijklm}	3.2 ^{m}	33.7 ^{def}	44.6 ^{abcde}	56.5 ^{ab}
NPKS	95.2 ^{a}	71.2 ^{b}	42.4 ^{c}	13.4 ^{hi}	6.6 ^{i}	7.1 ^{i}
	df	F-values		df	F-values	
Soil type (S)	2	166.1***		2	98.7***	
Fertilizer (F)	9	319.2***		9	159.8***	
S x F	18	24.4***		18	11.1***	

#Values within the next three columns followed by the same letter do not differ significantly at P<0.05 by Tukey's HSD multiple range test. ***Significant at P<0.001.

The high N: S ratio, notably on the *bottom* soil, and the increased values by the N application without S also implies the unbalanced supplies between N and S (Table 2). The ratios

reduced only by adding S as a result of the substantial increases in biomass production. In a conventional study, Yoshida and Chaudhry (1979) suggested a critical N: S ratio for S deficiency at 14 in straw, and they demonstrated 50% of biomass reduction at the value of 40. The other study also indicated the similar value as a critical N: S ratio, and slightly higher value for grains (Islam and Ponnamperuma, 1982). Our results indicated that sulfur shortage severely hampers rice growth as well as maximum efficiency of N from either inherent soils or fertilizers in the target floodplain. The limiting S supply was also apparent in the S concentration values, that were all below the critical deficiency level at 0.06% (Dobermann and Fairhurst, 2000), among the rice plants in the Experiment 1. The other macro- and micro- nutrient concentration values were above the critical deficiency level except four of the 18 plots for the P level (data not shown).

Conclusions

This study revealed steep gradients in soil fertility within a gentle slope of a river floodplain in the northern Ghana. The phytometrical experiments suggested that the spatial variances in soil fertility for rice growth were mainly represented by the N- and S- supplying capacity of the soils. Once the limit of S is lifted, rice production is expected higher as closer to water sources due to exponentially greater amounts of N-supplying capacity of the soils. A further study requires identifying spatial distributions in S-supplying capacity of the soils, so that efficient fertilizer management practices can be developed on a basis of balancing N and S applications. The development of toposequence-specific fertilizer management will certainly help effective extension of rice production within the target floodplain environments.

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Varietal Differences of Rice (*Oryza sativa* L.) Genotypes for Aleurone Traits Contributing to Lipid Content

Ohn Mar Khin[a], Masao Sato[b], Tong Li-Tao[a], Yuji Matsue[b], Atsushi Yoshimura[b], Toshihiro Mochizuki[b*]

[a]*Graduate school of Bioresource and Bioenvironmental sciences, Kyushu University, Fukuoka, Japan*
[b] *Faculty of Agriculture, Kyushu University, Fukuoka, Japan*
**Corresponding author: Experimental Farm, Kyushu University, Harumachi 111,*
Kasuya-machi, Kasuya-gun, Fukuoka 811-2307, Japan
Tel.:+81 92 612 2865; Fax: + 81 92 612 2865.
mochizuki@farm.kyushu-u.ac.jp

Abstract

The increasing demand for oil is more than the increasing production of oil seed crops. Rice bran oil has been used extensively in Japan, Korea, China, Taiwan, Thailand and Pakistan. Interest in rice oil is increasing because it is identified as "healthy oil" that reduces serum cholesterol. In rice seed, the lipid content deposited in the aleurone layer and embryo has been reported from 17.5 to 21.7%. To explore the useful genetic resources for aleurone traits closely related to the lipid content, 333 varieties were investigated. Brown rice seeds were cut in cross around center with razor blade and the outermost region of the cut surface stained with oil red O were measured with a fluorescence microscope. As a result, there were wide variations in stained area among varieties and many varieties with large stained area were found in Japonica group. Twenty varieties were selected according to their stained area to cover the whole range among varieties used and confirmed their thickness of aleurone layer by using micro thin section that was made by Leica cryostat with slight modification of the Kawamoto's film method (2008). The photos of sections were taken under a light microscope. The aleurone traits were measured by using Image J software. The amount and content of triacylglycerol (TAG) of brown rice without embryo were also measured. Both amount and content of TAG were significantly correlated with the area, the average thickness and the percentage of aleurone layer. These results suggest that the aleurone traits will be able to use as good indicators for selection of rice varieties with high TAG amount and content.

Keywords: aleurone layer, lipid content, rice bran oil, triacylglycerol, varietal differences.

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops, primarily in East and Southeast Asia. Rice bran is a valuable by-product of rice milling that contains a high concentration of nutritional compounds including edible lipids. Rice bran oil is used in foods, feed and industrial applications. More recent efforts have emphasized the nutritional benefits of rice bran oil. It can aptly be concluded that rice bran oil is an important future source for edible and essential oils in all over the world.

Rice bran oil has been extensively used in Asian countries such as Japan, Korea, China, Taiwan, Thailand and Pakistan (Kahlon, *et al.*, 1992). The use of rice bran oil in Japan, where it is the largest volume domestically produces vegetable oil, is as a frying oil (Orthoefer, 2005). In brown rice, the lipid content is from 2.3 to 3.9% (Juliano, 1977, Fujino, 1978). Embryos and aleurone layers in rice are major tissues to deposit lipids and the content in these tissues has been reported from 17.5 to 21.7%. The content in rice embryos and aleurone layers is equivalent to that of soybean and cotton seeds. The physiological role played by the aleurone layer is to provide a reserve site for minerals to deposit, which are crucial as an essential nutrient when seeds germinate

Tanaka *et al*., 1973). In the cells, oil bodies and aleurone particles are most predominant and seem to play a more important physiological function than starchy endosperm.

Because rice is primarily used for food, starchy endosperm or milled rice is more important. Little effort has been made to study about rice bran layer. There is some information about the thickness of aleurone layer of some varieties. Genes for enlargement of embryos and aleurone layers can contribute to an increase in the oil content of rice grains (Omura and Satoh, 1981). For such reason, characterization of genetically broad rice germplasms for thickness of aleurone layer is of special importance in identifying potential varieties. Therefore, the objectives of this study were to evaluate the thickness of aleurone layer and to compare it with the storage lipid of the seed.

Materials and Methods

Half–seed staining method

All experiments were conducted at Kyushu University in 2010-2011. Three hundred and thirty three rice varieties including 176 of IRRI Core Collection, 59 of World Rice Collection and 96 of Core Collection stored at Kyushu Uniersity, were used. The brown kernels were cut in half around center with razor blade and then stained with oil red O for 5 -10 minutes and then rinsed well in 70% ethanol for 3 - 4 times. The total cut surface area and the area of the stained region were measured by using a fluorescence microscope (BZ 9000, Keyence Co. Ltd Osaka,Japan). Then the percentage of the stained area and the average thickness of the stained region can be calculated by using the following formula.

$$\text{The percentage of the stained area (\%)} = \frac{\text{the area of the stained region } (\mu m^2)}{\text{the total cut surface area } (\mu m^2)} \times 100$$

$$\text{The average thickness of the stained region } (\mu m) = \frac{\text{the area of the stained region } (\mu m^2)}{\text{the circumference } (\mu m)}$$

Sectioning method

To confirm that the relationship between the thickness of the stained region and the real thickness of the aleurone layer, we selected 20 varieties according to their stained area to cover the whole range among varieties tested. The measurements were made by using micro thin section that was made by Leica cryostat with slight modification of the method of Kawamoto (2009). The sections were stained with Hematoxylin and Eosin and examined under a light microscope. The aleurone traits were measured using Image J software. Then the percentage of the aleurone layer and the average thickness of aleurone layer were calculated by the same formulae in half-seed staining method.

Measurement of Triacylglycerol (TAG)

The storage lipids (triacylglycerol: TAG) of selected varieties were analyzed. Hundred brown kernels without embryos were milled (Degree of milling: 90%) in a mall-scale rice mill. The lipids were extracted from the rice bran by the method of Folch *et al.* (1957). The TAG content was measured using enzyme assay kits (Triglyceride E test; Wako Pure Chemicals Osaka, Japan).

Results and Discussion

In each trait, the varieties were significantly different. Varietal groups were also significantly different in all traits except in percentage of stained area. Most of the *indica* varieties have smaller cut surface area. The smallest one was found in wild rice and the largest one in

tropical *japonica*. The mean of total cut surface area was 4.01 mm^2. There was wide variation from 0.07 to 0.35 mm^2 for area of the stained region and the mean was 0.18 mm^2. There were many varieties with large stained area in *japonica* group (Figure1). The smallest stained area was found in wild rice. Some varieties with large stained area were also found in *indica*, intermediate hybrid and tropical *japonica*. Although the percentage of stained area (%) was significantly different among varieties, the varietal groups were not significant and the mean was 4.45 %. In *japonica* group, many varieties with large thickness of stained region were found (Figure1). But there were also some *indica* varieties with large thickness of stained region. The mean of the thickness of stained region was 20.15μm. The results revealed that the varieties with smallest ones were found in *indica* and wild groups.

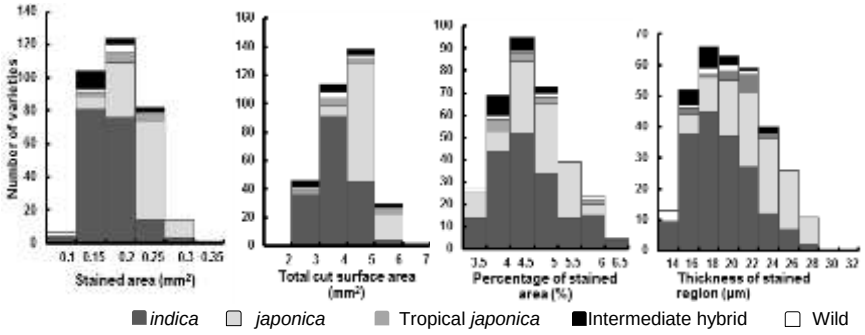
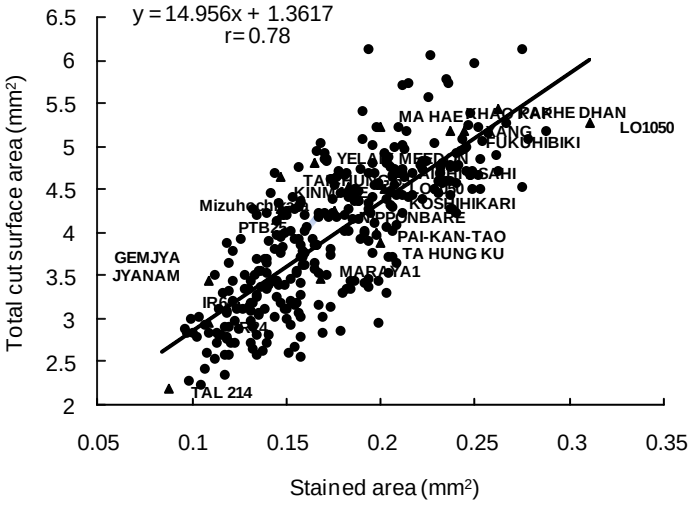


Figure 1. Frequency distribution of aleurone traits of different varietal groups.

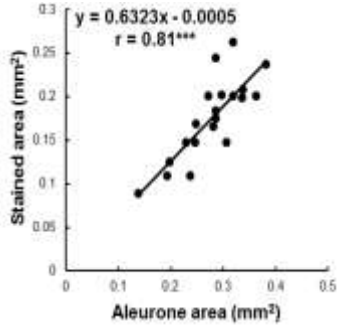


▲ indicates the selected varieties to check the aleurone layer, *** indicates the significance at 0.001 level.

Figure 2. The relationship between the stained area (mm^2) and total cut surface area (mm^2) of 333 rice varieties.

We selected 20 varieties according to their value of stained area to cover the whole range of varieties tested (Figure 2). The total cut surface area was nearly the same in half seed staining method and sectioning (Table.1). The mean of aleurone area was 0.27 mm^2 and the range was 0.12 - 0.42 mm^2 (Table 1). It was larger than the area of stained region. This may be due to the fact

that the stained region may become thinner than the real one depending on the time taken to wash the seed in 70% ethanol after staining. The correlation between these two parameters was significantly high (r=0.81***) (Figure 3). We can, therefore, use the area of stained region as a good indicator for selecting varieties for thickness of aleurone layer. The range of the thickness of aleurone layer was 22.88 - 48.78 µm (Table 1). The thickness of aleurone layer was larger than and significantly correlated with that of stained region (r=0.59**, data not shown). The percentage of aleurone layer was higher than that of the area of stained region and the correlation between these two parameters was not significant (r=0.24, data not shown).



*** indicates significance at 0.001 level.

Figure.3. The relationship between the stained area (mm^2) and the aleurone area (mm^2) of 20 rice varieties.

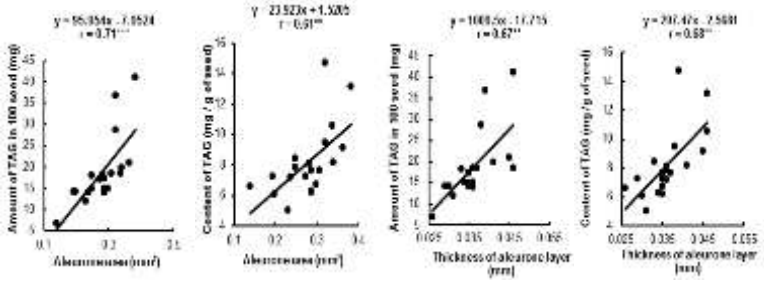
Table 1. Mean with the standard deviation of aleurone traits and Triacylglycerol (TAG) content of 20 rice varieties

	Sectioning method	Half-seed staining method
Total cut surface area (mm^2)	4.19 ± 0.99 (1.93 - 5.60)	4.24 ± 0.86 (1.97 - 6.00)
Aleurone area (mm^2)	0.27 ± 0.07 (0.12 - 0.42)	0.18 ± 0.05 (0.07 - 0.32)
Percentage of aleurone area (%)	6.45 ± 0.95 (0.20 - 9.00)	4.14 ±0.83 (2.30 - 6.49)
Thickness of aleurone layer (mm)	35.26 ± 6.12 (22.88 - 48.78)	19.23 ± 4.28 (10.00 - 29.14)
Amount of TAG (mg) in 100 seed	18.78 ± 7.91 (6.89 - 41.1)	
Content of TAG (mg /g of seed)	8.19 ± 2.3 (5.02 - 14.72)	

The values in parentheses indicate the range of each parameter.

The amount of TAG increased with increasing aleurone area. The range of amount of TAG without embryo in 100 brown seed ranged from 6.89 to 41.1 mg among 20 varieties tested. It was highly correlated with the area (r=0.71***) and thickness of aleurone layer (r=0.67**) (Figure 4). However, the percentage of aleurone area was not correlated with the amount of TAG. The content of TAG also increased with increasing the aleurone area. The range of content of TAG without embryo in 1g of brown kernel was from 5.02 to 14.72 mg/g among 20 varieties tested. It was highly

correlated with the area (r=0.61**), percentage (r=0.58**) and average thickness of aleurone layer (r=0.68**) (Figure 4).



** and *** indicate significant level at 0.01 and 0.001 respectively.

Figure 4. Relationship between TAG and aleurone traits of 20 rice varieties.

Conclusions

There was wide variation in aleurone traits among the genetically diverse rice varieties. The correlation between the aleurone area by sectioning method and the stained area by half-seed staining method was significantly high. Therefore, thestained area could be used as a good indicator for selecting varieties for thickness of aleurone layer. Furthermore, there were high correlation between aleurone area and aleurone thickness, and TAG. So, selection of varieties with high lipid content could be done without measuring directly lipid content of the varieties. A great potential was obtained to produce rice bran oil and it did not require any special cultivation since it is a by-product of the rice milling process.

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Development of New Cytoplasmic Male Sterile Lines with Good Flowering Behavior for Hybrid Rice Breeding

Indrastuti A Rumanti[a], Bambang S Purwoko[b*], Iswari S Dewi[c], H Aswidinnoor[b]

[a] *Indonesian Center for Rice Research, Ph.D. Student at Graduate School, Bogor Agricultural University*
[b] *Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
[c] *Indonesian Center of Agricultural Biotechnology and Genetic Resources Research and Development*
**Corresponding author: Jalan Meranti, Darmaga, Bogor 16680 Indonesia.*
Tel.: +62 251 8629353.
bambangpurwoko@gmail.com

Abstract

Intensive use of single source of male sterile cytoplasm (i.e. wild abortive or WA) in developing hybrid rice might lead to genetic vulnerability associated with susceptibility to pests and diseases. The research aim was to develop new cytoplasmic genetic male sterile lines (CMS) from 3 different cytoplasmic sources (WA, Gambiaca and Kalinga). Anther culture and successive backcrosses were conducted to develop new CMS. The screening of bacterial leaf blight to each CMS was done through artificial inoculation using three isolates i.e. pathotype III, IV and VIII. The results showed that combination of anther culture and backcrosses obtained 6 CMS lines of WA type, 3 CMS lines of Kalinga type, and 1 CMS line of Gambiaca type. The lines showed complete and stable sterility from first to fifth backcross generations. Some of them also showed resistance to certain *Xanthomonas oryzae* pathotypes. The new CMS lines with WA, Kalinga and Gambiaca cytoplasm were early in maturity with flowering behavior better than IR58025A (check CMS), such as larger stigma, higher stigma exertion, wider angle and longer duration of glume opening during anthesis. The accumulation of good flowering behavior increased seed set of the new CMS lines yielded from outcrossings. It ranged from 4.75–25.9%, meanwhile IR58025A seed set only reached 2.98%. Corellation analysis showed high positive and significant value between seed set of the new CMS lines and stigma width (r = 0.44*), stigma exertion (r = 0.54*) and angle of glume opening during anthesis (r = 0.42*), respectively. The seed set was also affected by some flower characters of the maintainer lines such as filament length and angle of glume opening during anthesis. The results indicated that the new CMS lines with diferent cytoplasm sources could be used in hybrid rice breeding.

Keywords: rice, cytoplasmic male sterility, flowering behavior, resistance, bacterial leaf blight

Introduction

Hybrid rice is able to increase rice productivity about 15-20% higher than the best commercial inbred varieties. Currently, hybrid rice technology is used in a large scale cultivation in several Asian countries. Hybrid rice covers more than 50% of rice total area in China (You *et al*., 2006). Three line system is generally used in Indonesia. This system needs three parental lines: cytoplasmic male sterile line (CMS/A line), maintainer line (B line) and restorer line (R line). Hybrid is the first filial generation of a cross between cytoplasmic male sterile line as female parent and restorer lines as male parent. The hybrid often exhibits heterosis or hybrid vigor, where hybrid progeny shows superior growth characteristics relative to one of the parental lines (Eckards, 2006). The commercial hybrid refers to a superior F_1, which not only performs better than its parents but also shows significant yield superiority over the best high yielding inbred variety of similar growth duration (Virmani & Kumar, 2004). Based on those, superior parental lines i.e. CMS needs to be developed.

CMS as female parent is a key factor for hybrid rice breeding. CMS is a condition under which a plant is unable to produce functional pollen (Eckardt, 2006). This line provides a valuable tool in production of hybrid seed in self-pollinating crop species including rice. Development of CMS in three line systems of hybrid rice breeding faces problems among others instability of pollen

sterility, flower behaviour not fully supporting outcrossing rate, and susceptibility to pests and diseases. Unstable pollen sterility causes difficulty in achieving high heterosis of the hybrid rice and its seed production. The low seed yield has been implicated in higher price of the hybrid seed. *Wild Abortive* (WA) CMS is mainly used in hybrid rice breeding in Indonesia (Suwarno *et al*., 2003) and elsewhere. This might lead to genetic vulnerability associated with susceptibility to pests and diseases (Li *et al*., 2007). Therefore, breeding of new CMS having stable pollen sterility derived from diverse cytoplasm sources and resistant to pests and diseases are warranted.

Commercial CMS should have good outcrossing rate, complete and stable sterile pollen, easy restorability, good and wide combining ability with any restorer lines, short plant, good exsertion of panicle, more than 70% of stigma exsertion, good quality, resistant to pests and diseases and adapted to target environment (Yuan *et al*., 2003). Dewi *et al*. (2007) identified 19 doubled haploid of maintainer line candidates through anther culture from F_1 of crossing between introduced CMS and donor lines resistant to bacterial leaf blight. The doubled haploid lines are potential maintainers to be converted to CMS because these lines were homozigous and having male sterile gene with normal cytoplasm. Rumanti *et al*. (2009) tested 48 F_1s, derived from testcross between cytoplasmic male sterile sources with those doubled-haploid maintainer lines, and 14 F_1s were completely sterile (100 % pollen sterility). The objectives of this research were to develop stable and completely sterile CMS lines from three different cytoplasmic sources, to evaluate resistance of the new CMS to bacterial leaf blight and study of flower behavior of the new CMS that support outcrossing ability.

Materials and Methods

Development of cytoplasmic male sterile lines through succesive backcross

Genetic material in this research were backcross population (F_1BC_n) between CMS candidates with their maintainer doubled haploid lines. CMS from International Rice Research Institute (IRRI) were used as cytoplasm donors, i.e. 3 CMS of wild abortive type (IR58025A, IR62829A, dan IR68897A), one Gambiaca type (IR80154A) and one Kalinga type (IR80156A). Succesive backcrosses were done 5 times. Each CMS candidate or female parent (F_1BC_n) was planted in 2 rows with 12 plants each, while male parent or their maintainer were planted in 3 rows with 12 plants each. Flowering syncronization was achieved by staggered planting of male parents in a 3-day intervals. Selection was based on pollen sterility in each F_1BC_n. Observation were done on each F_1BC_n generation for pollen sterility (%) (IRRI, 2002).

Resistance evaluation of the new cytoplamic male sterile lines to bacterial leaf blight

Ten new CMS' were planted in the field in 2 rows with spacing of 20 x 20 cm. Plants were inoculated with 10^8 cfu concentration of *Xanthomonas oryzae* pathotype using artificial method (leaf cutting/clipping). Disease severity was observed following modified IRRI method (IRRI, 2002) when susceptible check achieved 90% severity. Score of disease severity is shown in Table 1.

Table 1. Score of disease severity

Scale	Ratio of lesio length and leaf length (%)	Reaction
1	1-5	R (Resistant)
3	>5-12	MR (Moderate Resistant)
5	>12-25	MS (Moderate Susceptible)
7	>25-50	S (Susceptible)
9	>50-100	VS (Very Susceptible)

Evaluation of flowering behaviour which supports outcrossing ability between new CMS and their maintainer

Five new CMS lines (BI485A, BI599A, BI619A, BI639A dan BI665A) and their maintainers selected from the first experiment were evaluated for flowering behaviour. IR58025A was used as check line. The field experiment was conducted using randomized complete block design with three replicates. The days to flower, panicle exertion, stigma exertion, duration of glume opening during anthesis, angle of glume opening and seed set were observed based on SES (IRRI, 2002). Statistical analysis was conducted using SAS 9.0.

Results and Discussion

Development of cytoplasmic male sterile lines through succesive backcross

The successful use of hybrid vigor in rice depends on the availability of local cytoplasmic male sterile (CMS) and restorer lines. Cytoplasmic male sterile line is a rice genotype having abnormal anther. CMS anther has no pollen or has pollen, but easy to rupture, then there is no seed set yielded by self fertilization (Yuan *et al*., 2003). Male sterile character facilitates natural crossing thus ease the hybrid seed production.

Table 2 shows pollen sterility character of ten CMS from first backcross generation (F_1BC_1) to fifth generation (F_1BC_5). From the research, we identified six CMS candidates of Wild Abortive, three candidates of Kalinga and one candidate of Gambica types. Backcrosses formed segregating population and gave chance for breeder to select based on spesific target, in this case pollen sterility. The three cytoplasm types gave different pattern of pollen sterility in the five generations of backcrosses. In the WA type, there were unique pattern shown by BI497A and BI703A lines, in which both lines were completely sterile (100%) in the first and second generation of backcrosses, but reversed to be partial fertile on third and fourth generation. This phenomenon indicated that both lines showed unstable pollen sterility. Backcross should be done in several generations to determine pollen stability of both lines. The other lines, i.e. BI485A, BI543A, BI571A and BI599A showed positive increase in sterility percentage from first to fifth generation of backcrosses. The CMS with Gambiaca and Kalinga cytoplasm sources also increased pollen sterility as backcross progressing.

Table 2. Pollen sterility character of new cytoplasmic male sterile lines on F_1BC_1 - F_1BC_5 generation

CMS Lines	Pollen sterility on each generation (%)				
	F_1BC_1	F_1BC_2	F_1BC_3	F_1BC_4	F_1BC_5
CMS WA type:					
BI485A	98.0 ± 0.1	98.6 ± 1.8	99.8 ± 0.4	100.0 ± 0.1	100.0 ± 0.0
BI497A	100.0 ± 0.0	100.0 ± 0.0	98.4 ± 3.7	99.8 ± 0.4	100.0 ± 0.0
BI543A	94.3 ± 8.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
BI571A	92.0 ± 6.8	98.4 ± 2.0	98.8 ± 2.6	100.0 ± 0.0	100.0 ± 0.0
BI599A	93.8 ± 5.6	96.3 ± 5.3	97.6 ± 3.9	99.9 ± 0.2	100.0 ± 0.0
BI703A	100.0 ± 0.0	100.0 ± 0.0	99.8 ± 0.4	100.0 ± 0.1	100.0 ± 0.0
CMS Kalinga type					
BI639A	98.8 ± 2.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
BI665A	96.3 ± 4.2	94.3 ± 14.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
BI669A	85.3 ± 11.2	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
CMS Gambiaca type					
BI855A	95.2 ± 4.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Note: WA= *wild abortive*; value after ± was standard deviation

All completely sterile CMS' with WA cytoplasm source were achieved in the fifth generation (F_1BC_5). In the fourth backcross generation, four lines showed 100% pollen sterility (*completety sterile*), while other lines ranged from 99.8 to 99.9%. Different patterns is shown by CMS of Kalinga and Gambiaca types. The pollen stability of both CMS types were achieved earlier. All of CMS with Kalinga and Gambiaca cytoplasm had more stable pollen sterility than that of Wild Abortive. A commercially usable CMS line needs to have complete and stable male sterility, independent from environmental changes specially temperature (Yuan & Fu, 1995), adaptive to tropical rice growing conditions and possess good outcrossing potential to affect an economically viable hybrid seed production (Virmani & Kumar, 2004).

Resistance evaluation of the new cytoplamic male sterile lines to bacterial leaf blight

Male sterile lines should have good adaptation to their rice growing conditions and resistant to rice pest and disease. The three type of CMS were converted from dihaploid maintainer (DH_2) lines. The maintainers were obtained from anther culture of F_1 derived from crossing between elite maintainer lines and donor lines having high resistance to bacterial leaf blight. Both of maintainer lines and donor lines were elite breeding lines originated from Indonesia. Therefore, in the fifth backcross, the new CMS already had cytoplasm from introduced lines, but having 98.4375% nucleus genes from their male parents (*recurrent parent*). The new CMS' are expected be more adapted to Indonesian environment.

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most important disease of rice which cause substantial yield losses (Lee *et al*., 2000). We tested 10 new CMS lines for reaction to three Indonesian pathotypes. The new CMS shows variation in resistance to bacterial leaf blight (Table 3). The new CMS of wild abortive cytoplasm and Kalinga cytoplasm showed to be resistant to moderate resistant to pathotype III, while Gambiaca cytoplasm was resistant to pathotype III. In Indonesia, the pathotype IV and VIII of bacterial leaf blight were more virulence than pathotype III. The results showed variation in the reaction of all CMS from three cytoplasm sources to different pathotypes. One CMS of Kalinga type (BI665A) was resistant to both of pathotypes. The BI485A, BI497A, BI599A and BI855A showed moderate resistance to bacterial leaf blight pathotype IV. Reaction of CMS to patotype VIII also varied. BI543A and 571A were resistant to the pathotype VIII, while BI703A and BI669A were moderate resistant to the pathotype VIII.

Table 3. Reaction of cytoplasmic male sterile lines to three Indonesian pathotype of bacterial leaf blight

Cytoplasmic Male Sterile Lines	Resistance to Bacterial Leaf Blight (BLB)		
	Pathotype III	Pathotype IV	Pathotype VIII
CMS WA type:			
BI485A	3	3	5
BI497A	3	3	5
BI543A	3	7	1
BI571A	3	5	1
BI599A	3	3	5
BI703A	1	5	3
CMS Kalinga type:			
BI639A	3	7	5
BI665A	3	1	1
BI669A	1	5	3
CMS Gambiaca type:			
BI855A	1	3	5

Note: 1 Resistant, 3 Moderate Resistant, 5 Moderate Susceptible, 7 Susceptible, 9 Very Susceptible

Sudir & Suprihanto (2006) reported that virulence of the bacterial *Xanthomonas oryzae* pv. *oryzae* (Xoo) was significant in affecting disease severity of BLB. The elite line donor having *xa5* gene which has responsibility to disease resistance. The *xa5* gene is recessive and constitutively expressed at the same level as the dominant susceptible allele, *Xa5*, and neither allele is induced or suppresed in response to Xoo (Bart *et al*., 2006). Availability of CMS lines stable in sterility, good in agronomic characters, and resistant to disease will increase the adaptability of the hybrid developed from those CMS lines.

Evaluation of flowering behaviour that support the outcrossing ability between new CMS and their maintainer

Table 4 shows the observation of several flower behavior of five CMS lines. IR58025A was used as check line because most rice hybrids in Indonesia were derived from this line. New CMS lines were earlier to flower than IR58025A, except BI665A. The maintainers flowered 1–4 days before their respective CMS pairs. The different days to flower between CMS and their respective maintainers were in the threshold and important for flowering syncronization when produced CMS seed (Virmani *et al.* 1997).

Table 4. Flowering behavior of five new CMS and their maintainers

Cytoplasmic Male Sterile Lines	DF (days)		PE (%)	GOD (minutes)		GOA (°)		SE (%)	Seed Set (%)	
	A	B	A	A	B	A	B	A	A	B
CMS *Wild Abortive type* :										
BI 485	76.7 ^{d}	75.0 ^{c}	77.1 ^{b}	143.9 ^{ab}	38.4 ^{b}	34.9 ^{bc}	40.3 ^{a}	74.7 ^{a}	19.4 ^{ab}	90.8 ^{a}
BI 599	79.7 ^{c}	75.0 ^{c}	80.0 ^{ab}	162.9 ^{a}	52.1 ^{a}	39.8 ^{a}	35.6 ^{b}	71.8 ^{ab}	4.8 ^{c}	74.4 ^{c}
CMS Gambiaca type:										
BI 855	81.7 ^{c}	77.0 ^{c}	82.1 ^{a}	132.5 ^{bc}	28.6 ^{b}	36.0 ^{b}	36.5 ^{ab}	75.7 ^{a}	10.8 ^{bc}	91.5 ^{a}
CMS Kalinga type:										
BI 639	81.0 ^{c}	76.0 ^{c}	80.9 ^{a}	120.6 ^{c}	54.3 ^{a}	40.6 ^{a}	35.9 ^{b}	67.7 ^{b}	25.9 ^{a}	89.4 ^{ab}
BI 665	91.0 ^{a}	91.7 ^{a}	75.5 ^{c}	135.9 ^{bc}	41.9 ^{ab}	33.7 ^{bc}	38.3 ^{ab}	73.4 ^{ab}	15.7 ^{b}	65.2 ^{d}
Check (WA):										
IR58025A	88.0 ^{b}	88.3 ^{b}	81.0 ^{a}	121.8 ^{c}	54.3 ^{a}	32.8 ^{c}	29.3 ^{c}	43.3 ^{c}	2.9 ^{c}	81.7 ^{ab}
CV (%)	1.7	1.4	2.0	8.5	15.6	3.5	5.8	5.2	18.5	5.7

Note: A: cytoplasmic male sterile lines, B: maintainers; PE: panicle exsertion, SE: stigma exsertion, GOD: duration of glume opening during anthesis, GOA: angle of glume opening during anthesis; seed set formed in CMS (A) was yielded by natural outcrossing, pollen came from their respective maintainers; seed set of each maintainer (B) was obtained by selfing

All new CMS' showed good panicle exsertion (more than75%), but BI485A (WA) and BI665 (Kalinga) were more enclosed than IR58025A. Duration of glume opening during anthesis of BI485A and BI599A (WA type) was significantly longer than IR58025A. The other new CMS lines also showed longer duration of glume opening than that of IR58025A but not statistically significant. BI599A, BI855A and BI639A showed wider angle of glume opening than that of IR58025A i.e. 39.8; 36.0 and 40.6 degrees, respectively. The better duration and angle of glume opening would increase the opportunity of new CMS stigmas to receive pollen from maintainers.

Stigma exsertion of the three types of CMS was higher than IR58025A. It ranged from 67.7 to 74.7% compared to that of IR58025A (43.3%). The better flowering behavior caused higher seed set of the new CMS than IR58025A. The new lines set seed at about 4.8–25.9%, while IR58025A achieved 2.9% of seed set. Corellation analysis showed positive high and significant value between seed set of new CMS lines with stigma width (r=0.44*), stigma exertion (r=0.54*) and angle of glume opening during anthesis (r=0.42*). The seed set was also affected by some flower characters of maintainer lines such as filament length and angle of glume opening during anthesis (data not shown). The results indicated that the new CMS lines with different cytoplasm source could be used in hybrid rice breeding.

Conclusions

Combination of anther culture and backcrosses obtained 6 CMS lines of WA type, 3 CMS lines of Kalinga type, and 1 CMS line of Gambiaca type. The lines had stable sterility and resistance to 1-3 *Xanthomonas oryzae* pathotypes. The new CMS showed better flowering behavior which increase the seed set.

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Genetic Analysis of Superior Double Haploid Rice Lines Developed from Anther Culture

Muhammad Syafii[a,*], Bambang S. Purwoko[b], Iswari S. Dewi[c]

[a] *Postgraduate of Plant Breeding and Biotechnology, Bogor Agricultural University, Bogor, Indonesia*
[b] *Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia*
[c] *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Cimanggu, Bogor, Indonesia*
** Corresponding author: Postgraduate of Plant Breeding and Biotechnology, Bogor Agricultural University, Bogor, 16680-Indonesia.*
Tel.: +62 251 862 9353; fax: +62 251 862 9353.
syafii_m@yahoo.com

Abstract

Rice is the most important food commodity in Indonesia. The need for rice continues to increase with the population increase. Plant breeding's objective to obtain high-yielding varieties is an attempt to increase production plateu. Biotechnology is one of the breeding aproaches using tissue culture and genetic engineering techniques. The availability and diversity of plant genetic resources are important factors to develop high yielding varieties with desirable traits. This study was aimed to investigate the agronomic characters of double haploid lines developed from anther culture, evaluate genetic diversity, phenotypic variations and broad sense heritability of double haploid lines developed from anther culture, and to obtain rice genotypes potential as superior lines. Analysis of 18 genotypes showed there was diversity of agronomic characters among the rice double haploid genotypes. Characters of dry weight-based grain (yield) per hectare and the number of empty grain per panicle had high level of genetic variability, while other characters had rather low to low level of genetic variability. Yield per hectare in KP44223 line was the highest (4537.8 kg / ha or 4.5 tons / ha) among the lines, even higher than that of conrol varieties, Ciherang and Celebes. The characters of plant height, flowering age, harvesting time, panicle length, number of empty grain/ panicle, 1000 grain weight and yield per hectarehad high broad sense heritability, suggested that those characters can be used as selection characters to improve crop performance

Keywords: anther culture, double haploid, rice

Introduction

Rice is the most important food commodity in Indonesia. In addition, rice is also a major source of carbohydrate material for Indonesian people. The need for rice continues to increase with population increase. (Prasetyo 2008), Plant breeding to obtain high-yielding varieties is one attempt to overcome rice production plateu. Utilizing biotechnology through tissue culture technique and genetic engineering could be one of the appraches in plant breeding (Somantri *et al*. 2003). The availability and diversity of rice genetic resources is an important factor in developing high yielding varieties with desirable traits. Development of new high yielding varieties of rice in Indonesia has long been conducted since the early 1970s. After the 1980's breeding toward new high yielding varieties (VUB) started to be intensified to obtain varieties that are responsive to fertilizer and high quality of rice, such as IR64, Membramo, Cisadane and Ciherang (Samaullah 2007). Conventional breeding to develop new rice varieties require a long time (7-10 years), especially the time for selection process to obtain pure lines. Utilization of modern technologies (biotechnology) such as anther culture is expected to shorten the selection process thus saving time, effort, and cost. Dewi and Purwoko (2001), states that the researchers have applied the anther culture technique to obtain rice lines that are resistant to pests and diseases, quality of rice as well as tolerant of environmental

stress. Kim (1986), also reported that anther culture-based breeding approach have also been applied in Korea, Vietnam, China and India.

Materials and Methods

The study was conducted in January-May 2011 at IPB laboratory, Dramaga, Bogor. The materials used were 18 rice double haploid lines developed from anther culture (KP1-3-1-2, KP3-7-2-1, KP3-18-1-1, KP3-18 -1-2, KP3-18-1-3, KP3-19-1-1, KP3-19-1-2, KP3-19-1-3, KP3-19-1-4, KP4-19-1 -3, KP4-19-2-3, KP4-42-2-1, KP4-42-2-2, KP4-42-2-3, KP4-43-1-2-43-KP4 1-4 , KP4-43-2-3, KP4-43-2-4), and 2 control varieties (Ciherang and Celebes). The experiment was conducted at the Experimental station Babakan IPB, Darmaga, Bogor, using Randomized Block Design with four replicates. The treatments used were 18 double haploid rice genotypes and two control rice varieties. The 21 day old seedlings of each genotype were planted in field plots (0.8 x 5 m) with plant spacing of 20x20 cm having 2 seedlings/hole. Plants were fertilized with 200 kg/ha of Urea, 100 kg/ha of SP36 and 100 kg/ha of KCl applied three times at 10, 25 and 45 days after planting (DAP). Plants were maintained based on the paddy rice cultivation. The observations were performed on parameters of plant height (cm), total number of tillers (stems), number of productive tillers (stems), flowering date (days), harvesting date (days), panicle length (cm), number of filled grain per panicle (grains), number of empty grain per panicle (grains), total grain number per panicle (grains), weight of 1000 grains (g), and grain yield (kg/ha).

The data were analyzed using analysis of variance, Duncan multiple range test and analysis variance components (Singh and Chaudhary 1979). Variance component and heritability were determined based on Singh and Chaudhary (1979) as follow:

$$V_g = \frac{KTg - KTe}{r};\ V_P = V_g + Ve\ ;\ h^2_{bs} = \frac{Vg}{Vp}\ ;\ KVG = \left(\frac{\sqrt{Vg}}{X}\right).100\%\ ;\ KVP = \left(\frac{\sqrt{Vp}}{X}\right).100\%$$

Which was:
Vg: various genotypes; Vp: various phenotypes; r: replication; X: average common genotype; KVG: coefficient of genetic diversity; KVP: coefficient of phenotypic diversity; KTg: sum square of genotype; KTE: sum square error. Broad sense heritability values according to Stanfield (1983) is high (12:50 <h2 <1.00), moderate (12:20 <h2 <0.50) and low (h2 <0.20).

Results and Discussion

The results showed that the genotype effect was significant to all characters observed. This suggested that there was variability among genotypes for all the observed characteristics.

Plant Height

KP44323 and KP44324 lines were genotypes having highest average plant height (112.6 and 112.3 cm, respectively), while KP31812 and KP31813 lines had shortest average of the plant height (83.6 and 84.95 cm, respectively). Ciherang was classified as medium plant height (103 cm), while Celebes was classified as relatively short plant height (82.8 cm). From anther culture, the characters inheriteged from parent was the plant height, both on descent Ciherang and Celebes. This indiated that the plant height was dominant.

Total Productive Tillers and Tillers

Although not signicantly different among genotype, number of tillers and productive tillers per plant were varied among genotypes ranging from 14.1 to 19.55 and 11.55 to 15.25, respectively. KP31913 and KP44223 were the genotypes producing the highest and the lowest tillers. While KP1312 and KP44314 were the genotypes having the highest and the lowest productive tillers. The genotypes tested showed moderate number of tillers.

Flowering and Harvested

Flowering KP31812 of genotype lines and varieties Cilebes (control) i.e 63.25 consecutive days and 57 days compared to genotype lines KP41913, KP44312, KP44221, and KP44324 the consecutive 76 days and 75.75 days. This indicated that the lines have descended from varieties Ciherang age slower than lines flowering varieties descent Celebes. To determine the general harvesting age, the age of the rice crop can be classified into three groups calculated from the day after the scatterplot (HSS), namely age genjah (90-104 HSS), age was 105-120 HSS and age in at more than 120 HSS) (BB-padi 2009a). The average age of crop genotypes tested genjah belonging to moderate, with harvest age genjah of lines KP31811 and KP31813 was 91.75 day and most varieties genjah in Celebes (control) to 89.75 days old. While the maximum harvest age on genotype KP41913 and KP41923 a consecutive were 106 days and 105.75 days, respectively.

Panicle length

Panicle length characters in genotypes tested was quite short to medium. Longest panicle was found at KP44323 which was 24.96 cm. This was slightly shorter than that of varieties Ciherang at 25.82 cm, while the shortest panicle of KP44222 was 22.82 and 21.82 cm resulted from Celebes varieties (Table 3). DMRT test showed that all genotypes were not significantly different between the lines tested, except in the control varieties and Celebes Ciherang.

Number of Grain per Panicle

Line KP3721 had highest total amount of grain per panicle of 178.85 points, while the lowest was found in line KP31914 and KP31813 at 121.55 points and 121.88 points, respectively. Thus these genotypes were ssified as moderate (Table 4). All the genotypes tested were not significantly different with the second control test DMRT at 5%, except in line KP3721 and KP31914. Lines KP44312 and KP44324 had high in grain content / panicle at 100.35 points, respectively and 100.1 points higher than the control plants respectively 90.45 and 90.1 for the Celebes and Ciherang, while the lowest was found in line KP41923 had 56.45 points lower than the control varieties. KP41923 line had the most empty grain number/panicle at 106.3 points, while the lowest in line was KP31914 had 33.75 points. Although the line had a number of grain hollow KP31914/panicle, this line produced the lowest total amount of grain. That affected the yield per hectare. Calculation of the percentage (%) content of grain/panicle to total grain/panicle produced in line KP31914 was the highest at 72.23%, this value was higher than that of the varieties of Celebes (control) atf 70.06%. The lowest percentage was found at lines KP41923 and KP44314 which were 34.6 and 46.04%, respectively.

1000 Grain Weight

Weight of 1000 grains of rice indicated the size of grains of rice and beans level. The results showed that the highest weight of 1000 grains of rice was found at KP1312 line at 39 925 g. This value was higher than both the control varieties and Celebes Ciherang consecutive which were 36 025 and 30,575 g, respectively. The weight of 1000 grains of line KP3721 was the lowest at 26 325 g. This value was lower than the control Celebes varieties which was 30 575 g. KP3721 line had the lowest weight of 1000 grains allegedly due to low resistance to various diseases in the field, either by bacteria or viruses. Value of 1000 grain weight assessed with DMRT test at 5% level is shown in Table 3.

Grain Yield (kg /ha)

Grain yield per plot can be used to measure the amount of the plant production. Dry grain yield per plot was then converted to a hectare (kg/ha). The highest production was found at line KP44223 (no.entry 14) and KP44221 (no. entry 12) in a row having 4.5 and 4.3 t/ha, respectively, this significantly different with both the control varieties and Celebes Ciherang at 3:50 and 3:43 t/ha, respectively. The lowest dry grain was found at line KP41923 (no.entry 11) and KP31811 (no.entry 3) consecutive at 1:03 and 1.82 tonnes/ha, respectively. This differed markedly lower than the control based on test DMRT 5%. DMRT test at 5% for the grain yield/ha showed that all lines tested had distinct intangible, except in line KP44223 (highest) and line KP41923 (lowest) (Table 3).

Variety Component

Various components and heritability estimation is performed to determine the proportion of diversity caused by genetic factors and environmental factors. Heritability determines the success of selection for the appropriate environment, because it indicates the heritability of a trait is influenced by genetic or environmental factors. High heritability indicates the relative importance of genetic influences that can be used from the elders to their offspring as well as useful to determine the most appropriate selection method for improving a plant characters (Falconer and Mackay 1996). The value of plant genetic parameter estimation is shown in Table 3. Coefficient value of genetic diversity (KVG) ranged between 3.25 and 27.31 and phenotypic diversity coefficient (KVP) ranged between 4.51 and 36.83. Value KVG KVG 0.00-27.31% was the absolute value, that value was determined from the relative value of KVG. Absolute value of 27.31% KVG KVG was a relative value of 100%. According Moedjiono and Mejaya (1994), KVG relative value of the following criteria: low (0 <x <25%), somewhat lower (25%<x<50%), high (50%<x<75%) and high (75%x<100%). Based on the above criteria, it can be determined KVG absolute criteria in this trial, namely low (0.00 <x <6.83%), somewhat lower (6.83% <x <13.66%), high (13.66% <x <20:48%), and high (20:48% <x <27.31%). Murdaningsih *et al*. (1990) stated that the value was low and rather low KVG classified as narrow genetinya character variability, while quite high and high KVG classified as character mempynyai wide genetic variability.

Table 3. The value range of components and heritability estimates the value of agronomic characters and yield in rice

Agronomic character	mean	Vg	Vp	KVG (%)	KVP (%)	h^2bs (%)
Plant hight	93.79	87.61	103.04	9.98	10.82	85.03
Productive tillers	13.55	0.24	5.44	3.62	17.22	4.42
Total tiller	16.43	0.37	7.54	3.70	16.71	4.89
Flowering	69.59	30.54	31.56	7.94	8.07	96.79
Harvested	98.00	18.59	19.53	4.40	4.51	95.17
Panicle lenght	23.79	0.60	1.19	3.25	4.59	50.13
Number of grain/panicle	85.53	116.29	255.69	12.61	18.70	45.48
number of empty grain/ panicle	55.37	228.61	415.72	27.31	36.83	54.99
Total grain/panicle	140.90	165.78	372.94	9.14	13.71	44.45
1000 grain weight	34.43	9.46	14.01	8.93	10.87	67.53
Yield (ton/ha)	2.94	5.64	11.24	25.58	36.12	50.17

Remarks: Vg=varian genotype, Vp=varian phenotype, KVG=coefisien varian genotype, KVP=coefisien varian phenotype, h^2bs= boad sense heritability

Based on the criteria of absolute KVG, there were two characters having relatively high KVG ie grain yield / ha and the number of empty grain per panicle, five characters have a rather low KVG, namely plant height, flowering age, number of grain content per panicle, total grain number per panicle and 1000 grain weight, and the four characters have low KVG ie the number of productive tillers, total number of tillers, harvesting age, and length of panicle. Thus there are two characters having a broad genetic variability, and there are nine characters that have a narrow variability. Nugraha Lestari (2007) mentioned the opportunities for improvement of grain yield can be done with the selection of the character of the number of grain per panicle and grain yield dry. Broad sense heritability value of the characters observed was between 04:42 and 96.79% (Table 3). According to Stanfield (1983), broad sense heritability values can be grouped into three high heritability (12:50 <h2 <1.00), the heritability of moderate (0.2 <h 2 <0.50) and low heritability (h2 <0.20). Based on the above criteria that broad sense heritability values for characters: plant height, flowering age, age of harvest, panicle length, number of empty grain / panicle, 1000 grain weight and dry grain yield per hectare was high, whereas the character of grain number per panicle and the number of content total grains per panicle classified as being; then to the character of productive tillers and total number of tillers per hill had a low broad sense heritability values. Therefore, the improvment of grain yield can be done through the selection of plant height, flowering age, age of harvest, panicle length, number of empty grain / panicle, 1000 grain weight and grain yield per hectare dry.

Conclusions

Analysis of agronomic characters of 18 genotypes indicated that there was diversity in agronomic characters and character among the double haploid genotypes of rice anther culture results. Dry grain yield per hectare and the number of empty grain per panicle had a high level of genetic variability, agronomic characters and yield while others had a rather low level of genetic variability to low. Dry grain yield per hectare obtained from line KP44223 was the highest of 4.5 ton/ha than varieties Ciherang and Celebes. High broad sense heritability value generated on the characters were plant height, flowering age, age of harvest, panicle length, number of empty grain/panicle, 1000 grain weight and grain yield per hectare dry Therefore, the characters can be used as benchmarks for improving the character of the next generation of this crop.

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QTLs on Chromosome 12 Responsible for Expressing Root Plasticity under Transient Soil Moisture Fluctuation Stress in Rice

Jonathan M. Niones, Yoshiaki Inukai, Akira Yamauchi*

Graduate School of Bioagricultural Sciences, Nagoya University
**Corresponding author: Nagoya University, Chikusa, Nagoya 464-8601 Japan*
Tel : +81-52-789-4021; Fax : +81-52-789-4022
ayama@agr.nagoya-u.ac.jp

Abstract

Soil moisture fluctuations (i.e. transient waterlogged to drought) frequently occur in rainfed rice fields due to erratic rainfall pattern, and irrigated fields due to wetting and drying system practices and inefficient irrigation system. Such conditions are stressful for growth and development of rice. With the utilization of chromosome segment substitution line (CSSL), we previously demonstrated the significant roles of root plasticity as expressed by higher lateral root production in response to soil moisture fluctuation stress. Moreover, our molecular mapping analysis showed the presence of QTL that is associated with root plasticity, at short arm of chromosome 12 region. This QTL was found to control the productions of L-type lateral roots with the increase effect from Kasalath allele. In this study, we attempted to quantify the significant functions of QTL (qLLRn-12) on chromosome 12 in relation to the shoot dry matter productions and root system development. CSSL genotypes with QTL or substituted segment of Kasalath allele on chromosome 12 were evaluated and subjected to 38 days of transient soil moisture fluctuation stress. Results revealed that CSSL genotype with qLLRn-12 showed greater root system development as expressed its greater total root length than those without qLLRn-12 in response to moisture fluctuation stress. Longer total root length was attributed to higher production of L-type lateral roots, which can effectively maintain water and nutrient uptake in the soil. Such plastic root responses resulted in increased stomatal conductance and photosynthesis, eventually increased shoot dry matter production by 37% compared to its Nipponbare parent. Comparison of the location of the QTL across rice cultivars showed that there is no QTL reported in this region related to lateral root production. This may possibly be a putative QTL and potentially be used in the marker-aided breeding program to improve adaptation under fluctuating soil moisture environment.

Keywords: chromosome segment substitution lines (CSSL), lateral root, rice, root plasticity, soil moisture fluctuation

Introduction

With the scarcity of water for irrigation and continuing increase of world population, future increase in rice production will more heavily rely on rainfed rice ecosystem. Rainfed lowland conditions are characterized by frequent fluctuation in soil moisture at varying degrees throughout the cropping season, which have stressful effects on the growth and development of rice (Niones *et al*., 2009). Plant roots play an important role in water and nutrient acquisition. Both constitutive and adaptive root growth have been implicated in the improvement of plant performance under rainfed conditions (Kamoshita *et al*., 2002). A plant has an ability to change its phenotype, in response to soil's heterogeneous environment (Yamauchi *et al.,* 1996), which is termed as plasticity, and root plasticity is a key trait for plant adaptation to stressful conditions. Moreover, we reported that traits associated with developmental root plasticity triggered by mild drought stress played significant roles in water uptake and dry matter production in rice (Kano *et al*., 2011).

With the use of chromosome segment substitution lines (CSSL), we previously identified one line CSSL47 that consistently showed more plastic root growth than Nipponbare in terms of branching of lateral roots and aerenchyma formation at early vegetative stage (Suralta *et al.,* 2008,

2010) and until maturity (Niones *et al*., 2009) under transient soil moisture fluctuations. This line contains 10 substituted segments from Kasalath allele with Nipponbare genetic background. Niones *et al.* (2010) identified the location of the QTL at short arm of chromosome 12 region, which are responsible for controlling root developmental plasticity specifically the L-type lateral root production. The expression of such root plasticity QTL and its effect on dry matter production and root system development when rice plants are grown under transient soil moisture fluctuation conditions have not yet been further verified.

In this study, we examined the significant function of the QTL or substituted segment from Kasalath allele on chromosome 12 in relation to the shoot dry matter production at vegetative stage. Furthermore, we tested the variation in root plasticity expression of parents and CSSL genotype with QTL or substituted segment of Kasalath allele on chromosome 12 under 38 days of transient soil moisture fluctuation stress.

Materials and Methods

Plant materials

Four genotypes (Nipponbare, CSSL47, CSSL27, CSSL52) were used in this study. Nipponbare, an irrigated lowland japonica variety and recurrent parent of the 54 chromosome substituted segment line (CSSL) (Nipponbare/ Kasalath cross) population genotypes. CSSL47 genotype has ten substitute segments across genome from Kasalath allele with a unique characteristics of having greater plastic responses in root development under soil moisture fluctuations than the other CSSLs (Suralta *et al*., 2010; Niones *et al.* (2009). The CSSL27 and CSSL52 genotypes were selected from the 54 CSSL population. CSSL27 genotype contained no substituted segment of Kasalath in short arm of chromosome 12 region, and thus is hereon referred as ***- SL***, while CSSL52 (referred as ***+ SL***) has substituted segment of Kasalath in short arm of chromosome 12 region only. The seeds of CSSL population was provided by the Rice Genome Research Center of the National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Treatment conditions

Seedlings were grown in soil-filled plastic root box and exposed to three different soil water conditions; the well-watered (WW), transient waterlogged to drought (W-D) and transient drought to waterlogged (D-W) conditions. Plants were exposed to waterlogged conditions for 17 days and thereafter the water was allowed to drain to the target soil moisture content (SMC) of 20%, which was then maintained for 21 days by watering every two days. Plant samplings were done at 38 DAS.

Root phenotyping

Lateral roots (LRn) were manually counted. Each nodal root was cut into 5-cm segments keeping the lateral roots intact. In this way, the number of each type of lateral root; L and S type of lateral roots in rice (Yamauchi *et al*., 1996) can be determined and expressed as linear frequency (number of lateral roots per unit length root axis; Ito *et al*., 2006). One of their major noticeable differences is that L type is branching while S type is non-branching. For total root length (TRL) measurements, root samples from the FAA were rinsed with running water and spread on transparent sheet without overlapping. The digitized images were taken using Epson scanner (ES2200) at 300 dpi resolution. TRL was analyzed using a NIH image software (ver.1.60), a public domain released by the National Institute of Health, USA.

Statistical analysis

The experiments were laid out in split-plot randomized complete block design with three replicates. The significance differences of four genotypes were compared at LSD > 0.05 significance level using the Cropstat software.

Results and Discussion

Plastic root development was mainly attributed to enhanced lateral root production particularly the L-type lateral (Bañoc *et al.,* 2002; Suralta *et al*., 2010), which is a key trait for the plant adaptation under transient soil moisture production. Lateral roots generally comprised the greater proportion of the whole root system (Yamauchi *et al.,* 1987), and thus promoted lateral root development in response to moisture stress directly reflects the performance of the entire root system. Enhanced lateral root development results in increased root surface area, and soil water extraction and water use. Therefore, the maintenance of stomatal conductance and photosynthesis could be a consequence of greater supply of water to leaves due to the enhanced lateral root development as a result of their plastic responses.

QTL reported by Niones *et al.* (2010) was located at the short arm of chromosome 12 region, which is hereon referred as qLLRn-12. This was associated with root developmental plasticity with the increase effect from Kasalath allele. The function of this QTL was to regulate the production of L-type lateral roots, which is associated with plastic root system development. It is assumed that the possible location of the qLLRn-12 may be near TG156 marker locus with the approximate distance of 17.6 cM between the adjacent markers RM6296.

In this study, greater lateral root development of ***+ SL*** genotype over Nipponbare and – ***SL*** genotypes was shown in higher linear frequency of lateral root number in response to transient soil moisture fluctuation stress. This result showed the evidence the importance of QTL or Kasalath segment that was present in chromosome 12 (Figure 1). Figure 2 shows the significant positive correlation between total root lengths and the production of lateral root under both transient soil moisture fluctuation stress treatments, with r-value of 0.624 for D-W and 0.646 for W-D. This result indicates that under transient fluctuations of soil moisture, higher production of lateral root had a significant contribution to greater root system development that eventually leads to enhanced water and nutrient uptake in the soil.

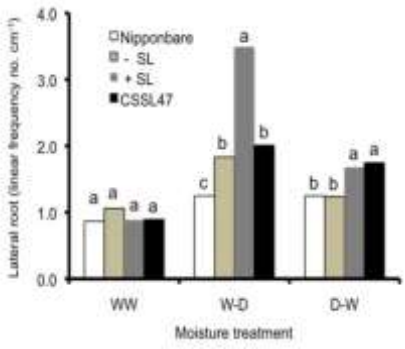

Figure 1. The linear frequency of lateral root number per cm of the four genotypes grown under different soil moisture conditions: i.e. WW, well-watered; W-D, transient waterlogging to drought; D-W, transient drought to waterlogging. (□)、Nipponbare; □□) - SL indicates the CSSL genotypes that do not contain QTL or introgressed segment from Kasalath, (□) + SL indicates the CSSL genotypes that do not contain QTL or introgressed segment from Kasalath and (□)、CSSL47. Same letter means that values are not significant at LSD > 5% level of significant level.

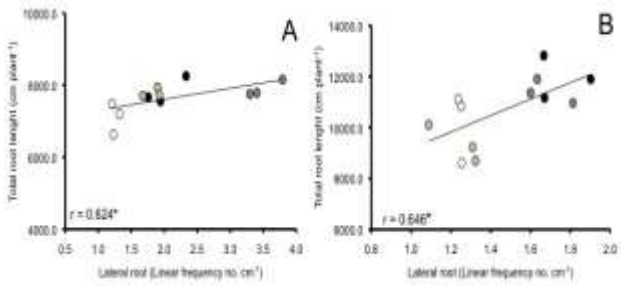

Figure 2. Relationships of linear frequency of lateral root with total root length under different soil moisture conditions; A, transient waterlogging to drought; B, transient drought to waterlogging. (□), - SL (CSSL28); (□), CSSL47; (□), + SL (CSSL52); (○), Nipponbare.

To further demonstrate the effect of the existence of QTLs in response to transient soil moisture fluctuation stress, ***+ SL*** genotypes were evaluated for plastic root system development in relation to shoot dry matter and root system development as shown in Figure3. The ***+ SL*** genotype showed significantly greater shoot dry matter production with the increase of 30% in W-D and 43% in D-W than Nipponbare. The increase in shoot dry matter of ***+ SL*** genotype was associated with a 10% (W-D) and 17% (D-W) increase in total root length compared to Nipponbare. On the other hand, these parameters were not significantly different between ***– SL*** genotype and Nipponbare parent. These facts clearly suggest that the function of QTLs at chromosome 12 was to regulate the production and branching of lateral roots (*i.e.*, L-type lateral root), which resulted in greater plastic root system development. This eventually led to the enhanced shoot dry matter production at vegetative stage under fluctuating soil moisture conditions.

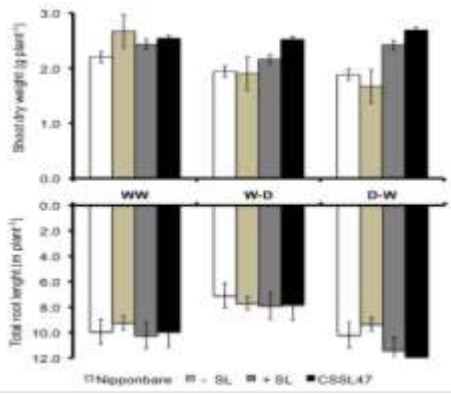

Figure 3. Effect of the presence of QTL at short arm of chromosome 12 on the shoot dry matter production and total root length under different transient soil moisture fluctuation conditions. Same letter means that values are not significant at LSD > 5% level of significant level.

We identified in short-arm of chromosome 12 region, the location of QTL that is responsible for regulating the production of L-type lateral roots with the increase effect from Kasalath allele. The use of the CSSL genotypes that contain substituted segment of Kasalath in chromosome 12 only further validated this QTL function. There have been reports on QTLs that are associated with root length, root number, root dry weight and branching index traits (Price *et al*., 2002; Horii *et al*., 2006; Gowda *et al*., 2011), however, with the best of our knowledge, there have been no reports on QTL in this region, which are related to lateral root production under soil moisture fluctuation stress. Fine mapping is necessary to narrow down the distance of linked

markers to the target trait. Then, this could be potentially used in the marker-aided breeding program for enhanced plant adaptation under such environment.

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Yield and Related Traits in Two *Japonica* Rice Lines Carrying *Ur1* Gene

Stanis Malangen[a], Takumi Iwakura[b], Toru Hata[b], Takeshi Akaoka[b], Amol Dahal[b], Masayuki Murai[b,] *

[a] *United Graduate School of Ehime University, Matsuyama, Ehime, 790-8566, Japan*
[b] *Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi, 783-8502, Japan*
** Corresponding author: Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi, 783-8502, Japan*
Tel.: +81- 088-864-5120; Fax: +81- 088-864-5200 (common)
muraim@kochi-u.ac.jp

Abstract

Ur1 (Undulate rachis-1) is an incompletely dominant gene on chromosome 6 in rice, being characterized by undulation of primary and secondary rachis branches. *Ur1* increases not only the number of secondary branches per panicle but also spikelet number per single secondary branch, resulting in a large spikelet number per panicle. This genic effect can increase grain yield by enlarging sink size. We examined the yielding abilities of the two *Ur1*-carrying lines under field conditions (mid-April sowing and early-May transplanting in two experimental years). The two *Ur1*-carrying lines (MR79 and MR53) were selected from 108 recombinant inbred lines (F8 generation) originating from 108 F2 plants of the cross of 'Nishihikari' × an isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d*. 'Hinohikari', a leading variety in southern Japan was the check variety in the present study, being denoted by "Hi". Additionally, 'Nishihikari' ("Ni"), a high yielding variety adaptable to fertile land, was employed. They were grown under three fertilizer levels (4, 8 and 16 of Ng/㎡ in total) and two fertilizer levels (12 and 21 of Ng/㎡ in total), together with P and K elements, in 2003 and 2005, respectively. Yield and other traits were measured. The results at 21 of N g/㎡in 2005 are described as follows. In yield (brown rice, g/㎡), the lines-varieties were ranked in the order MR79 (728) > MR53 (723) > Ni (695) > Hi (558). MR79 and MR53 had 170 g/㎡ (30%) and 165 g/㎡ (30%) higher yields, respectively, than Hi. Regarding spikelet number per panicle, they were in the order MR79 (132.7) > MR53 (116.0) > Hi (94.5) > Ni (90.5). As for panicle number per ㎡, Ni and Hi were higher than MR79 and MR53, although the difference is smaller than that in the previous trait. Regarding ripened-grain percentage, Ni (88.9%) and MR53 (85.0%) were higher than MR79 (79.8%) and Hi (79.2%). Regarding sink size (single-grain weight × fertilized-spikelet number per ㎡), they were in the same order as in yield. The yield of MR79 (728 g/㎡) was outstandingly high as compared with yield levels of ordinary *japonica* varieties in southern Japan. This high yield was due to its more spikelets per panicle caused by *Ur1*, its ripened-grain percentage being maintained at almost 80%. Moreover, MR79 had significantly higher yield than MR53, Ni and Hi at 12 of N g/㎡ in 2005 as well as at the three fertilizer levels in 2003. Hence, MR79 had outstanding high yields at the low to high fertilizer levels. MR79 was 14-18 days later in 80%-heading date than the rather late variety Hi in all cultivated conditions in the two years. MR79 was higher-yielding than the rather–early line MR53 in every environmental conditions. The lateness of MR79 might be advantageous to attain high yield. MR79 has high eating quality comparable to that of Hi, one of high eating-quality varieties in Japan. Consequently, MR79 could be a super-high yielding variety with exceptionally late maturity in southern Japan.

Keywords : *rice, Ur1 gene, spikelet number per panicle, sink size, yield*

Introduction

Ur1 (Undulate rachis-1), located on chromosome 6 (Sato & Shinjyo, 1991), is an incompletely dominant gene of rice, being characterized by undulation of primary and secondary rachis branches. *Ur1* increases not only number of secondary branches per panicle but also spikelet number per secondary branch, resulting in a large spikelet number per panicle (Nagao *et al*., 1958; Murai & Iizawa, 1994). This genic effect can increase grain yield by enlarging sink size

(Murai & Iizawa, 1994; Murai *et al.*, 2002 and 2005 b). However, *Ur1* decreases both ripened-grain percentage and 1000-grain weight.

Murai *et al.* (2005 a) preliminarily reported that a *japonica Ur1*-carrying line Murai 79 (hereafter "MR79") had a higher yield than the levels of ordinary *japonica* varieties in southern Japan, due to its higher sink size caused by more spikelets per panicle, which was derived from the cross of a Japanese commercial variety and an isogenic line of Taichung 65 carrying *Ur1*. MR79 and another *Ur1*-carrying line "MR53" were used in the present study, which possess extremely late and rather early heading times, respectively. The two lines, a representative variety of southern Japan 'Hinohikari' and a *japonica* high-yielding variety 'Nishihikari' were grown under three fertilizer levels in 2003 and two fertilizer levels in 2005. Yield, its components and other traits were measured for the lines-varieties. On the basis of two-year data, characteristics of the two *Ur1*-carrying lines were examined. We discuss utility of the lines in southern Japan.

Materials and Methods

Development of two Ur1-carrying lines

The highest-yielding F1 in the yield tests for various F1 hybrids with the *Ur1* / + genotype (Murai *et al*., 1997 and 2003) was used for developing recombinant inbred lines with and without *Ur1*. Its maternal and paternal parents were 'Nishihikari' and an isogenic line of Taichung 65 carrying both *Ur1* and *sd1-d* (dee-geo-woo-gen dwarf), respectively. The F2 population was grown in 1992, and the generation was progressed to F8 generation without selection in glasshouse condition. In 1999, the 108 F9 lines originating from the respective 108 F2 plants were grown in a paddy field; the two most well-ripened lines carrying *Ur1*, viz. MR53 and MR79 were selected from *Ur1*-carrying lines by field observation. The uniformity (non-segregation) of MR53 and MR79 was confirmed from 2000 to 2003 (F14 generation).

Field experiments

Besides MR53 and MR79, 'Nishihikari' and 'Hinohikari', (denoted by "Ni" and "Hi" respectively) were used for the field experiments. Ni is a short-culm and panicle-number type variety possessing the highest lodging tolerance in southern Japan (Nishiyama, 1982). Hi is a leading variety in southern Japan, which possesses rather-late heading, rather long culm, rather many panicles.

The two *Ur1*-carrying lines, Ni and Hi were seeded in mid April and transplanted to a paddy field of the faculty of Agriculture, Kochi University, Japan in early May, in the two experimental years (Table 1). Two seedlings per hill were transplanted at a spacing of 30.0 x 15.0 cm. They were grown under three fertilizer levels (4, 8 and 16 of N g/㎡ in total) and two fertilizer levels (12 and 21 of N g/㎡ in total) together with P and K elements, in 2003 and 2005, respectively (Table 1). The randomized block design with three replications was adopted for all combinations of the lines-varieties and fertilizer levels in each year.

Measurements of yield and other traits

All panicles of about 30 hills were sampled from each plot at maturity, and the panicle weight of each hill was checked after cutting just at their panicle bases after air-drying. Out of nine hills randomly selected from about 30 hills of each plot, five hills having intermediate panicle weights were selected. The panicles in the five hills were threshed, and all spikelets in each hill were counted. Grains after hulling (hereafter "grain") were sieved at 1.7 mm to select ripened grains by thickness. All ripened grains in each of the five hills were counted, and were weighed. The percentage of ripened-grain weight to panicle weight in the five selected hills of each plot was

calculated; then, the ripened-grain weight (yield) of 30 hills of each plot was estimated from this percentage.

Table 1. Fertilizer application, dates of sowing and transplanting for MR78, MR53, Ni and Hi in the experiments in 2003 and 2005

Year	Total amount of fertilizer applied (N g/m^2)[2)]	Basal dressing (N g/m^2)[2)]	Top dressing (N g/m^2)[2)]
2003 [1)]	16.0	8.0[3)]	8.0[5)]
	8.0	4.0[4)]	4.0[5)]
	4.0	2.0	2.0[5)]
2003 [2)]	21.0	7.0[6)]	14.0[7)]
	12.0	4.0	8.0[7)]

[1)] Dates of sowing and transplanting were April 16 and May 9 in 2003, and April 20 and May 8 in 2005.
[2)] P2O5 and K2O elements were applied at the same level as N element
[3)] The 6.0 g/m^2 of each nutrient element was applied with LONG® 100 type, Chisso Asahi Fertilizer Co., Ltd. (about 7% of each nutrient element is readily available) six days after transplanting. In additional to 2.0 g/m^2 of each nutrient element applied with an ordinary chemical fertilizer before puddling.
[4)] The 2.0 g/m^2 of each nutrient element was applied with LONG® 100 type, in addition to 2.0 g/m^2 of each nutrient element applied before puddling.
[5)] LONG® 100 type was applied on June 30 for MR53, July 27 for MR 79, July 14 for Ni ('Nishihikari') and July 9 for Hi ('Hinohikari').
[6)] The 3.0 g/m^2 of each nutrient element was applied with LONG® 100 type six days after transplanting. In additional to 4.0 g/m^2 of each nutrient element applied with the ordinary chemical fertilizer before puddling.
[7)] Another slow-release coated fertilizer, LONG® 100 type, Chisso Asahi Fertilizer Co., Ltd. (about 3% of each nutrient element is readily available) was applied on May 20 for MR53, June 2 for MR79, June 9 for Ni and June 5 for Hi.

Results and Discussion

In yield, the lines-varieties were ranked in the order MR79 > MR53 > Ni > Hi at every fertilizer level in the two years: MR79 and MR53 were 30 to 70% and 23 to 48%, respectively, higher than Hi in the five environments (Table 2). Regarding spikelet number per panicle, they were in the order MR79 > (or ≒) MR53 > Hi > (or ≒) Ni at each of the fertilizer levels in the two years. In this trait, MR79 and MR53 were 24 to 40% and 21 to 30%, respectively, higher than Hi in the five environments. As for panicle number per m^2, MR79, MR53 and Ni were 95-110%, 92-100% and 103-111%, respectively, of Hi in the two years, although the difference among the four lines-varieties in this trait was smaller than that in the previous trait in every environment. In ripened-grain percentage, Hi was around 80% and the lowest among the lines-varieties in every environment. MR53 was similar to or not so different from Ni in every environment. MR79 was not significantly different from Ni in all environments except at 21 of N g/m^2 in 2005. In terms of 1000-grain weight, Ni was heavier than the other lines-variety, and either MR53 or MR79 was similar to or not so different from Hi in both years. Regarding sink size, they were in the same order as in yield. In culm length, MR79 was similar to Hi. MR53 was rather longer than low-height Ni in both years. They had panicle lengths within the ranges of 2.1 and 2.5 cm in 2003 and 2005, respectively, although varietal difference was detected. MR53 and MR79 were 11-17 days earlier and 14-18 days later, respectively, in 80% heading date than Hi in the two years.

The higher the fertilizer level, the higher the yield in each line/variety in each year (Table 2). Similar fertilizer responses were noticed in both spikelet number per panicle and panicle number per m^2, although the latter trait was more responsive than the former trait in each line/variety in both years. All line-varieties except MR53 had the lowest ripened-grain percentage at 4 of N g/m^2 in 2003, whereas they had higher values at 12 of N g/m^2 than at 21 of N g/m^2 in 2005. However, range caused by fertilizer effect within each line/variety was less than 5.1% inclusive in each of the years. Every line/variety had little higher 1000-grain weight at 16 of N g/m^2 than at 4 of N g/m^2 in 2003, but such consistent response was not detected in 2005.

Regarding sink size (single-grain weight × fertilized-spikelet number per m^2), they were in the same order as in yield (Table 2). The yield of MR79 at 21 of N g/m^2 in 2005 (728 g/m^2) was outstandingly high as compared with yield levels of ordinary *japonica* varieties in southern Japan. This high yield was due to its more spikelets per panicle caused by *Ur1,* and its ripened-grain percentage being maintained at almost 80%. Furthermore, MR79 had significantly higher yield than MR53, Ni and Hi at the other four environments. Hence, the high yielding ability of MR79 was stable over the low to high fertilizer levels.

Table 2. Yield and other traits of MR79, MR53, Ni and Hi at the three fertilizer levels in 2003 and two fertilizer level in 2005

Trait	year	Fertilizer Level (N g/m^2)	MR79		MR53		Ni ('Nishihikari')		Hi ('Hinohi-kari')	LSD
Yield	2003	16	673 ^{a}	(149)	612 ^{b}	(136)$^{2)}$	554 ^{c}	(123)	451 ^{de}	37
		8	626 ^{b}	(165)	549 ^{a}	(145)	478 ^{a}	(126)	379 ^{a}	
		4	553 ^{c}	(170)	481 ^{a}	(148)	442 ^{a}	(136)	326 ^{a}	
	2005	21	728 ^{a}	(130)	723 ^{a}	(130)	695 ^{a}	(124)	558 ^{a}	32
		12	662 ^{c}	(131)	626 ^{a}	(123)	572 ^{a}	(113)	507 ^{a}	
Spikelets/ panicle	2003	16	103.8 ^{ab}	(124)	107.4 ^{a}	(128)	80.6 ^{de}	(96)	83.6 ^{d}	5.2
		8	101.4 ^{bc}	(130)	101.8 ^{bc}	(130)	76.5 ^{ef}	(98)	78.3 ^{ef}	
		4	102.0 ^{b}	(137)	96.9 ^{c}	(130)	74.1 ^{f}	(100)	74.3 ^{f}	
	2005	21	132.7 ^{a}	(140)	116.0 ^{c}	(123)	90.5 ^{de}	(96)	94.5 ^{d}	6.3
		12	123.8 ^{b}	(134)	111.2 ^{c}	(121)	85.2 ^{e}	(92)	92.1 ^{d}	
Panicle/ m^2	2003	16	338 ^{b}	(107)	309 ^{c}	(97)	336 ^{a}	(106)	317 ^{b}	14
		8	317 ^{b}	(110)	288 ^{cd}	(100)	314 ^{b}	(109)	287 ^{cd}	
		4	289 ^{c}	(105)	268 ^{e}	(98)	304 ^{b}	(111)	274 ^{de}	
	2005	21	354 ^{bcd}	(95)	360 ^{bc}	(96)	389 ^{a}	(104)	374 ^{ab}	20
		12	326 ^{ef}	(96)	312 ^{f}	(92)	349 ^{cd}	(103)	339 ^{de}	
Ripened grain percent-tage	2003	16	94.0 ^{ab}	(115)	91.6 ^{b}	(112)	94.1 ^{ab}	(115)	82.0 ^{c}	2.7
		8	95.3 ^{a}	(117)	93.0 ^{ab}	(114)	93.2 ^{ab}	(114)	81.6 ^{cd}	
		4	92.4 ^{b}	(117)	92.4 ^{b}	(117)	92.7 ^{ab}	(117)	78.9 ^{d}	
	2005	21	79.8 ^{c}	(101)	85.0 ^{b}	(107)	88.9 ^{a}	(112)	79.2 ^{c}	2.4
		12	84.9 ^{b}	(104)	88,9 ^{a}	(109)	85.5 ^{b}	(105)	81.3 ^{cb}	
1000-grain weight$^{1)}$ (g)	2003	16	20.4 ^{cde}	(98)	20.2 ^{e}	(97)	21.7 ^{a}	(105)	20.8 ^{c}	0.4
		8	20.4 ^{cde}	(99)	20.2 ^{e}	(98)	21.3 ^{b}	(103)	20.6 ^{cd}	
		4	20.3 ^{de}	(100)	20.1 ^{cde}	(99)	21.1 ^{b}	(104)	20.3 ^{de}	
	2005	21	19.5 ^{d}	(97)	20.4 ^{b}	(102)	22.2 ^{a}	(111)	20.0 ^{c}	0.4
		12	19.3 ^{d}	(97)	20.3 ^{bc}	(101)	22.5 ^{a}	(112)	20.0 ^{c}	
Sink size$^{3)}$ (g/m^2)	2003	16	695 ^{a}	(141)	640 b	(130)	567 c	(115)	492 de	38
		8	640 ^{b}	(151)	566 c	(134)	492 de	(117)	422 f	
		4	577 ^{c}	(155)	496 d	(133)	458 ef	(123)	373 g	
	2005	21	827 ^{a}	(131)	780 b	(123)	733 c	(116)	631 de	32
		12	726 ^{c}	(129)	659 d	(117)	618 e	(110)	562 f	
Culm length (cm)	2003	16	73.5 a	(97)	65.9 b	(87)	63.6 c	(84)	75.6 a	2.1
	2005	21	77.0 a	(191)	66.1 b	(87)	64.6 b	(85)	75.9 a	2.4
Panicle length (cm)	2003	16	20.3 ab	(110)	19.7 b	(107)	20.5 a	(111)	18.4 c	0.8
	2005	21	22.8 a	(112)	21.6 b	(106)	20.5 c	(101)	20.3 c	0.9
80%-heading date	2003	4-16	Aug.22		July 28/29		Aug.13		Aug.9	
	2005	12-21	Aug.23/24		July 21		Aug.11		Aug.7	

$^{1)}$ Brown rice weight after sieving at 1.7mm, being adjusted into 15% moisture. $^{2)}$ Percentage to H in parentheses $^{3)}$ Single-grain weight x fertilizer-spikelet number per m2. Values followed by the same letter within each year in a trait are not significantly different at the 0.05 level being determined by LSD in the table.

MR79 was 14-18 days later in 80% heading date than the rather late variety Hi in all environmental conditions in the two years. MR79 was higher-yielding than the rather–early line MR53 in every environmental condition. The lateness of MR79 might be advantageous to attain

high yield. MR79 has high eating quality comparable to that of Hi, one of excellent-taste varieties in Japan (Murai, unpublished). Consequently, MR79 could be a super-high yielding variety with exceptionally late maturity in southern Japan.

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Oxidative Stress and its Relation to Radiation Use Efficiency in Rice Growing under Rainfed Condition in Northeast Thailand

Kohtaro Iseki[a,*], Koki Homma[a] , Boonrat Jongdee[b]

[a] *Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
[b]*Ubon Rice Research Center, Ubon Ratchathani, Thailand*
** Corresponding author: Graduate School of Agriculture, Kyoto University, Sakyo-ku, 6068502, Kyoto, Japan*
Tel.: +81 75 753 6042; fax: +81 75 753 6065.
kiseki@kais.kyoto-u.ac.jp

Abstract

In rainfed cultivation of rice, the scarcity of available water often inhibits plant growth. Under such conditions, the drought induces oxidative stress which damages to photosynthetic cells such as protein degradation, lipid peroxidation and enzyme inactivation. In this study, we evaluate oxidative damage for 10 rice genotypes under rainfed condition in relation to radiation use efficiency (RUE). Although the drought stress was relatively mild as shown in the slightly decreases of stomatal conductance and leaf water potential, RUE under rainfed condition was significantly decreased from that under flooded condition. The oxidative damages evaluated by chlorophyll fluorescence and lipid peroxidation were also increased under rainfed condition. The genotypic variation of RUE in both water conditions was negatively correlated to the value of midday Fv/Fm and it was thought to be affected by the canopy structure of each genotypes. Damage of leaf cells evaluated by membrane stability index under rainfed condition was not significantly different from that under flooded condition, but the larger photoinhibition and lipid peroxidation seems to cause severe cell damage when the water scarcity continues for long term.

Keywords: rice, photoinhibition, chlorophyll fluorescence, drought, oxidative stress

Introduction

Plant oxidative stress is induced by various abiotic stresses and damages to leaf cells (Mittler, 2002). Under drought and high light conditions, decreasing of energy use for Calvin cycle leads to over excitation of energy which causes generation of reactive oxygen species (ROS) and promote oxidative damage to photosynthetic cells (Murata *et al*., 2007). Oxidation of cell membrane increases water leakage and decreases leaf water potential. In addition, severe oxidative stress is thought to be a cause of metabolic limitation in Calvin cycle, inducing non-stomatal inhibition of photosynthesis (Lawlor, 2002). Drought is an increasingly important constraint of rice production: the scarcity of water resource for agriculture provides the opportunity for rice to have water deficit even for irrigated production as well as for rainfed production. However, the relation between oxidative stress and drought stress has not been examined in terms of crop response under field conditions with various environmental stresses.

To clarify the relationship between drought and oxidative stress under field condition, we firstly focused oxidative stress of rice in relation to plant water status. Effect of the oxidative stress on plant growth was evaluated in relation to RUE.

Materials and Methods

Field growth conditions

Ten rice genotypes from Surin1 backcross introgression lines with IR68586-FA-CA-143 (BC_3) were used. 3 weeks seedlings were transplanted with the plant density of 25 plants m^{-2} in

rainfed and flooded field on 12th August 2010 at Ubon Rice Research Center, Northeast Thailand. Fertilizer was applied with 3-3-1.5 g m^{-2} of N-P_2O_5-K_2O for each field. The items below were measured from 2 to 9 weeks after transplanting.

Radiation use efficiency

Total shoot dry weight was measured at 2 and 9 weeks after transplanting, and the difference of dry weight was defined as total shoot growth. Radiation use efficiency (RUE) was calculated as RUE (g MJ^{-1}) = (total shoot growth)/(total intercepted solar radiation). To determine canopy light absorbance rate (kc), photosynthetic active radiation (PAR) at the top and bottom of the canopy in each plot was measured with a linear PAR intercept-meter (AccuPAR, Decagon, USA) under diffused solar radiation conditions once every week. The weekly accumulation of incident solar radiation was multiplied by the weekly value of kc, and summarized during the period as total intercepted solar radiation..

Chlorophyll fluorescence, gas conductance (gs) and leaf water potential (LWP)

Maximum quantum yield (Fv/Fm) and effective quantum yield (Φ_{PS2}) of photosystem Ⅱ was measured by chlorophyll fluorometer (OS-30p, Opti Science, USA) in early morning and midday of a clear sunny day every week. The topmost fully expanded leaves were used for the measurement. Simultaneously, gs and LWP of the topmost fully expanded leaves were also measured.

Membrane stability and lipid peroxidation

The topmost fully expanded leaves were sampled at 8 weeks after transplanting and used for the following analyses. The leaf membrane stability index (MSI) was determined according to Sairam and Saxena (2000). Leaf discs were soaked in distilled water at 40 °C for 60 min and its electrical conductivity recorded (C1). Subsequently the same samples were kept in boiling water for 10 min and its electrical conductivity also recorded (C2). MSI was calculated as MSI (%) = [1–(C1/C2)] × 100.

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation. Fresh leaf sample was homogenized in 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 12000 G for 10 min. The 0.5 ml aliquot of the supernatant was added to 1.5 ml of 0.5% thiobarbituric acid in 20% TCA. The mixture was heated at 95°C for 30 min and then quickly cooled in ice. After centrifugation at 3000 G for 10 min, the absorbance of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its extinction coefficient of 155mM^{-1} cm^{-1} and expressed as nmol MDA g^{-1} fresh weight.

Results and Discussion

Most genotypes decreased their total shoot growth under rainfed condition from that under flooded condition. The smaller shoot growth under rainfed condition was mainly caused by the lower RUE (Figure 1). The Surin1 which showed higher RUE under flooded condition also showed higher RUE under rainfed condition. Another parent line, IR68586-FA-CA-143 showed lower RUE under both water conditions (Table 1). However, except these two parent genotypes, the relationship of RUE between rainfed and flooded condition was not obvious. It implies that the effect of water limitation on RUE was different among genotypes. In the theory, RUE can be explained as the function of canopy photosynthesis (Sinclair and Horie, 1989, Reynolds *et al*., 2000). In terms of single leaf, photosynthetic rate is closely related with stomatal conductance, which is governed by LWP (Medrano *et al*., 2002). The slightly but significantly lower value of gs and LWP under rainfed condition suggested that mild drought stress was occurred (Table 1). The drought stress might decrease RUE.

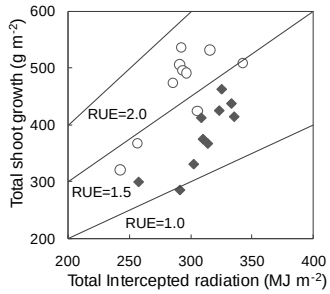


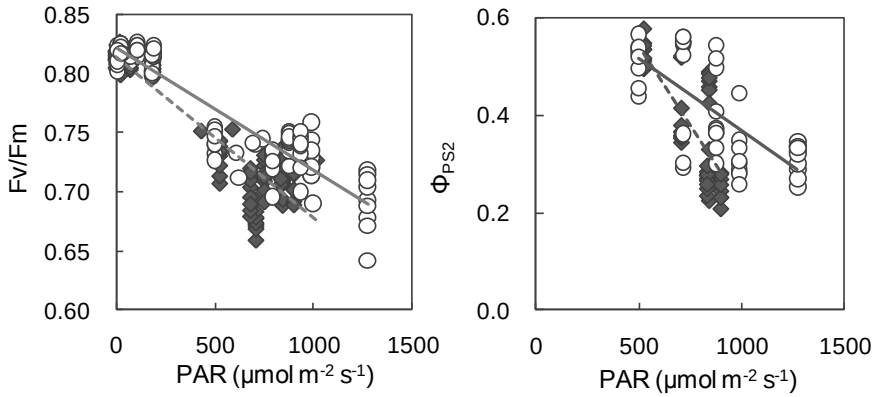
Figure 1. Genotypic variation in total shoot growth, total intercepted solar radiation and RUE under rainfed and flooded conditions. Open circle and closed diamond represents the genotype grown under flooded and rainfed condition, respectively. Data are average of three replicates.

Table 1. Radiation use efficiency, stomatal conductance and leaf water potential of 10 genotypes under flooded and rainfed conditions

	RUE (g MJ $^{-1}$)		g_s (mmol m^{-2})		LWP (Mpa)	
	Flooded	Rained	Flooded	Rained	Flooded	Rained
IR68586-FA-CA-143	1.42	0.97	218	184	-0.796	-0.836
Surin 1	1.83	1.34	214	224	-0.797	-0.864
IRUBN030055-5-87	1.38	1.43	239	244	-0.808	-0.906
IRUBN030055-5-112	1.73	1.17	261	192	-0.808	-0.856
IRUBN030055-5-190	1.68	1.25	286	230	-0.819	-0.853
IRUBN030056-10-42	1.49	1.32	280	220	-0.789	-0.850
IRUBN030056-10-107	1.65	1.32	229	189	-0.822	-0.847
IRUBN030062-1-9	1.68	1.20	249	222	-0.806	-0.864
IRUBN030063-9-4	1.33	1.14	311	229	-0.847	-0.847
IRUBN030070-9-32	1.66	1.09	229	194	-0.861	-0.861
Average	1.59	1.22	252	213	-0.803	-0.858
Genotype (G)	*		ns		ns	
Water condition (E)	***		***		***	
G x E	*		ns		ns	

Data are average of three replicates. * and ** represent statistically significant at 0.05, 0.01 level, and ns represents not significant.

The water limitation, even mild drought, decreases carbon fixation of rice and induces oxidative damage (Zhou *et al*., 2007). Measurement of chlorophyll fluorescence can easily evaluate photosynthetic capacity, and is widely applied for environmental stresses studies. The maximum quantum yield of photosystem Ⅱ (Fv/Fm) is an indicator of photoinhibition which relate to oxidative stress (Maxwell and Johnson, 2000). Generally, Fv/Fm decreases with increasing of exposed light intensity at midday. Decrease in Fv/Fm by high PAR was larger under rainfed condition than that under flooded condition (Figure 2). The same tendency was observed for the effective quantum yield (Φ_{PS2}) but the difference was not significant between rainfed and flooded conditions. It is known that the carbon fixation rate is closely related to the value of Φ_{PS2} under the environment of constant CO_2 concentration (Kato *et al*., 2003). Decrease of Φ_{PS2} induces over reduction in electron transport chain and increases oxidative damage. The lower value of Fv/Fm under rainfed condition is thought to be resulted by increased oxidative damage that derived from inhibition of carbon fixation. The genotypic variation in Fv/Fm and Φ_{PS2} was not significant, because of large residual variation in this study.



Open circle and closed diamond represents the genotype grown under flooded and rainfed condition, respectively. Data are average of four replicates.

Figure 2. Decrease of Fv/Fm and Φ_{PS2} with increasing of PAR in midday. Fv/Fm was measured in early morning and midday, and Φ_{PS2} was measured only in midday.

MDA content was higher under rainfed condition, indicating that damage of cell membrane by reactive oxygen species was larger under rainfed than that under flooded condition (Table 2). On the other hands, difference of MSI, another indicator of membrane damage, was not significant between the two water conditions. MDA is a product of lipid of peroxidation, while MSI indicates water leakage from cell induced by oxidative damage to cell membrane (Mittler, 2002). Accordingly, the result in this study may suggest that lipid peroxidation proceeded under rainfed condition but the damage was alleviated by antioxidation mechanisms.

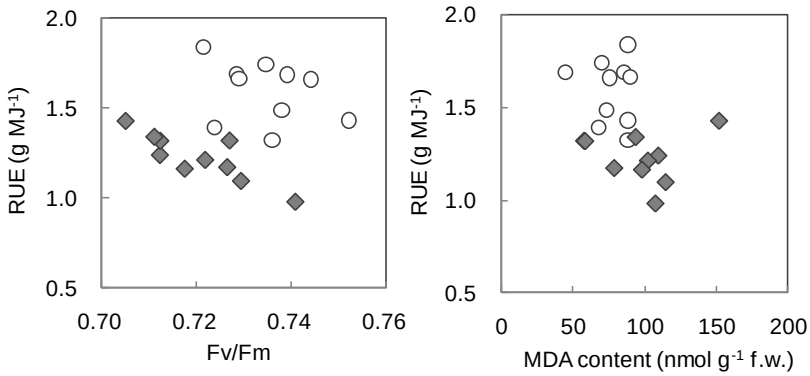
Table 2 Malondialdehyde content and membrane stability index of 10 genotypes under flooded and rainfed conditions

	MDA (nmol g^{-1} f.w.)		MSI (%)	
	Flooded	Rainfed	Flooded	Rainfed
IR68586-FA-CA-143	88.4	107.3	81.7	76.1
Surin 1	88.3	93.5	81.5	76.2
IRUBN030055-5-87	67.7	151.9	79.7	78.6
IRUBN030055-5-112	70.2	78.4	77.8	75.7
IRUBN030055-5-190	85.5	109.3	75.7	77.1
IRUBN030056-10-42	73.1	57.2	79.6	76.0
IRUBN030056-10-107	75.3	58.0	78.5	79.0
IRUBN030062-1-9	44.2	102.0	82.3	80.7
IRUBN030063-9-4	88.1	97.8	83.1	78.1
IRUBN030070-9-32	89.6	114.5	76.5	80.0
Average	77.0	97.0	79.7	77.7
Genotype (G)	***		ns	
Water condition (E)	***		ns	
G x E	***		ns	

Data are average of three replicates. * and ** represent statistically significant at 0.05, 0.01 level, and ns represents not significant.

Drought stress decreases RUE and Fv/Fm, implying the RUE was positively correlated with Fv/Fm. However, the relationships for each water condition showed negative correlations (Figure 3). This suggests that the genotypes which showed larger canopy photosynthesis were suffered with larger photoinhibition. Because RUE is not determined only by single leaf

photosynthesis but also canopy structure, further study is necessary to reveal the effect of photoinhibition on plant production.



Open circle and closed diamond represents the genotype grown under flooded and rainfed condition, respectively. Fv/Fm is the average of three measurement days from sixth to eighth weeks after transplanting.

Figure 3. Relationships of RUE to Fv/Fm and to MDA content.

In this study, oxidative stress in rice was evaluated under field environment of rainfed condition during wet season where drought stress was relatively mild. The drought stress decreased RUE and, increased photoinhibition and lipid peroxidation. The results suggest that the oxidative stress was associated with dry matter production. To elucidate the relation between drought and oxidative stress and to apply it for breeding program of drought tolerance, further studies are necessary.

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The Growth Characteristics of Some Varieties of Aceh's Local Rice (*Oriza sativa* L.) on Acid Soils

Elly Kesumawati[a,*], Erita Hayati[a], Muhafaz Zulusfitri[a]

Faculty of Agriculture Department of Agrotechnology, Syiah Kuala University
Banda Aceh, Aceh Province, Indonesia
** Corresponding author: Faculty of Agriculture Jl.Tgk.Hasan Krueng Kalee, Kopelma Darussalam,*
Banda Aceh 23111, Aceh Province, Indonesia
Tel.: +0651-7552223; fax: +065-17555269
ekesumawati@yahoo.com

Abstract

To identify the agronomic and morphological characteristic of some local rice varieties in Aceh, to describe based on the classifications of rice and to observe the varieties resistant to soil acidity, it was needed to conduct a study of 18 Aceh's local rice varieties and one "Dupa" variety as a comparison. The results showed that there were 6 local varieties of Aceh tolerant to Al toxicity and 12 were somewhat tolerant varieties, as well as varieties had tolerant nature to "Dupa". There were differences in flowering age, counted from planting rice to grew panicle by 10%. This age difference could be grouped into three, namely: age of flowering under 100 days was found in 2 varieties, age of flowering ranged from 100 to 119 days was found in 12 varieties (including Dupa), while flowering more than 120 days was found in 5 varieties. There were 15 varieties of rice belonging to the class Indica and 4 varieties in Javanica group. The largest stem diameter by Sigupai Wangi variety and smallest by Pade Cut Kresek variety. Longest panicle varieties belonged to Pade Rangan and shortest Sigupai Nagan Raya. There were 14 varieties which did not possess grain hair-tip, while shortest grain hair-tip by 4 varieties and 1 varieties of grain had long hair-tip. The widest seed variety was found in Dupa and the smallest was from Pade Cut Kresek. The thick seed was Dupa variety and the thinnest was Ramos Tihion. Based on length, the longest seed character was found in 17 varieties and other 2 variety was just long.

Keywords: characteristics, variety, local Aceh's rice, acid soils

Introduction

Rice is a strategic commodity plays an important role in the economy and national food security and become a major base in the revitalization of agriculture in the future. About 95% of national rice production depends on the production of rice paddies and only 5% of upland rice. Increased productivity of rice reached only 0.40% per year while the land use field reaches 0.77% per year (Puslitbangtan, 2007).

Expansion of the current wetland is difficult because suitable land for new rice fields are very limited, the cost of irrigation system development and printing of new rice fields are very expensive. Alternative increase rice production can be done by the expansion of upland rice cropping on dry land. Dry land area reached 54.4 million ha, but the 45.8 million ha of land of which is acid (BPS, 2007), and 11 million hectares more of them has the potential to be developed as upland rice cropping land (Puslitbangtan, 2007). However, the development of upland rice on dry land faced with the problem of Al toxicity and nutrient deficiency phosphate appearing simultaneously on acid soils of land (Kochian *et al.,* 2004). Liming and P fertilization are used to overcome these problems, but this way is not economical for areas that are far from sources of lime, not a good means of transportation, the availability of P fertilizers is often not continuous and expensive. It would require the development of varieties that grace Al and efficient in the use of P.

Aceh is a province in Indonesia rich in diversity of rice local varieties, which needs to be preserved and utilized for sustainable development in order to improve the welfare of all people.

Characterization of genetic resources will provide added value in enriching the "gene pool", the new diversity of local varieties is useful for the assembly of new varieties (Neereja *et al.,* 2005). These genetic resources continue to decline due to lack of attention, their utilization, and the changes in traditional farming practices. Introduction of high yielding varieties called genjah to rice production centers in various regions may cause genetic erosion of local varieties existing in certain areas (Mishra *et al.,* 2009). The existence of genetic erosion due to modern farming practices led to increasingly felt the importance of germplasm collection and conservation. To anticipate the erosion of plant genes, it is necessary to preserve the plant genetic material through exploration, characterization, and documentation (Hanarida *et al.,* 2005). Genetic diversity can be determined through characterization and identification. Improved varieties can be done either through conventional breeding or modern breeding program utilizing biotechnology assemblies germplasm using seeds from existing genetic resources. Each variety has certain important characteristics which can be used for crossing among varieties (Liu *et al.,* 2007). Aceh local rice varieties are still widely used by farmers in many districts in Aceh, but in relatively small quantities. Until now, local rice germplasm has not been characterized and evaluated, therefore, this requires the identification, conservation and classification which are useful for the in breeding programs and serve for the protection of local plant varieties (Menkumham, 2000; Bhuyan *et al.* 2007; Silitonga, 2008).

This study aimed to identify the agronomic properties and morphology of 18 local rice varieties in Aceh, based on the classification of rice varieties and to observe their resistant to soil acidity.

Materials and Methods

The research was conducted at the Garden Experiments Faculty of Agriculture, University of Syiah Kuala Banda Aceh from January to June 2010. Rice seeds were 18 local varieties (varieties Ramos Merah, Rom Mokot, Sigupai Wangi, Sigupai Nagan Raya, Sambei, Rasi Singke, Kepala Gajah Kinco, Bo Padang, Rasi Kuneng, Pade Mangat Bu, Bo Santeut, Ramos Tihion, Pade Cut Kresek, Pade Pineung, Cantek Puteh, Itam Tangke, Pade Rangan, Sigupai Blang Pidie), obtained from farmers from several districts of Aceh, and a control varieties of varieties of Incense originally from stone Estuary Borneo leaf blast-resistant and tolerant to Al toxicity.

The research was conducted using a randomized block design (RGD), non-factorial with 3 replicates. Treatment of rice varieties (V) consisted of 19 varieties, and 57 units experiment. Each experimental unit consisted of four plants, so that overall there were 228 units observation. Soils used were soil type ultisol (pH 4.35) at 950 kg obtained from Jantho Aceh Besar district. The soil weight was 8 kg/bucket. Fertilizers used were Urea, KCl, and SP 36 at 2 kg of each. Seeds were germination on paper soaked in water to thei field capacity, then left for 5 days. Each bucket consisted of four seedlings of rice plants. A seeding was planted in each planting hole. Furadan at 0.5 g/bucket was applied to soil surface in each bucket. Urea, SP 36 and KCl at 1 g / bucket of each were given one day before planting. At 15 DAT urea at 1 g/bucket were applied, at 45 DAT Urea and KCl were again applied at 1 g / bucket. At 55 DAT Urea applied at 1 g/bucket. Plant maintenance is carried out by watering, weeding, as well as controlling pest and disease.

The characters observed were agronomic trait (plant height and number of tillers per hill at the age of 30, 45 and 60 DAT, level of tolerance, and the age of flowering) and morphology (diameter of the stem segment below, panicle length, feather edge grain, seed width, seed thickness, and length of seed). The data were described based on Characterization and Evaluation System Guide Rice with scoring for each character observed (Deptan, 2003). Data were calculated for the relative values (RV) to determine differences in plant height and number of tillers of the soil acidity. Grouping level of tolerance to soil acidity was based on the criteria modified from Sarkarung (1986) then grouped to tolerant (when the value of RV was greater than 75%), somewhat tolerant

(when the value of RV was between 50 to 74%), sensitive (when the value of RV was less than 50%).

$$\text{The relative value (RV) (\%)} = \frac{\text{Plant height or number of pups at acid conditions}}{\text{Plant height or number of pups at normal conditions}} \times 100\%$$

Results and Discussion

Agronomic characteristic

The results showed that rice varieties significantly affected the relative values for plant height at 30 DAT, but not at 45 and 60 DAT (data not showed). The relative value of number of tillers per hill of rice plants was not significantly different at 30, 45, and 60 DAT (data not showed). The Aceh local rice varieties Sigupai Nagan Raya (V4) and Cantek Manis (V15) were taller than other local varieties such as Dupa (V19) variety. The shortest rice plant varieties was Red Ramos (V1). The plants high in rice plants had a positive and negative values on the cultivation system. The tall rice plants can be used as an elder male in producing hybrid seed. Tall rice plants will be more easily to spread its pollen to the shorter one (female elders). Hybrid rice production needa the stud taller than 20 cm for the elder females. However, tall rice plants are susceptible to fall down (Samac and Tasfaye, 2003).

Grouping of local rice varieties tolerant to acid soil in Aceh was based on the relative value (RV). According to Sarkarung (1986), local rice in Aceh produces 6 varieties having a relative value of more than 75% (tolerance type), and 12 varieties having a relative value between 50-74% (somewhat tolerant type) similar to the control Dupa tolerant nature (Table 1). Varieties tolerant to high acid soil may also tolerant to stress of Al. This was shown by the relative value of more than 75%. Tolerant plants allegedly had the ability to suppress the adverse influence of Al by reducing the Al^{3+} ion uptake by roots and neutralize toxic Al in the network, so that plant growth is not disturbed (Watanabe and Osaki, 2002; Sopandie *et al.,* 2003). Variations in the nature of resistance 18 local varieties of Aceh on Al toxicity in this study presumably because the genes resistant to Al toxicity did not work together (Nguyen *et al.,* 2002).

Age of flowering plants in Aceh local rice was varied. Sambei (V5) and Rom Mokot varieties (V2) were flowering under 100 days. Varieties flowerring at 100-119 days were 11 varieties, including control variety Dupa (V19), varieties flowerring at more than 120 days were 5 varieties (Table 1).

Morphological characteristic

The results showed that local rice varieties based on morphological characters of rice were grouped in two, namely the Indica and Javanica groups. There were 15 varieties *Indica* type, and 4 varieties of *Javanica*. Indica rice (paddy cere) had several characteristics of : having a lot of roots, smooth leaf surfaces, long panicle, litle hair on the the surface of the grain, no tail on the end grain, slender grain shape, grain size was small to moderate and harvest age was faster. Characteristics of the group Javanica rice (paddy fur) were : the number of tillers was less, leaves had a rough surface, long panicle, hairy grain surface, a tail on the end grain, medium to large grain size and the age old crop (Grubben and Partohardjono, 1996).

The largest diameter stem segment was found in Sigupai Wangi (V3), while the smallest diameter of the rod segment was found in Pade Cut Kresek variety (V13) (Table 2). The longest panicle variety was found in Pade Rangan (V17) and the shortest panicle variety was found in Nagan Raya Sigupai (V4) (Table 2). According to the AAK (1990), panicle length is divided into three different sizes which are short panicle (less than 20 cm), medium panicle (20-30 cm), and long panicle (more than 30 cm).

Table 1. Grouping of local rice varieties for tolerance to soil acidity based on the relative value (NL) and by age of flowering

Varieties of rice	Relative value (%)	Criteria	Flowers age
V1 (Ramos Merah)	66,40	somewhat tolerant	113
V2 (Rom Mokot)	75,52	Tolerant	98
V3 (Sigupai Wangi)	65,71	somewhat tolerant	112
V4 (Sigupai Nagan Raya)	79,53	Tolerant	112
V5 (Sambei)	72,75	somewhat tolerant	96
V6 (Rasi Singke)	68,09	somewhat tolerant	120
V7 (Kepala Gajah Kinco)	72,44	somewhat tolerant	130
V8 (Bo Padang)	77,37	Tolerant	130
V9 (Rasi Kuneng)	68,69	somewhat tolerant	130
V10 (Pade Mangat Bu)	73,43	somewhat tolerant	120
V11 (Bo Santeut)	74,98	somewhat tolerant	101
V12 (Ramos Tihion)	73,03	somewhat tolerant	112
V13 (Pade Cut Kresek)	69,19	somewhat tolerant	115
V14 (Pade Pineung)	77,00	Tolerant	110
V15 (Cantek Puteh)	75,23	Tolerant	101
V 16 (Itam Tangke)	75,06	Tolerant	119
V17 (Pade Rangan)	65,54	somewhat tolerant	115
V18 (Sigupai Blang Pidie)	70,71	somewhat tolerant	116
V 19 (Dupa)	77,21	Tolerant	100

Table 2. Classification of rice based on morphological characters, diameter of rod segment and panicle length of rice plants

Varieties of rice	Group	Diameter of rod segments (cm)	Length of panicle (cm)
V1 (Ramos Merah)	*Indica*	0.51	24.03
V2 (Rom Mokot)	*Indica*	0.53	23.50
V3 (Sigupai Wangi)	*Indica*	0.91	20.65
V4 (Sigupai Nagan Raya)	*Indica*	0.58	18.85
V5 (Sambei)	*Indica*	0.55	23.28
V6 (Rasi Singke)	*Indica*	0.55	29.70
V7 (Kepala Gajah Kinco)	*Indica*	0.59	24.03
V8 (Bo Padang)	*Javanica*	0.61	24.23
V9 (Rasi Kuneng)	*Javanica*	0.70	30.17
V10 (Pade Mangat Bu)	*Indica*	0.43	24.63
V11 (Bo Santeut)	*Javanica*	0.52	28.85
V12 (Ramos Tihion)	*Indica*	0.61	26.50
V13 (Pade Cut Kresek)	*Indica*	0.42	21.75
V14 (Pade Pineung)	*Indica*	0.53	29.65
V15 (Cantek Puteh)	*Indica*	0.50	24.50
V 16 (Itam Tangke)	*Javanica*	0.54	20.18
V17 (Pade Rangan)	*Indica*	0.60	34.88
V18 (Sigupai Blang Pidie)	*Indica*	0.50	23.78
V 19 (Dupa)	*Indica*	0.60	21.68

Grain hair-tip was not found in 13 local rice varieties and the control Dupa (V19). Four varieties had short grain hair-tip and some furry at Rasi Singke (V6), Bo Padang (V8), Rasi Kuneng (V9) and Itam Tangke (V16), while Bo santeut (V11) varieties had a long grain hair-tip and all furry (Table 3).

Differences in width and thickness of seeds in each variety are presented in Table 3. Control varieties (V19) had the widest seed, meanwhile the Pade Cut Kresek varieties (V13) was the slimmest variety. Varieties of Dupa (V19) also had the thickest seeds and variety Ramos Tihion (V12) was a thin variety. Character of the length scale 1 (seed longer than 7:50 mm) was found at 17 varieties, including varieties of Dupa (V19). Character length scale 2, (seed length of 6.61-7.50 mm) was found in Singke Rasi variety (V6) and Itam Tangke (V16).

A number of rice varieties characteristic were difficult to distinguish. Abdullah *et al.,* (2006) stated that the differences will affect the nature of plant varieties. Each plant has the characteristics for special properties that may differ from each other, so it will show the diversity of characters. According to Lesmana *et al.,* (2004) morphological features often used to distinguish varieties of rice are plant height, stem color, leaf color, leaf surfaces, grain shape, grain color and grain surfaces. The character of grain morphology on the presence of feathers covering the tip of grain, seed thickness, seed width and seed length. According Aldair *et al.,* (1966) *in* Grist (1986), grain shape consisting of three kinds, namely rounded, medium and lean. In addition, the inflorescence characters can distinguish rice varieties (Wet *et al.,* 1986).

Table 3. The presence of feather tip of grain, seeds character width, thickness and length of seed grain Aceh local

Varieties of rice	The presence of grain hair- tip	Width seeds (mm)	The thickness of the seeds (mm)	Length of seeds
V1 (Ramos Merah)	Lint	2.10	1.50	Very long
V2 (Rom Mokot)	Lint	3.00	2.00	Very long
V3 (Sigupai Wangi)	Lint	2.10	1.90	Very long
V4 (Sigupai Nagan Raya)	Lint	2.10	1.90	Very long
V5 (Sambei)	Lint	2.70	2.00	Very long
V6 (Rasi Singke)	Short and partly feathered	2.49	1.79	Long
V7 (Kepala Gajah Kinco)	Lint	3.04	2.00	Very long
V8 (Bo Padang)	Short and partly feathered	2.15	1.68	Very long
V9 (Rasi Kuneng)	Short and partly feathered	2.70	2.00	Very long
V10 (Pade Mangat Bu)	Lint	2.15	1.92	Very long
V11 (Bo Santeut)	Length and all hairy	2.10	2.00	Very long
V12 (Ramos Tihion)	Lint	2.06	1.50	Very long
V13 (Pade Cut Kresek)	Lint	1.90	1.52	Very long
V14 (Pade Pineung)	Lint	2.90	2.00	Very long
V15 (Cantek Puteh)	Lint	2.50	1.90	Very long
V 16 (Itam Tangke)	Short and partly feathered	2.20	1.79	Long
V17 (Pade Rangan)	Lint	4.10	2.00	Very long
V18 (Sigupai Blang Pidie)	Lint	2.20	1.88	Very long
V 19 (Dupa)	Lint	4.50	2.10	Very long

Acknowledgement

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Effect of NPK Fertilizer and Biochar Applications on Growth and Yield of Irrigation Rice

Zaitun[a*], Khairun Nisa[b], Sufardi[a], Chairunas[c], Anischan Gani[d], Peter Slavich[e], Malem McLeod[e]

[a] *Agriculture Faculty, Syiah Kuala University, Banda Aceh, Indonesia*
[b] *Agency Implementation of Extension and Food Security of West Aceh, Indonesia*
[c] *Provincial Agricultural Service (BPTP NAD), Banda Aceh, Indonesia*
[d] *Indonesian Centre for Rice Research, Sukamandi, West Java, Indonesia*
[e] *New South Wales Primary Industries, Australia*
* *Corresponding author: Agriculture Faculty, Syiah Kuala University, Darussalam, Banda Aceh, Indonesia 23111.*
Tel.: +62 81360301372; fax: +62 651 7555214
zaitundara@gmail.com

ABSTRACT

The objective of experiment was to investigate the effect of NPK and Biochar applications on paddy rice growth and its production. The research was conducted at Empetrieng village, Aceh Besar district, Aceh Province, Indonesia. The experimental was arranged in a randomized complete block design with two factors and four replicates. First factor was NPK at ratio of 15:15:15 as fertilizer application (0; 60; and 120 kg ha^{-1}, respectively) and second factor was Biochar application at 0 and and 10 ton ha^{-1}). The results showed that the application of Biochar affected significanly on plant height at 28 and 90 DAP (day after planting), percentage of unfilled grain per peanicle; whereas aplication of NPK affected significanly on plant height at 45 and 90 DAP, number of tiller 45 DAP, number of peanicle per clump, number of total grain per peanicle, number of filled grain per peanicle, and yield ton per ha, respectively.

Keywords : NPK fertilizer, Biochar, paddy rice

Introduction

Rice is the main food of Indonesian people. The rice field has dominant contribution in yield of paddy rice because generally rice paddy is planted in wet land. Irrigation is very important for the rice field paddy because this influences the yield of paddy rice productivity (Adiratma, 2004). Biochar or charcoal represents one option to soil management. Biochar had been applied traditionally by some farmers at many places. Several reports showed that biochar has the role in improving fertility soil. Biochar has a lot of pores because it has large surface, so it has high water holding capacity. Although biochar is not fertilizer, it can be used as mixed fertilizers (Gani, 2009).

Materials and Methods

The field experiment was established at Empetring Village, Darul Kamal Subdistrict, Aceh Besar District, Aceh Province, Indonesia on December 2009 to May 2010.

This research was designed as experiment field continued in laboratory with the the following step: (a) field trial was done by planting Ciherang varietas paddy rice. NPK fertilizer and biochar treatment were applied as according to each plot combination treatment; (b) observation was done by looking at the plant growth i.e. plant height, and number of tiller; (c) paddy rice growth was measured by determining the plant height at 28, 35, 45 and 90 day after planting (DAP) and number of tiller 28, 35, and 45 day after planting (DAP). Measurement of final plant height and number of tiller were done, at 1-2 days before harvest paddy rice; and (d) estimation to yield component of paddy rice was done by determining the number of panicle per clump, number of total

grain per panicle, percentage of unfilled grain per panicle, number of filled grain per panicle, weight of 1.000 grain at water content 14%, and yield ton per hectare at water content 14%.

The experiment was arranged in a randomized complete block design with two factors and four replicates. First factor was NPK at 15:15:15 fertilizer application. F0 was without fertilizer; F1was 60 kg ha^{-1} NPK which was equal to 150 g $plot^{-1}$; and F2 was 120 kg ha^{-1} NPK which was equal to 300 g $plot^{-1}$. Second factor was Biochar application; B0 was without biochar; and B1 was 10 ton ha^{-1} which was equal to 25 kg $plot^{-1}$.

Results and Discussion

Plant Height and Number of Tiller of Paddy Rice Plant

Vegetative growth with parameter of plant height and tiller number were not significantly affected by biochar and NPK treatments (Tables 1 and 2), eventhough, plant height and tiller number tend to increase with biochar 10 ton ha^{-1} and NPK 120 kg ha^{-1} treatments. Biochar and NPK applications can improve soil fertility by repairing soil chemical properties in the form of content improvement and in the N, P, K nutrient availibility. Improvement of N, P, K nutrient availibility would affect the nutrient availibility, so that can this would improve the growth of plant height and tiller number.

Table 1. Average of plant height effect biochar and NPK treatment

Treatment Combination	Plant height (cm)			
	28 DAP	35 DAP	45 DAP	90 DAP
B0 F0	42,70 a A	52,40 a A	66,45 a A	69,88 a A
B0 F1	44,45 a A	55,09 a A	71,30 a A	71,18 a A
B0 F2	43,68 a A	54,85 a A	71,73 a A	74,80 a A
B1 F0	45,23 a A	45,27 a A	65,63 a A	71,05 a A
B1 F1	46,28 a A	48,35 a A	72,80 a A	76,93 a A
B1 F2	46,75 a A	48,74 a A	76,88 a A	78,23 a A
BNT 0,05	3,206	15,341	4,627	5,700

Tabel 2. Average of tiller number effect biochar and NPK treatment

Treatment Combination	Number of tiller (bar)		
	28 DAP	35 DAP	45 DAP
B0 F0	14,08 a A	12,70 a A	11,98 a A
B0 F1	12,50 a A	13,05 a A	13,68 a A
B0 F2	14,35 a A	14,53 a A	16,23 a A
B1 F0	13,05 a A	13,05 a A	11,65 a A
B1 F1	13,70 a A	15,03 a A	14,83 a A
B1 F2	14,15 a A	14,83 a A	17,18 a A
BNT 0,05	1,802	2,269	1,937

Mori and Marjenah (1993) reported that biochar or husk chrocoal can be used as soil material repairing with increasing air permeability as well as the water percolation. Purnomo (2009) stated that paddy rice plant responded to single NPK and compound NPK. Compound NPK fertization significantly improves paddy rice plant height at the primordial stage. Improvement of this growth is caused of by soil chemical properties may increase the rate of N and P in soil.

Kaderi (2004) stated that number of tiller per clump was higher after the addition of NPK compared to control plant. This showed that compound of NPK added the nutrient element for plant. Growth of tiller number is related to N sufficiency and efficacy to primordial forming.

Yield of Paddy Rice Plant

Yield component of paddy rice plant did not significantly effected either by Biochar or NPK treatments (Table 3), eventhough the yield component of paddy rice plant tend to increase with the application of Biochar at 10 ton ha^{-1} and NPK at 120 kg ha^{-1} compared to the control treatmentwithout Biochar and without NPK.

Tabel 3. Average of yield component effect biochar and NPK treatment

Variable	Treatment combination						BNT 0,05
	B0 F0	B0 F1	B0 F2	B1 F0	B1 F1	B1 F2	
Number of panicle per clump (panicle)	9,67 Aa	11,04 aA	12,54 aA	9,56 aA	10,96 aA	11,75 aA	1,249
Number of total grain per panicle (grain)	146,72 aA	130,75 aA	117,09 aA	145,14 aA	126,61 aA	122,65 aA	20,425
Percentage of unfilled grain per panicle (%)	20,37 aA	22,84 aA	23,14 aA	16,68 aA	19,11 aA	17,86 aA	4,569
Number of filled grain per panicle (grain)	116,29 aA	100,81 aA	89,78 aA	120,61 aA	102,44 aA	100,63 aA	14,190
Weight of 1.000 grain at water content 14% (gram)	24,78 aA	24,93 aA	24,74 aA	25,02 aA	25,28 aA	24,21 aA	1,576
Yield ton per hectare at water content 14% (ton ha^{-1})	4,32 aA	5,88 aA	6,20 aA	4,33 aA	5,89 aA	6,79 aA	0,748

Biochar at 10 ton ha^{-1} tend to increase the yield component of paddy rice plant as is compared to without Biochar treatment. This is because Biochar at 10 ton ha^{-1} gave the growth media of soil microorganism. Consequently this may improve the amount of soil microorganism but this was not followed with the increase of nutrient availibility in soil. Therefore, soil microorganism exploit the nutrient source to energy that give in the emulation between soil microorganism with the root plant in fulfilling nutrient. This may cause competition between soil microorganism and the root plant.

Increase of NPK dose tend to to improve the yield component of paddy rice plant compared to that without NPK treatment. This is caused by NPK fertilize that can directly gave the nutrient element required by plant. So that with the existence of nutrient element provided by NPK can fulfill the nutrient required by the plant growth. This may also repaire soil chemical properties with sufficiency of nutrient element so that this affected the increase of yield component of paddy rice plant.

Conclusions

Treatment of biochar affected significantly onthe plant height at 28 and 90 day after planting (DAP), percentage of unfilled grain per panicle. Meanwhile, treatment of NPK affected significantly on the plant height at 45 and 90 DAP, number of tiller 45 DAP, number of panicle per clump, number of total grain per panicle, number of filled grain per panicle, and yield ton per ha.

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Effects of New Foliar-Application Fertilizer Containing 5-Aminolevlinic Acid on Yield Increase of Direct-Sowing Rice Plants

Ryuji Yoshida[a*], Keitaro Watanabe[b]

[a]*Toyama prefectural University, Toyama, Japan*
[b]*Cosmo Oil Co., Ltd., Japan*
[*]*Corresponding author: Toyama Prefectural University, Imizu, 5180, 939-0398, Japan*
Tel.: +81 766 56 7500; Fax: +81 766 56 0396
yoshida@pu-toyama.ac.jp

Abstract

In Japan, the cultivation methods for rice plants (*Oryza sativa* L.) in paddy field can be mainly classified into two types, i.e. mechanical transplanting cultivation and direct-sowing. However, in the case of direct-sowing cultivation the grain number per m^2 becomes often an excess of 30,000 grains. As the result, the decrease of percentage of ripened grains takes places in inferior grains than in superior ones and, as a consequence, the grain yield decreases. In the present experiment, effects of foliar application fertilizer containing 5-aminolevulinic acid (5-ALA fertilizer: commercial name; Penta-keep) on the percentage of ripened grains in direct-sowing rice plants were examined. This 5-ALA fertilizer was sprayed at the heading stage of cvs. Koshihikari and Tenkomori, the promotive effect on the yield increase of grains was found in both the cultivars tested. It should be noted that the high yielding effects were at least in part supported by the increasing of grain filling in inferior grains and superior, especially in inferior grains. On the other hand, the excellent effect of 5-ALA fertilizer was found in the aerial spray by small automated helicopter. From all results, we emphasize that the application of foliar application fertilizer containing 5-ALA are very useful to achievehigh yield of direct-sowing rice plants.

Keywords: 5-aminolevulinic acid (5-ALA), direct-sowing of rice plants, new foliar-application fertilizer (-ALA fertilizer; Penta-keep), yield-increase

Introduction

In Japan, the cultivation methods for rice plants (*Oryza sativa* L.) in paddy field can be mainly classified into two types, i.e. mechanical transplanting cultivation and direct-sowing. In recent year, the growing area using the latter cultivation type progressively tends to the extension because of the establishment of labor-saving cultivation for rice. However, the grain number per m^2 of rice plants cultivated by direct-sowing becomes often an excess of 30,000 grains. As the result, the decrease of percentage of ripened grains take places in inferior grains than in superior ones and, as a consequence, the grain yield decreases.

Yoshida *et al*. (1996) reviewed that 5-aminolevulinic acid (5-ALA) has often promoting effects on yield increase of crops. Hotta *et al*. (1997) reported that ALA has plant growth regutating properties at low concentration (<30ppm) and the foliar application of this compound enhanced the growth and yield of kidney beans, barley, potato and garlic. However, there is insufficient information about the promotive effect of ALA on the grain filling of rice plants. The supply of large amount for 5-ALA having high-quality and inexpensive price has already been successfully achieved by Tanaka (1995). A new foliar-application fertilizer containing 5-aminolevlic acid using the extension in agriculture field was also developed by Cosmo Oil Research Group (Tanaka *et al*. 2006). Commercial name for this fertilizer is PENTAKEEP® super or 5-ALA fertilizer.

This experiment, therefore, was carried out to clarify whether the foliar application of 5-ALA fertilizer was able to alter the percentage of ripened grains, especially in direct-sowing rice plants. The utility for achieving high yielding grains of direct-sowing rice cultivation was discussed.

Materials and Methods

Experiment I (2009)

Rice cultivation. Rice cultivars used were Koshihikari (medium maturing variety) and Tenkomori (late maturing variety) in Toyama, Japan. The coating pregerminated seeds with calcium peroxide (CaO_2 of 2-fold) were sown in paddy soil by using direct-shooting seeder. The sowing rate of both the cultivars was 2.5～2.8 kg per 0.1ha.The controlled availability fertilizer (LPs, N:P:K=18:12:12) was basally dressed at rate of 30～35kg per 0.1ha. When the growth of rice plants reached the full heading time, 5-ALA fertilizer was sprayed to the whole plant.

Spray of 5-ALA fertilizer. Fertilizer 5-ALA was composed of N at 8.0%, P_2O_5 at 5.0%, K_2O at 3.0%, MgO at 3.0%, Fe at 0.29%, Mn at 0.12% and other micro-elements, but the concentration of 5-ALA was not only open. At the full heading time, 5-ALA fertilizer was sprayed weekly twice to whole plants. The concentration and application volume of 5-ALA fertilizer were 2000-fold solution and 200 liter per 0.1ha, respectively. The percentage of ripened grains, such as superior and inferior gains of panicle, was determined at the harvesting time. Yield component of rice plants and quality of grains was also observed.

Experiment II (2010)

Cv. Koshihikari was grown in paddy field. The rates of basal-dressing fertilizer were the same as those used in Experiment I. The application of 5-ALA fertilizer was sprayed by small automated helicopter. In this case of aerial application, the concentration and application volume of 5-ALA fertilizer were 8-fold solution and 0.8 liter per 0.1ha, respectively. The effects of spraying 5-ALA fertilizer on the percentage of ripened grains and yield component were evaluated at the harvesting stage.

Results and Discussion

In Japan, the yield decrease of direct-sowing rice plants is attributed to the excess of grain number per m^2 (more than 30,000 grains). This excess of unfulled grains per m^2 takes place the increase of sterile and imperfect grains in panicle. In practical producing field of direct sowing cultivation, the percentage of ripened grains in cv. Koshihikari was well known to vary the range of 65～74%. For achieving high-yielding grains in direct-sowing rice cultivation, therefore, understanding the effects of 5-ALA fertilizer was particularly important. In this experiment, the unfulled grains of panicle were divided into superior and inferior grains (Figure.1).

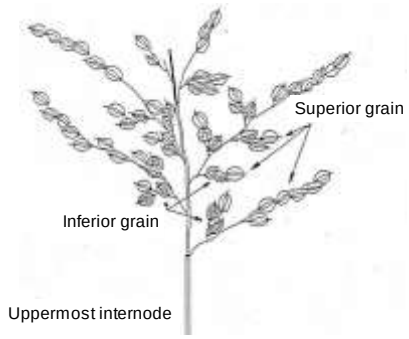

Figure 1. Classification of superior and inferior grains in panicle.

All grains attached directly to primary branches and the terminal grains of secondary branches were defined as superior grains and the grains on secondary branches, excepting the terminal one, were defined as inferior grains. The following data (Tables 1, 2, 3) were obtained from Experiment I. Table 1 shows the effects of spraying 5-ALA fertilizer on yield component. In the case of cv. Koshihikari, the rate of grain-straw was 1.1 in both the control plants (untreated-treated with 5-ALA fertilizer) and the plants treated with 5-ALA fertilizer. The unfulled grain number per m^2 was about 33,000 in both the plants. In the case of cv.Tenkomori, the grain-straw ratio and unfulled grain number were 0.9 and 30,000～33,000, respectively. The yield components in both the cultivars did not vary with the spraying 5-ALA fertilizer.

Table 1. Effects of 5-ALA fertilizer on yield component

Cultivar	Treatment 5-ALA fertilizer	Unfulled grain weight (g/hill)	Straw weight (g/hill)	Unhulled grain - straw ratio	Panical number per hill	Unhulled grain number per hill	Unhulled grain number per m^2
Koshihikari	Untreated (control)	51	48	1.1	22.9	1.839	33.470
	Treated	52	49	1.1	23.0	1.806	32.860
Tenkomori	Untreated (control)	45	48	0.9	21.2	1.654	30.103
	Treated	46	51	0.9	21.7	1.786	32.504

1. The density of direct-sowing rice plants is 21~24 per m^2
2. The value show the means of 3 replications. The number of hills tested is 240.

The percentage of ripened grain in rice plant treated with 5-ALA fertilizer is shown in Tables 2. As was expected, the 5-ALA fertilizer enhanced the percentage of ripened grains. This increase was much more marked in the inferior grains than in the superior ones. The extent of the increase in both the cultivars brought about by the spraying treatment amounted from 13.6 to 29.9% (cf. control) in the inferior grains. Thus, the spraying treatment of 5-ALAfertilizer significantly affected the grain filling of inferior grains ($p < 0.05$).

Table 2. Effect of 5-ALA fertilizer on percentage of ripened gains

Cultivar	Treatment 5-ALA fertilizer	Unhulled grain number per panicle	Ratio of grains per panicle (%)		Percentage of ripened grain (%)	
			Superior grains	Inferior grain	Superior grains	Inferior grain
Koshihikari	Untreated (control)	80.3±45	77.9±0.7	22.1±0.7	$82.7±3.0_a$	$54.9±2.1_b$
	Treated	78.5±5.5	82.8±2.3	17.2±2.3	$88.8±2.8_a$	$84.8±4.6_a$
Tenkomori	Untreated (control)	78.0±7.0	71.8±2.6	28.2±2.6	$94.0±3.3_a$	$79.8±4.4_b$
	Treated	82.3±2.8	72.7±3.0	27.4±3.0	$95.7±1.4_a$	$93.4±1.5_a$

1.Date are means±SE of 150 panicles.
2.Different letters within columus in each cultivar indicate significant differences by Tukey's multiple range test ($p<0.05$)

As for the 1,000 grain weight, the cv. Tenkomori showed higher value in inferior grains than that in superior ones (Table 3). In the case of cv. Koshihikari, the fulled grain yield of the plants treated with 5-ALA fertilizer was 627.5kg per 0.1ha, this value was higher than that of control plants (Table 4). The effect of spraying 5-ALA fertilizer also was much more marked in the case of cv. Tenkomori. The degree of yield increased was 145.5kg per 0.1ha. These findings showed that 5-ALA fertilizer acts as a regulator for yield increase of rice plants through a steady increase of grain filling, especially in inferior grains. Regarding the physiological function of 5-ALA, Tanaka (1995)

has been reported in radish plants that 5-ALA enhanced the activity of photosynthesis and suppressed the rate of respiratory activity. Yoshida (1996) showed that the accumulation fractan in rakkayo and shallot plants was increased by 5-ALA application. From the results obtained here and these findings, the steady increase of the percentage of ripened grains by the application of 5-ALA fertilizer seemed to be dependent on the activity of photosynthesis during the grain filling period. However, the physiological functions of this fertilizer during maturation of rice grains are still almost unknown at the present.

Table 3. Effect of 5-ALA fertilizer on yield and quality of gains (hulled grains)

Cultivar	Treatment 5-ALA fertilizer	1000 grain weight (g)	Grain yield (kg/0.1ha)	Quality of grain (%)		
				Protein content (%)	Amilose content (%)	Index of paratability
Koshihikari	Untreated (control)	22.2.0±0.5	506.6	6.1	18.7	73
	Treated	22.0±0.5	627.5	5.9	18.7	74
Tenkomori	Untreated (control)	22.3±0.3	583.3	6.0	18.9	74
	Treated	23.7±0.5	728.8	6.0	19.0	75

1.means±SE
2.quality of grains is determined by Satake rice*grain paratability tester

Table 4. Effects of 5-ALA fertilizer sprayed by the helicopter on the percentage of ripened grains in cv. Koshihikari

Treatment 5-ALA fertilizer	1000 grains weight (g)	Percentage of ripened grains (%)		Grain yield (kg/0.1ha)
		Superior grains	inferior grams	
Untreated (control)	$21.2\pm0.3_a$	$83.5\pm3.9_a$	$68.7\pm5.1_b$	670.0
Treated	$20.8\pm0.2_a$	$86.2\pm5.0_a$	$79.2\pm4.3_a$	696.2

1.Data are means±SE of 150 panicles
2. Different letters within columns indicate significant differences by Tukey's multiple range test (p=0.05)

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Yield Stability Evaluation of Upland Rice Lines Obtained from Anther Culture

Deni D Sulaeman[a], Bambang S Purwoko[b*], Iswari S Dewi[c], Muhamad Syukur[b], Desta Wirnas[b], Heni Safitri[d]

[a]*Graduate Student of Plant Breeding and Biotechnology Major, the Graduate School of Bogor Agricultural University,* [b]*Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural Universit,* [c]*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor,* [d]*Indonesian Center for Rice Research, Bogor*
**Corresponding author: Jln Meranti, Darmaga, Bogor 16680 Indonesia.*
Tel: +62 251 8629353.
E-mail address: bambangpurwoko@gmail.com

Abstract

The objective of this research was to study the adaptation and yield stability of upland rice lines obtained from anther culture. Ten upland rice doubled-haploid (DH) lines were tested for their potential yield in eight different locations (in Provinces of Lampung, West Java, Central Java, Yogyakarta, East Java - Indonesia) in the rainy season of 2010/2011 along with two check varieties (Way Rarem and Batutegi). In each location, the design was Randomized Complete Block Design with four replicates. The method of Francis & Kannenberg, Finlay & Wilkinson, Eberhart & RusseII and AMMI (Additive Main Effect Multiplicative Interaction) were used to analyze the adaptation and yield stability of the tested DH lines. The results indicated that the line showing the most stable yield in different environment was I5-10-1-1 followed by WI-44, and IG-38. I5-10-1-1 produced 4.01 tons of dry grain per hectare. The line showing the highest yield was WI-44, and this line produced 4.72 tons of dry grain per hectare. Visualization with AMMI showed that IW-56 and IW-67 lines were specifically adapted in Purworejo, O18-b-1 is specifically adapted in Bogor, and IG-19 is specifically adapted in Malang, respectively.

Keywords: upland rice, DH lines, yield stability

Introduction

Efforts to increase the food production, especially rice, through the utilization of dry land can be reached by breeding upland rice, since the high-yielding upland rice varieties may be accepted by farmers. Anther culture has been reported to produce doubled-haploid (DH) plants or pure lines in a short time (Dewi *et al*., 1996). On the contrary, conventional breeding will require a long period of time (more than 5 years) in obtaining pure lines as a result of combining the desirable traits of different varieties or parents. Through anther culture of F1, several number of upland rice DH lines were obtained from previous experiment, i.e. IW-56, IW-67, IG-19, IG-38, and GI-8 lines tolerant to low light intensity or shade (Sasmita *et al*., 2006); III3-4-6-1 and I5-10-1-1 lines tolerant to aluminum toxicity (Herathwati *et al.*, 2009); while O18-b-1 and B13-2e lines tolerant to both shade and Al toxicity (Purwoko, 2007). Those DH lines need to be evaluated further.

Subandi (1981) asserted that in the establishment of superior varieties breeders need to pay attention to the yield stability in a systematic and continuous manner, starting from the formation of the basic population to test varieties. Adaptability evaluation in different growing environments need to be done because of the diversity of land, soil, cultivation, cropping patterns and planting season in Indonesia. The diversity of the growing environment will affect the yield per unit area, because the plant growth is a function of genotype and environment (Allard, 1960). Appearance of plants depends on the genotype and environmental conditions where the plant grew, as well as the interaction between the two. Yield stability evaluation through a series of multi location test is an important stage before the varieties can be released. Therefore, this research

was aimed to study the adaptation and yield stability of upland rice DH lines obtained from anther culture in 8 different environments.

Materials and Methods

The yield stability test of upland rice was held at eight locations in the rainy season during October 2010 until April 2011. Testing locations spread across Java and Sumatra, namely: the Taman Bogo - Lampung, Natar - Lampung, Cikarawang Bogor - West Java, Sukabumi - West Java, Indramayu - West Java, Purworejo - Central Java, Wonosari - Gunung Kidul, and Malang - East Java. A total of 12 genotypes were used as test material, which consisted of 10 upland rice DH lines obtained from anther culture, i.e. III3-4-6-1, I5-10-1-1, WI-44, GI-7, O18-b-1, IW-67, IG-19, IG-38, IW 56, and B13-2e, while Batutegi and Way Rarem.were two check varieties used for comparison.

The experimental design was Randomized Complete Block Design with four replicates. An experimental unit consisted of 4 x 5 m^2 plot. Each of the tested genotypes were planted with a spacing of 30 x 15 cm. Observations and data analysis were conducted on dry grain yield per hectare. The method of Francis & Kannenberg, Finlay & Wilkinson, Eberhart & Russell and AMMI (Additive Main Effect Multiplicative Interaction) were used to analyze the adaptation and yield stability of the tested DH lines.

Results and Discussion

The variance analysis of pooled data at eight test locations showed the occurrence of interaction between lines (genotype) and environment (G × E) (Table 1). The existence of such interactions will cause each lines responded differently to the environment in which testing was conducted. The response was mainly shown by the fluctuations in grain yield. A genotypes or varieties though, will not always produce the same great results if planted in different environments. This is caused by the large diversity of the macro geophysical environment that will provide the large environmental diversity for plant growth as well (Satoto *et al*, 2009).

Table 1. Analysis of variance of pooled data at 8 locations

Source of Variation	df	SS	MS	F value	
Location	7	123.18	17.60	30.68	**
Rep/Location	24	66.29	2.76	4.82	**
Lines	11	123.48	11.23	19.57	**
Lines × Location	77	230.93	3.00	5.23	**
Error	264	151.41	0.57		
Total	383	695.30			

*Notes : ** very significantly different*

The average of dry grain yield of WI-44 is able to outperform other lines and check varieties at two locations, namely Bogor and Malang (Table 2). The average dry grain yield showed that only the WI-44 and IW-67 was able to outperform Batutegi varieties, while Way Rarem varieties only surpassed by WI-44. The difference in average yields between WI-44 with Batutegi was 0.34 tons/ha, where WI-44 was about 7.67% higher than Batutegi. The difference in average yield between IW-56 with Batutegi was 0.01 tons/ha or approximately IW-56 was only 0.2% higher than

Batutegi. However, the difference in average yields between WI-44 with Way Rarem was 0.1 tons/ha or WI-44 approximately 17.2% higher than Way Rarem.

Tabel 2. The average dry grain yield (tons/ha) of upland rice DH lines in every test location

DH Lines	LOCATION								Yield Average (tons/ha)
	Indra-mayu	Wono-sari	Purwo-rejo	Suka-bumi	Bogor	Malang	Lam-pung	Taman Bogo	
III3-4-6-1	2.95	2.39	4.88	2.59	1.52	3.11	2.79	3.36	2.95
I5-10-1-1	2.88	3.44	4.62	4.70	4.81	4.11	2.99	4.51	4.01
WI-44	3.24	3.74	5.55	5.69	5.40	5.98	4.15	4.02	4.72
GI-7	2.72	2.60	2.53	3.73	5.16	5.10	2.68	3.69	3.52
O18-b-1	2.17	2.41	3.19	3.45	5.04	3.58	2.68	2.52	3.13
IW-67	3.14	4.35	6.94	5.08	4.14	3.90	3.76	3.85	4.39
IG-19	1.86	3.61	2.66	3.40	3.79	4.32	2.26	4.48	3.30
IG-38	2.06	3.38	3.28	3.70	2.89	4.51	2.31	4.13	3.28
IW-56	2.51	4.39	6.81	4.14	2.69	3.25	3.53	3.21	3.82
B13-2e	2.91	2.52	3.54	4.07	4.22	4.66	4.00	4.41	3.79
Batutegi	3.01	3.14	5.89	4.16	3.04	5.63	4.66	5.54	4.38
Way Rarem	3.79	3.87	4.35	6.95	3.46	5.33	4.99	4.24	4.62
Average	2.77	3.32	4.52	4.30	3.85	4.46	3.40	4.00	3.81
CV (%)	24.0	23.2	20.6	15.6	23.1	10.6	12.6	25.7	

The parameters of stability of dry grain yield of upland rice DH lines from eight locations are presented in Table 3. Lin *et al.*(1986) suggested three concepts of stability. A genotype is considered to be stable if: (1) the variance among environment is small, (2) the response to its environment is parallel to the mean response of all genotypes in the trial, (3) residual mean square (MS) from the regression model on the environmental index is small.

Tabel 3. Parameters of stability of dry grain yield of upland rice DH lines from eight locations

DH lines	Yield (t/ha)	SD_i	CV_i	b_i		δ_2	R_i^2
III3-4-6-1	2.95	1.09	32.51	0.64	*	1.04	0.31
I5-10-1-1	4.01	0.74	19.74	1.10	ns	0.73	0.64
WI-44	4.72	1.02	22.17	1.52	*	1.25	0.43
GI-7	3.53	1.21	31.16	0.77	*	1.40	0.29
O18-b-1	3.13	1.01	29.55	0.76	*	0.98	0.37
IW-67	4.39	1.00	26.56	1.31	*	1.58	0.32
IG-19	3.30	1.23	28.85	0.84	ns	1.05	0.37
IG-38	3.28	1.10	25.70	1.02	ns	0.82	0.47
IW-56	3.82	1.38	35.92	1.18	ns	2.19	0.20
B13-2e	3.79	1.04	19.71	0.78	*	0.65	0.67
Batutegi	4.22	1.20	27.98	1.17	ns	1.75	0.29
Way Rarem	4.62	1.15	24.42	0.90	ns	1.47	0.35
Rata-rata	3.81			1.00			

Notes : SDi= Standard deviation of genotype; CVi=Coeficient of variance of genotype;, bi= Coefficient of regression of genotype, value of b_i * (significantly different with 1), ns (not significantly different with 1); δ_2=parameter of deviation;, R_i^2=Coefficient of determination.

Francis & Kanennberg (1978)

Francis and Kanennberg (1978) measure the stability by using the coefficient of variability (% CV_i) of each genotype tested in multiple environments. The small values of its coefficient of genotype diversity meant that the genotype is more stable. Moedjiono and Mejaya (1994) categorized the value of the coefficient of genotype diversity in four groups, namely low (<25%), rather low (25 -50%), rather high (50-75%), and high (75-100%). Therefore, based on those criteria the DH lines tested in this study fell into of low and rather low groups. I5-10-1-1, WI-44, B13-2e, and Way Rarem have a low coefficient of genotype diversity, thus classified as stable.

Finlay and Wilkinson (1963)

Stability analysis of Finlay and Wilkinson (1963) is a method of measurement stability that is based on the regression coefficient (b_i) between the average yields of a genotype with a general average of all genotypes tested in all test environments. This analysis can explain the phenomenon of stability and adaptability of a genotype. Finlay and Wilkinson categorize standard b_i values of stability in the three groups, namely (1) the stability is below the average, if the value of $b_i > 1$, (2) stability is equivalent the average, if the value of $_{bi} = 1$, (3) stability is above the average, if the value of $b_i < 1$.

Based on those criteria, Batutegi and Way Rarem and also three DH lines had b_i values not significantly different from 1, i.e. I5-10-1-1, IG-19, IG-38, and IW-56. Those DH lines were categorized as a stable line, which meant the lines will be able to adapt to the large environment. Lines with the stability below the average were WI-44 and IW-67 with the b_i of 1.52 and 1.31, while the lines with the stability above the average were III3-4-6-1, GI-7, O18-b-1, and B13-2e with b_i values of 0.64, 0.77, 0.76, and 0.78, respectively. Lines that have below-average stability are sensitive to environmental changes and adapt to particular environmental benefit (favorable). The lines which have above average stability are generally able to adapt to marginal environments.

Eberhard dan Russel (1966)

Stability analysis of Eberhard and Russell (1966) is a measure of stability based on the deviation from the regression of the average value of genotype on the environmental index. A genotype is stable if residual mean square (MS) from the regression model on the environmental index is small. Parameter stability seen from the deviation value (δ_2) and coefficient of determination (R_i^2) of the tested genotypes. Model stability of a genotype is good if it has little δ_2 value and large R_i^2 value (approaching 1).

Based on these parameters, two DH lines namely B13-2e and I5-10-1-1 had the smallest value of δ_2, i.e. 0.65 and 0.73 (Table 3). Coefficient of determination (R_i^2) from these two lines also had the largest value among lines and other varieties, i.e. 0.67 and 0.64. Therefore, the regression model used to estimate the stability of those two lines are better than the other lines.

AMMI (Additive Main Effect Multiplicative Interaction)

Due to limited visualization of graphs that are only capable of displaying two-dimensional graph, then the model described in this paper is AMMI2. AMMI2 model can explain the diversity of the interaction effect up to 70.98%. Bi-plot of interaction affects AMMI2 model for the dry grain yield of upland rice DH lines are presented in Figure 1. The bi-plot of principal components 1 and 2 can explain the main components of lines which are stable at all location test or specific to a particular location. Mattjik and Sumertajaya (2000) states that a line or genotype is said to be stable when close to the axis or point (0,0). The lines or genotypes that are far away from the axis but close to the line location, then the lines were classified as specific location lines. Based on these, lines that are stable at all location test are I5-10-1-1, WI-44, IG-38, and Way Rarem. IW-56 and IW-67 were specific to the location of Purworejo, O18b-1 line-specific for the location of Bogor. IG-19 is specific to the location of Malang.

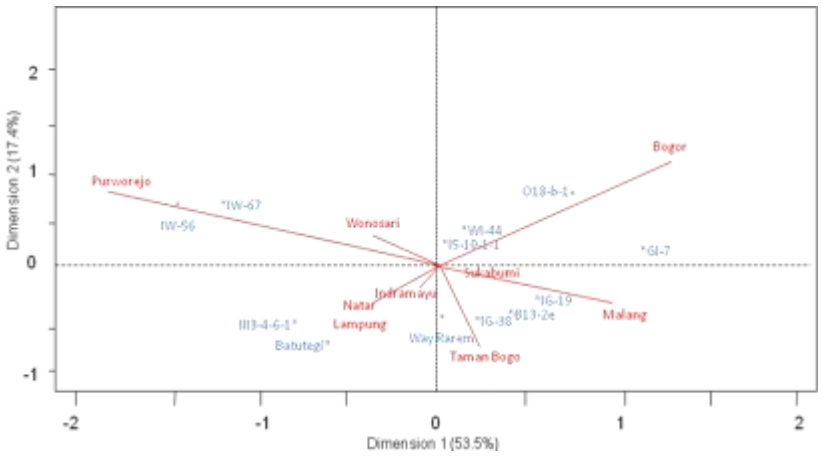

Figure 1. Bi-plot of interaction effects AMMI2 model for the dry grain yield of upland rice DH lines derived from anther culture

Conclusions

The DH line showing the most stable yield in different environment was I5-10-1-1 followed by WI-44, and IG-38. Visualization with AMMI showed that IW-56 and IW-67 lines were specifically adapted in Purworejo, O18-b-1 was specifically adapted in Bogor, and IG-19 was specifically adapted in Malang.

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Yield and Blast Resistance Evaluation of Upland Rice Lines with New Plant Type Characters

Jose MA Ornai[a] , Bambang S Purwoko[b*], Desta Wirnas[b], Iswari S Dewi[c], Heni Safitri[d]

[a] *Graduate Student, Plant Breeding and Biotechnology Major, the Graduate School, Bogor Agricultural University*
[b] *Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
[c] *Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Bogor*
[d] *Indonesian Center for Rice Research, Bogor*
** Corresponding author: Jln. Meranti, Darmaga, Bogor 16680 Indonesia.*
Tel/ Fax: +62 251 8629353.
bambangpurwoko@gmail.com

Abstract

The present studies were conducted to determine agronomic characters, genetic variability, broad sense heritability (h^2_{bs}) and blast resistance of doubled haploid rice lines and to obtain new plant type (NPT) of upland rice with high yield potential and blast resistant. The research was conducted in Bogor and Sukabumi, Indonesia started from November 2010 to June 2011. There were 14 genotypes evaluated namely the new lines of rice from anther culture (FG1-66-2-1, FG1R-108-1-1, FG1R-51-2-1, FM1-14-1-1, FM1-14-1-2, FM1-25-1-1, FM1-25-1-2, FM1-57-1-2, FM1R-32-1-1, FM1R-23-1-1, FG2-47-1-3, FM1R-19-2-4, FM2-12-1-1, FAT-4-1-2), as well as Fatmawati, Limboto and Batutegi as check varieties. The experimental design used was randomized block design (RBD). The results showed that there was variability on agronomic traits and blast resistance among lines evaluated. FG2-47-1-3 did not have complete characters of NPT, but the line had superior characteristics such as high percentage of filled grain, medium days to harvest, medium height, medium 1000-filled grain weight, stay green when harvested and high resistance to blast disease. The results also indicated that the tested population had high heritability (h^2_{bs}) in leaf blast and neck blast resistance, plant height, filled grain per panicle, days to harvest, number of grain per panicle and 1000-filled grain weight. They showed high variance coefficient for leaf blast and neck blast resistance, flag leaf length and percentage of filled grain.

Keywords: new plant type, upland rice, blast resistance, heritability.

Introduction

Rice is staple food in Indonesia. Population growth will increase national rice consumption (Prasetyo 2008). In the year of 2000-2006, population growth rate was 1.36% with per capita consumption of rice at 137 kg. It is estimated that in 2010, 2015 and 2020 consumption of rice and a population with declining population growth rate assumption of 0.03%, respectively was 32.13 million tons with a population of 235 million, 34.12 million tons with a population of 249 and 35.97 million tons with population of 263 million inhabitants (Puslitbangtan 2007).

Increase in national rice production must be continually pursued. One of the effort to increase national rice production is the use of dry land to grow upland rice so as to support national food security (Puslitbangtan 2007). Indonesia has 11.6 million ha of dry land (BPS 2005). Dry land used for planting upland rice in 2010 reached 1.13 million ha (Deptan 2011), so there are still opportunities to expand the area of upland rice cropping. Upland rice production reached 3.45 million tons with yield of 3.05 tons/ha (Deptan 2011). Contribution of upland rice to the national rice production is still low at 5% (Prasetyo 2008).

Increased productivity of upland rice can be done by developing a new plant type (NPT) of upland rice lines. NPT ideotype characters desired, among others, are plant height <150 cm, number of productive tillers > 6, number of grain per panicle > 150, short growth period (105-124 days), good filled grain (> 75%), 1000-filled grain weight more than 28 g (Herawati 2010).

Based on previous research, rice lines were obtained from crosses of a new plat type of lowland rice Famawati with local upland rice varieties from the island of Buru namely Fulan Telo Gawa (FTG) and Fulan Telo Mihat (FTM) through anther culture (Safitri 2010). Lines need to be further evaluated both morphological and agronomic characters/ performance and resistance to blast disease.

Blast disease is caused by fungus *Pyricularia grisea*. The disease can reduce the productivity of upland rice between 11-50% (Beker *at al*. 1997; Scardaci *et al*. 1997). Fungus *Pyricularia grisea* infects leaves at vegetative stage, called leaf blast, while in the generative stage, the pathogen also infects the panicle neck, called neck blast (BB Biogen 2009).

The present studies were conducted to obtain information on performance of agronomic characters and resistance to leaf blast and neck blast in the new rice lines. In addition, this study was also aimed to select a new plant type of upland rice lines with high yield and resistant to blast disease.

Materials and Methods

The research was conducted in Babakan Experiment Station, University Farm, Bogor Agricultural University and in the village of Bojong, Cikembar Sub-District, Sukabumi District, West Jawa, Indonesia (endemic blast region) from November 2010 to April 2011. The genetic materials were 15 doubled haploid upland rice lines obtained from anther culture of F1, crosses between Fatmawati and Fulan Telo Mihat or Fulan Telo Gawa (Safitri 2010). Fatmawati, Batutegi and Limboto were used as a check varieties.

This study used a randomized blocked design with lines as treatment and consisted of 3 replicates. Experimental unit in Bogor was done in the form of a plot of 3 x 3.6 m. Three seeds were planted per hill with spacing of 30 x 20 cm. In Sukabumi, each replicate was 10 x 2 m. One seed was planted per hill with spacing of 20 x 10 cm. Fertilizer use were 10 tons of manure/ha, 200 kg of Urea/ ha (given: 20% age 1 week, 40% at age 4 weeks and 7 weeks), 100 kg/ha of KCl and 100 kg/ha of SP36. Maintenance was performed in accordance with the conditions and crop needs in the field.

Observations were made on 5 plants per plot on variables: plant height, number of productive tillers, days to harvest, total grain per panicle, percentage of filled grain, 1000-filled grain weight, productivity, the scale and intensity of leaf blast and neck blast disease. The data obtained were analyzed by F test, if significantly different then followed by Dunnett test on a level of significance 5%, range estimation and analysis component of the correlation between characters.

The intensity of leaf blast disease was calculated with the formula: $I = \Sigma (n \times v) / (N \times V) \times 100\%$, where I = intensity of disease, n = number of plants infected, v = scale of plant infected, N = total number of plant observed, V = the highest scale of blast disease (9) (IRRI 1996). Resistance to blast disease were classified into resistant, if $I \leq 10\%$ and susceptible, if $I > 10\%$. Observation of the panicle neck blast was performed at harvest which includes the scale and intensity of the disease. The intensity was calculated by: $I = \Sigma n / N \times 100\%$, where I = intensity of blast disease, n = number of panicles infected, N = number of panicles observed. The scale of the disease was determined based on the evaluation system on panicle neck blast disease from IRRI (1996) as follows: 0 = no symptom, 1 = infection of less than 5%, 3 = 5-10% infected, 5 = 11-25% infected, 7 = 26-50% infected, 9 = more than 50% infected. Panicle neck blast resistance were classified into resistant (score 0-1), moderately resistant (score 3-4), moderately susceptible (score 5-6) and susceptible (score 7-9).

Results and Discussion

The variance analysis showed that the genotype effect was significant on all characters observed. This indicated that there were differences between doubled haploid lines of upland rice. The coefficient of variability (CV) ranged between 2.5 and 43.6% (Table 1). Plant height ranged from 73.8 to 124.0 cm (Table 1). Higher plant meets the criteria as a new plant type of upland rice lines. According to Herawati (2010) and Abdullah *et al*. (2005), height of new plant type of upland rice is under 150 cm and for a new plant type of lowland rice is 80-100 cm. The height of some lines tested (FG1R-108-1-1, FG1R-51-2-1, FM1-14-1-1, FM1-57-1-2-32-FM1R 1-1 FG2-47-1-3) were significantly different from and were higher than Fatmawati (Table 1). Nonetheless, plant height still meets the general criteria for the plant height of new types of upland rice lines.

The number of productive tillers of 14 lines tested ranged from 6.5-15.5. According to Peng *et al*. (2008), the number of productive tillers are ideal for a new pant type of rice (NPT) is 10-15 for lowland rice, while according to Herawati (2010) the number of productive tillers for a new plant type of upland rice lines is > 6. Fatmawati has the potential to produce 6-14 tillers. The number of tillers Fatmawati in upland conditions conducted in this study was 13.

Table 1. Agronomic performance of doubled haploid upland rice lines

Lines	PH	NPT	DH	TS	FG	PFG	B1000	YP
FG1-66-2-1	91.2	11.8	127.7	177.6	59.4	33.3	29.7^{e+}	0.52
FG1R-108-1-1	124.0^{a+}	6.5^{a-}	130.3	137.4^{e-}	11.6^{e-}	17.1	26.3	0.24^{e-}
FG1R-51-2-1	116.6^{a+}	7.9	130.0	154.0	7.5^{e-}	4.5^{e-}	29.3^{e+}	0.08^{e-}
FM1-14-1-1	102.7^{a+}	8.3	126.7	114.7^{e-}	18.4^{e-}	15.0	30.3^{e+}	0.41
FM1-14-1-2	97.6	10.4	128.3	100.5^{e-}	13.0^{e-}	11.7^{e-}	29.3^{e+}	0.26^{e-}
FM1-25-1-1	90.4	8.4	134.3^{a+}	122.3^{e-}	5.4^{e-}	3.9^{e-}	29.0^{e+}	0.12^{e-}
FM1-25-1-2	93.2	8.1	134.7^{a+}	115.1^{e-}	15.0^{e-}	2.1	25.3	0.65
FM1-57-1-2	112.1^{a+}	11.3	134.3^{a+}	133.6^{e-}	29.5^{e-}	8.3^{e-}	31.0^{e+}	1.48
FM1R-32-1-1	118.7^{a+}	10.7	130.0	128.7^{e-}	46.4	37.1	33.3^{e+}	1.06
FM1R-23-1-1	77.4	11.3	131.3^{a+}	115.8^{e-}	2.1^{e-}	2.3^{e-}	25.3	0.08^{e-}
FG2-47-1-3	110.3^{a+}	15.5	128.0	118.4^{e-}	80.1	68.3	25.3	2.00
FM1R-19-2-4	73.8	13.3	128.3	69.0^{e-}	2.5^{e-}	32.8^{e-}	25.7	0.04^{e-}
FM2-12-1-1	94.0	11.2	122.0	61.3^{e-}	22.3^{e-}	34.8	23.3^{e+}	0.31
FAT-4-1-2	79.1	11.1	128.3	112.7^{e-}	40.0	34.8	28.3	0.72
Fatmawati(a)	84.6	13.0	123.3	102.6	11.5	10.0	25.7	0.31
Batutegi (e)	118.6	10.7	126.0	213.3	114.2	53.8	26.3	1.88
CV (%)	6.6	12.5	2.5	9.9	43.6	40.0	3.4	26.3

Note: Numbers followed by the letters a+ and a- column differ significantly higher and differ or lower with Fatmawati. Numbers and e+ e- is significantly different higher or lower than Batutegi based on the Dunnett test at significant level of 5%. PH = plant height, NPT = Number of productive tillers, DH = days to harvest. TS = Total spikelet, FG = number of filled grain, PFG = Percentage of filled grain, B1000 = 1000-filled grain weight, YP = Yield potential (tons/ha), CV = coefficient of variability.

According to Herawati (2010), the days to harvest of new plant type of upland rice ranged from 105 to 124 days. Days to harvest of the lines tested ranged between 122-134.3 days. The line that meet the criteria was FM2-12-1-1. Fatmawati variety showed 123.3 days to harvest. Some of the lines tested (FM2-25-1-1, FM1-25-1-2, FM1-57-1-2, FM1R-23-1-1) had significantly different days to harvest and were longer than that of Fatmawati.

Batutegi was a variety with the highest number of spikelet per panicle (213.3 spikelets), but only had percentage of filled grain of 53.8 percent. The number of spikelet per panicle of the lines tested ranged between 61.3-177. Lines with the highest total number of spikelets was FG1-66-2-1 (177), but only 59.4 filled grain per panicle, that was 33.3%. Despite of only having a total number of spikelet 118.4, FG2-47-1-3 has the highest percentage of filled grain. According to Herawati (2010) the number of spikelet per panicle of NPT upland rice is more than 150 per panicle. Based on the number of spikelet per panicle, only FM2-12-1-1 and FG1R-51-2-1 meets the criteria. However, they had low percentage of filled grain. Weight of 1000 grain of rice ranged from 23.3 to 33.3 g

(Table 1). Variety has comparable weight of 1000 grain, ranging from 25.7 and 26.3 g. Of the 15 lines tested, 8 lines have weight of 1000 grain had more than 28 g (Herawati 2010) (Table 1).

Yield of 14 lines tested ranged from 0.04 to 2.00 tons/ha. Yield of varieties were 0.31 and 1.88 tons/ha. Batutegi had the highest yield among the check varieties (1.88 tons/ha). There were 3 lines having yield of more than one ton/ha i.e. FG2-47-1-3 (2.00 tons), FM1-57-1-2 (1.48 tons) and FM1R-32-1-1 (1.06 tons) (Table 1). The low yield of the lines and check varieties was due to blast and bacterial leaf blight and scarce rain during the reproductive period.

Based on the criteria of CGV, then there are two characters that have relatively high CGV, namely plant height and intensity of neck blast disease; two characters have the medium CGV i.e. the number of grain per panicle and the percentage of filled grain and number of filled grain per panicle; and several other characters have low CGV. Murdaningsih *et al*. (1990) stated that low CGV showed rather low genetic variability of the characters and was classified as narrow, while the medium and high value CGV were classed as broad genetic variability. Thus there were 4 characters that have broad genetic variability, and six have a narrow genetic variability.

Broad sense heritability value of the characters observed ranged from 33.4-91.2% (Table 2). According to Stanfield (1983), broad sense heritability values can be grouped into three high heritability ($0.50 < h^2 < 1.00$), medium heritability ($0.20 < h^2 \leq 0.50$) and low heritability ($h^2 < 0.20$). Based on the heritability values there are two characters that have moderate heritability (20-50%), i.e. the number of productive tillers and yield potential. another character has a heritability of 50% (Table 3). This means that agronomic characters were controlled by genetic factors more than environmental factors. Selection will be effectively carried out for characters with high heritability value.

Table 2. Variance components, heritability and genetic variability coefficient new plant type of upland rice lines

	Characters	σ^2_g	$\sigma^2 p$	$\sigma^2 e$	h^2_{bs}(%)	CGV (%)
1.	Plant height	315.9	357.4	41.4	88.4	17.0
2.	Days to harvest	18.8	29.1	10.4	64.4	3.8
3.	Number of productive tillers	0.1	0.3	0.2	44.3	2.0
4.	Number of filled grain per panicle	5.6	9.7	4.1	57.5	10.0
5.	Number of spikelet per panicle	3.0	4.1	1.1	73.0	5.3
6.	Percentage of filled grain	3.7	6.8	3.1	55.0	9.2
7.	The 1000-filled grain weight	9.0	9.9	0.9	91.2	5.7
8.	Yield	0.04	0.1	0.1	33.4	1.9
9.	Intensity of leaf blast disease	3.4	3.6	0.5	95.7	8.4
10.	Intensity of neck blast disease	12.4	13.2	2.2	94.4	15.0

Description: $\sigma^2 g$ =Genetic variance, $\sigma^2 p$ =Phenotypic variance , $h^2 bs$ = broad sense heritability, CGV = Coefficient of genetic variance .

Line FM2-12-1-2 was blast-resistant but only had yield potential of 0.31 ton per ha. The line was not tolerant to drought. FG2-47-1-3 line was resistant to leaf blast and neck blast and had the highest number of productive tillers than all other varieties and lines tested, thus the yield of FG2-47-13 was also the highest (2 tons/ha). There were ten other lines having yield less than one ton. Low yield tons was due to low resistance of the lines to leaf blast and neck blast and drought during reproductive period (Table 3 Location in Bogor).

Based on Dunnett tests the intensity of leaf blast disease in the endemic area in Sukabumi, of 15 lines tested, there were two lines showing intensity of leaf blast disease not significantly different from control resistant (Limboto), namely FG2-47-1-3 and FM2-33-1-1 (Table 3). Panicle blast resistance of the 15 lines tested showed that the lines with intensity of neck blast disease not different from controls resistant (Limboto) were FM1-25-1-1, FM1-25-1-2, FG2-47-1 -3, FM2-12-1-1, and FAT-4-1-2 (Table 3). Line showing both resistance to leaf blast and neck blast was FG2-47-13.

Table 3. The scale and intensity of leaf blast and neck blast in lines of upland rice in Bogor and Sukabumi

Lines	Bogor				Sukabumi			
	Leaf Blast		Neck Blast		Leaf Blast		Neck Blast	
	S	I (%)	S	I (%)	S	I (%)	S	I (%)
FG1-66-2-1	5	17.4^{aR}	9	86.3^{aR}	7	38.0^{aR}	9	74.1^{aR}
FG1R-108-1-1	5	12.6^{aR}	9	63.3^{aR}	5	23.4^{aR}	5	23.5^{R}
FG1R-51-2-1	5	38.5^{aR}	9	85.0^{aR}	5	25.4^{aR}	7	35.0^{aR}
FM1-14-1-1	7	57.8^{aR}	9	97.7^{aR}	7	55.5^{aR}	9	87.8^{aR}
FM1-14-1-2	7	84.8^{aR}	9	94.7^{aR}	7	44.8^{aR}	9	100.0^{aR}
FM1-25-1-1	3	5.6^{T}	9	53.0^{aR}	5	16.2^{aR}	5	16.8^{R}
FM1-25-1-2	1	2.9^{T}	9	73.7^{aR}	5	19.5^{aR}	5	13.2^{R}
FM1-57-1-2	3	4.1^{T}	5	14.7^{R}	7	53.2^{aR}	9	64.4^{aR}
FM1R-32-1-1	5	45.2^{a}	9	64.7^{aR}	5	28.7^{aR}	7	28.9^{R}
FM1R-23-1-1	7	50.0^{a}	9	80.7^{aR}	5	29.6^{aR}	9	88.4^{aR}
FG2-47-1-3	0	0.0^{T}	1	3.0^{T}	3	7.8^{T}	3	5.5^{MT}
FM1R-19-2-4	7	71.5^{a}	7	32.7^{R}	9	62.6^{aR}	9	96.3^{aR}
FM2-12-1-1	1	1.1^{T}	3	6.0^{MT}	9	66.8^{aR}	5	18.4^{R}
FAT-4-1-2	5	20.0^{aR}	7	49.7^{R}	7	34.1^{aR}	1	2.3^{T}
FM2-33-1-1	-	-	-	-	3	7.5^{T}	9	77.5^{aR}
Limboto (a)	0	0.0^{T}	1	3.7^{T}	3	4.8^{T}	1	0.0^{T}

Note: Figures followed by letter (a) showed higher intensity of blast disease than Limboto (resistant variety), based on the Dunnett test on a significant level of 5%. I = intensity of the blast disease, T = resistant, R = Susceptible, MT = Moderate resistant, MR = Moderate susceptible.

Conclusions

There was variability on agronomic traits and blast resistance among the lines evaluated. FG2-47-1-3 did not have complete characters of NPT, but the line had superior characteristics such as high percentage of filled grain, medium days to harvest, medium height, medium 1000-filled grain weight, stay green when harvested and high resistance to blast disease. The population had high heritability (h^2_{bs}) in leaf blast and neck blast resistance, plant height, filled grain per panicle, days to harvest, number of grain per panicle and 1000-filled grain weight. They showed high variance coefficient for leaf blast and neck blast resistance, flag leaf length and percentage of filled grain.

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Functional Analysis of Drought-Induced *Os*LEA3 Promoter Isolated from Batutegi Rice Cultivar

Agus Rachmat[1], Satya Nugroho[1], Bernadetta Rina Hastilestari[1], Kinasih Prayuni[1], Sudarsono[2]

[1.] *Research Center For Biotechnology, Indonesian Institute of Sciences*
[2]*Faculty of Agriculture, Bogor Agricultural University*
guess_btk@yahoo.com

Abstract

Drought is the most significant environmental stress in agriculture and many efforts have been made to improve crop productivity under water-limiting conditions. Improving yield under drought is a major goal of plant breeding. Late embryogenesis abundant (LEA) proteins have been implicated in many stress responses of plants. It has been found that LEA genes are a gene family and play important roles in the protection of water stress. Expression of *Os*LEA3 is induced by abiotic stresses, including drought and high salinity. The promoter of *Os*LEA3 was cloned from Batutegi, Rojolele and Nipponbare cultivars and its function was analyzed in transgenic Nipponbare plant by a GUS reporter gene. Transient assay was conducted in order to investigate GUS expression driven by *Os*LEA3 promoter. *Os*LEA3 promoter was expressed in embryogenic callus indicated by the appearance of blue spots. Differences in color produced in embryogenic callus showed different expression level.

Key words: *rice, drought, Batutegi, Rojolele, Nipponbare, GUS, OsLEA3 promoter*

Introduction

Rice (*Oryza sativa* L.) is one of the major staple foods. As the population growth goes up, the rice production must be increased. However, the field for rice cultivation has decreased because of the change of paddy field into housing and industry. Moreover, drought influences the rice production especially in North China and South Asian. Thus, rice becomes one of the target source of gene and genome sequence analysis project among cereal plants (Goff *et al.* 2002).

LEA proteins constitute a large group of proteins that specifically expressed during seed development. LEA proteins are located in seeds and pollen. Many vegetative organs can also express some LEA proteins under conditions of dehydration, osmotic stress, cold or exogenous ABA. First LEA proteins were identified during the desiccation phases of seed development. These proteins were reported to protect specific cellular structures or ameliorate the effect of drought stress by sequestering ions and maintaining minimum cellular water requirements (Hu, 2008).

Plant has physiological and molecular response mechanisms to tolerate drought. Those mechanisms are osmotic accumulation, scavenging ROS, and osmoprotectant protein production such as late embryogenesis abundant (LEA) and heat shock protein. LEA gene is identified as an expresed gene in the late phase of seed growth. Research on LEA genes has showed that LEA 3 gene is responsible for drought tolerance. A major problem under severe dehydration is that the loss of water leads to crystallization of cellular components during the course of dehydration and dormancy, which damages cell structures (Spelundm *et al* 1996). Some of the LEA proteins could essentially be considered compatible solutes that likely play the role similar to sugars in maintaining the structure of the cytoplasm in the absence of water (Hu 2008). The goal of this research is to study the function of LEA3 promoter isolated from rice cv. Batutegi.

Materials and Methods

Promoter OsLEA3

Promoter sequence primers were designed based on sequence of genes LEA3 HVA-like gene promoter from rice cultivar IRAT 109 (GenBank Acc. DQ837728) and has been adapted to the BAC sequence of chromosome No. 5 rice cultivar Nipponbare (GenBank Acc. AC104713). Primer sequences are also designed to include the start codon (ATG) of mRNA sequences LEA3 gene (GenBank Acc. DQ789359) and the addition sequence EcoRI (G↓ AATTC) and BglII (A↓GATCT) in order to add *EcoR*I and *Bgl*II restriction sites in the primers. The predicted promoter was amplified and isolated by PCR. The amplified fragments were sequenced to ensure fidelity.

Isolation of rice genomic DNA

Isolation of genomic DNA of rice cv. Batutegi was conducted by CTAB method (Saghai-Maroof *et al*., 1984) that has been optimized. Rice leaf samples were taken from 1-month-old plants. A total of 2 g leaf samples were prepared using aluminum foil wrapping. Leaf powder was put into polypropylene tubes containing 10 ml of buffer solution which has been has been preheated in the incubator at 65^{o}C for 30 minutes.

PCR analysis

All PCR reactions were done using 20 ng DNA template. PCR reaction mix contained 6.5 uL nuclease free water, 12.5 uL Green Go Taq 2x Master, 2.5 uL for each LEA F primer (0.1 µM) and LEA R (0.1 µM) promoter LEA3, and 1 µl template DNA. PCR conditions were: initial denaturation 95^{o}C for 3 minutes, followed by 35 cycles of denaturation at of 95^{o}C for 1 minute, annealing at 60°C for 1 minute, and initial extention at 72^{o}C for 1 minute. The cycle was terminated by final extention at 72^{o}C for 10 minutes.

Vector Construction

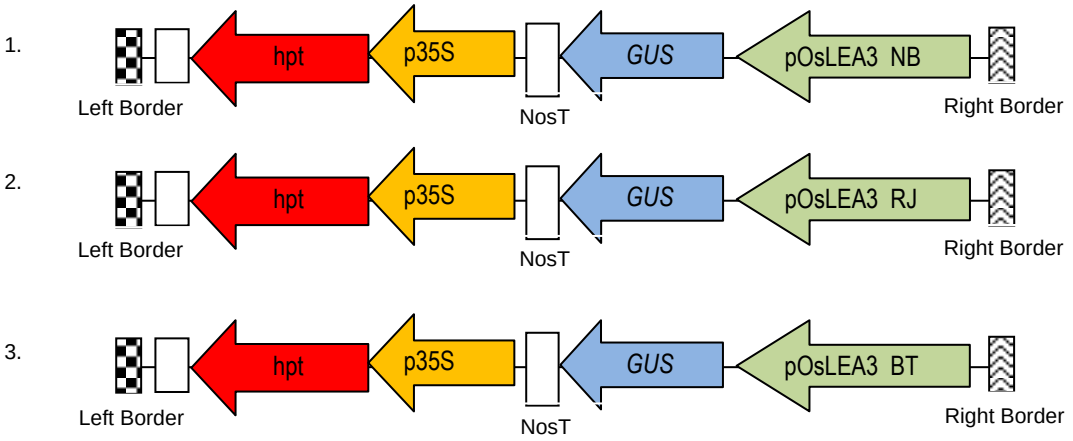

Figure 1. Vector Construction of OsLEA3 promoter::GUS.

Agrobacterium mediated transformation

Agrobacterium tumefaciens LBA4404 strain was transformed using recombinant plasmid pC1305 containing hygromycin phosphotransferase gene (hpt) controlled by CAMV 35S promoter, and GUS gene controlled by OsLEA3 promoter.

Histochemichal GUS assay

Water deficit treated and non treated plant tissues were vacuum infiltrated for 1 h in the GUS reaction mixture containing 1 mM 5-bromo-4-chloro-3-indolyl- b-D-glucuronide (X-gluc) and 50 mM sodium phosphate buffer and incubated at 37^{o}C overnight. The reaction was stopped by adding 75% ethanol, and the pigments and chlorophylls were removed by repeated ethanol treatment.

Results and Discussion

Amplification of OsLEA3 promoter

Isolation of 1.291 bp OsLEA3 gene promoter was conducted by amplification of DNA genome using PCR with specific primers and visualized with 0.8% agarose gel (Figure 2). The PCR fragment was then cloned into pGEM-T Easy and then used to construct pC1305 based recombinant plasmid.

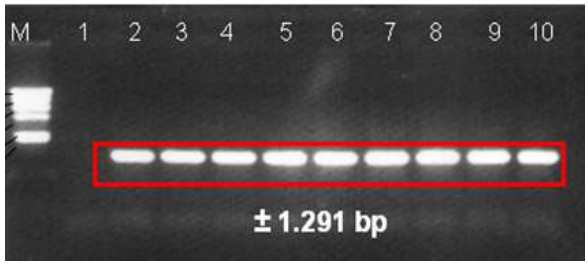

Figure 2. Amplification of OsLEA3 promoter.

Verification of the recombinant pGEM-T plasmid was conducted using *EcoR*I restriction analysis . The result showed 3 fragments, which were fragment with size 3.001 bp (pGEM-T Easy vector plasmid), 1.291 bp (gene OsLEA3 promoter), and 12 bp (nucleotides between EcoRI site of OsLEA promotor gene and EcoRI site of pGEM-T Easy) fragments (Figure 3).

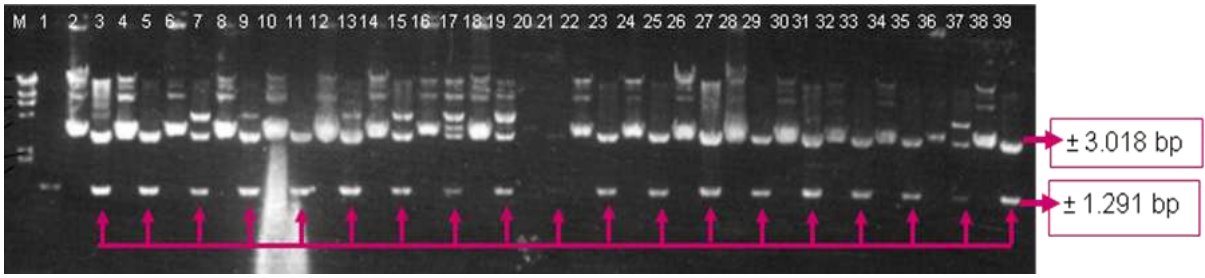

Figure 3. Electrophoresis of recombinant vector digested with *Eco*RI.

Rice Transformation using *Agrobacterium*

Recombinant vector containing OsLEA3promoter was successfully constructed and trasformed to rice cv. Nipponbare. Selection of Transformed plants was selected on hygromisin contained medium.

Figure 4. Successfull transformed plant in the selection medium.

Some transformed calli survived in medium containing hygroimisin. Hygromisin resistant celli were subcultured to regeneration medium. Calli with green spot grew into planlets. Table 1 showed low transformation efficiency. It needs to be increased by optimizing conditions of the culture. Factors that influence the low efficieny of transfomation are method of tranformation, type of explants and condition of culture.

Table 1. Genetic transformation of *Os*LEA3 ::GUS to rice cv. Nipponbare

No	Recombinant vectors	Rice Varieties	∑ infected calli	GUS assay	Number of Planlets
1.	*Os*LEA3 NB:: GUS	Nipponbare	600	Positive	32
2.	*OsLEA3* Rj:: GUS	Nipponbare	600	Positive	19
3.	*OsLEA3* BT:: GUS	Nipponbare	600	Positive	7

Histochemical Assay

Transient expression assaysof OsLEA3::GUS promoter of rice cv. Nipponbare, Rojolele and Batutegi were successfully conducted. GUS gene expression was identified with blue spot on the transformed calli .Figure 5 showed the difference intensity of blue spot in three cultivars. It means that the expression level of GUS gene differ among different OsLEA3 promoters. Real Time PCR will be conducted for further qualitative analysis.

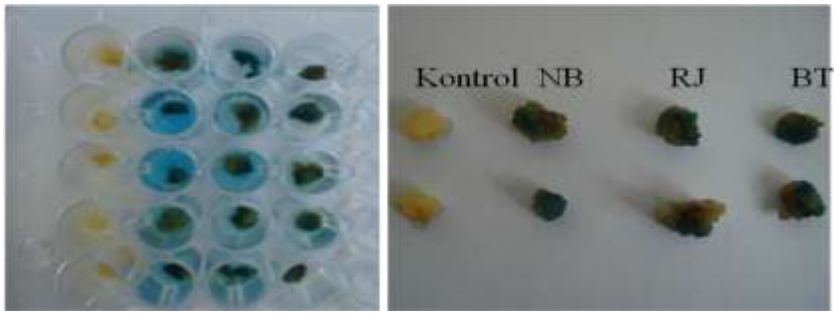

Figure 5. Histochemical assay of transformed calli, NB= Niopponbare, RJ= Rojolele, BT= Batutegi.

Conclusion

1. Recombinant plasmid pCambia1305 of OsLEA3::GUS of Batutegi, Rojolele and Nipponbare cultivars was succesfully constructed.
2. Promoters OsLEA3 of Batutegi, Rojolele and Nipponbare cultivars controlled GUS expression differently among those cultivars

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Evaluation of the Nutritional Environment for Rice in Cianjur, Indonesia for Development of an Advanced Basin Model for Asia

Koki Homma [a,*], Gunardi Sigit [b], Handarto [c], Masayasu Maki [d], Chiharu Hongo [e], Koshi Yoshida [f], Kazuo Oki [g], Hiroaki Shirakawa [h], Tatsuhiko Shiraiwa [a], Ritsuko Hara [a], Mitsuo Kambayashi [a], Yoshihiro Hirooka [a], Hiroki Iwamoto [a]

[a] *Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
[b] *Regional Office of Food Crops Service West Jawa Province, Indonesia*
[c] *Human Resource Development, Universitas Padjadjaran, Indonesia*
[d] *Graduate School of Global Environmental Studies*
[e] *Center for Environmental Remote Sensing, Chiba University*
[f] *Collage of Agriculture, Ibaraki University*
[g] *Institute of Industrial Science, the University of Tokyo*
[h] *Graduate School of Environmental Studies, Nagoya University*
** Corresponding author: Graduate School of Agriculture, Kyoto University, Sakyo-ku, 6068502, Kyoto, Japan.*
Tel.: +81 75 753 6042; fax: +81 75 753 6065.
homma@kais.kyoto-u.ac.jp

Abstract

Food production systems in tropical Asia face the following problems: 1) increased demand for production; 2) increasing environmental load; 3) water scarcity and 4) effects of climate change. To overcome these problems, strategies for better management of crop and environmental resources are recommended. Here, we describe our research in Cianjur, Indonesia under the project "Development and Practice of Advanced Basin Model in Asia – Toward Adaptation to Climate Change". The study area, Cianjur, is one of the main rice production areas in Indonesia, and has water reservoirs that supply water to Jakarta. However, excess nutrients from agricultural land cause environmental problems. In this situation, evaluation of the nutritional environment for rice growth is necessary. We have begun to evaluate the nutrient concentrations in the irrigation water, soil and plants. The relationships of these nutrient contents to rice growth were analyzed using a rice growth simulation model. The simulation model and remote-sensing technologies were used in combination to evaluate the geographic distribution of rice growth and nutritional environment. The research produced baseline information for developing the Advanced Basin Model.

Keywords: environmental problem, irrigation water, remote-sensing, simulation model, soil solution

Introduction

Food production systems in tropical Asia face the following problems: 1) increased demand for production; 2) increasing environmental load; 3) water scarcity and 4) effects of climate change. To overcome these problems, strategies for better management of crops and environmental resources are recommended.

Since the green revolution, modern agricultural technology has increased land productivity and supported population growth. However, this technology tends to institutionalize agricultural management and reduce diversity. Previous studies reported that such institutionalized management increased the risk of disasters. Tsuno (1995) analyzed cold summer damage to rice production in Japan in 1993 and concluded that the simplification of management in cultivars and cultivation methods increased the extent of damage. Shiraiwa *et al.* (2002) analyzed the variability of rice production in Thailand and concluded that the annual variation was larger in an intensive high yielding area (the Central Plain) than in an extensive low-yielding area (the Northeast). Yoshino *et al.* (2000) analyzed the relationship between the Southern Oscillation Index and rice production in Indonesia and reported that the negative effect was largest in Java, which was the

most productive area. These examples indicate that the present management is insufficient for coping with unfavorable conditions. Management variability may thus be the key to attaining production stability.

The high fertilizer input required by modern agriculture technology often exceeds an acceptable amount in the field and increases the environmental load. To optimize the input, the available nutrients in the soil and the requirements of the plant should first be evaluated. Because water scarcity is predicted in the future, the available water supply should also be evaluated.

The project "Development and Practice of Advanced Basin Model in Asia – Toward Adaptation to Climate Change"

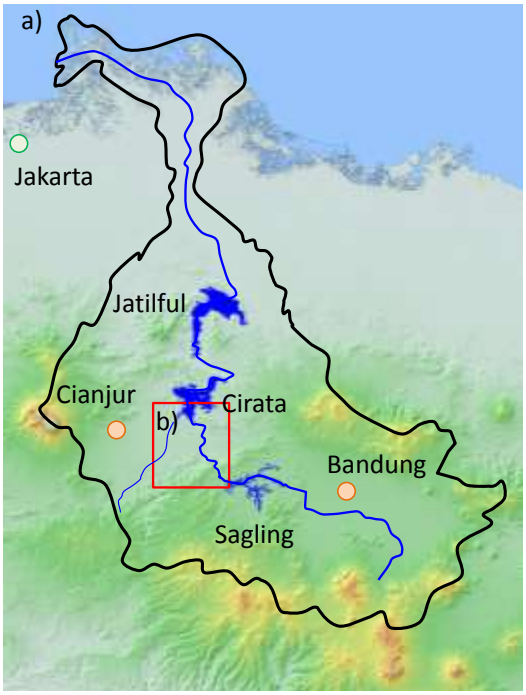
As mentioned above, the present situation demands not only a production increase, but also management optimization based on resource evaluation. Because the smallest water management unit is a basin and nearly all excess nutrients move with the water stream in the basin, a strategy to overcome these problems must be developed at the basin scale. Here, we established the project "Development and Practice of Advanced Basin Model in Asia – Toward Adaptation to Climate Change" supported by the Ministry of the Environment, Japan. The project includes the evaluation of environmental resources and crop production, proposals for better management and also the establishment of an environmental conservation community. The Citarum River in Indonesia and the Num Ngum River in Laos were selected as test sites (Oki, 2011). This manuscript describes one of the project activities in Indonesia, which were managed by the lead author. In the activities, the nutrient concentrations of the irrigation water, soil solution and plants are evaluated their relationships to rice growth will be analyzed using a rice growth simulation model. The relations in terms of rice growth would be analyzed by using rice growth simulation model. The simulation model and remote-sensing technologies will be used in combination to evaluate the geographic distribution of rice growth and then nutritional environment.

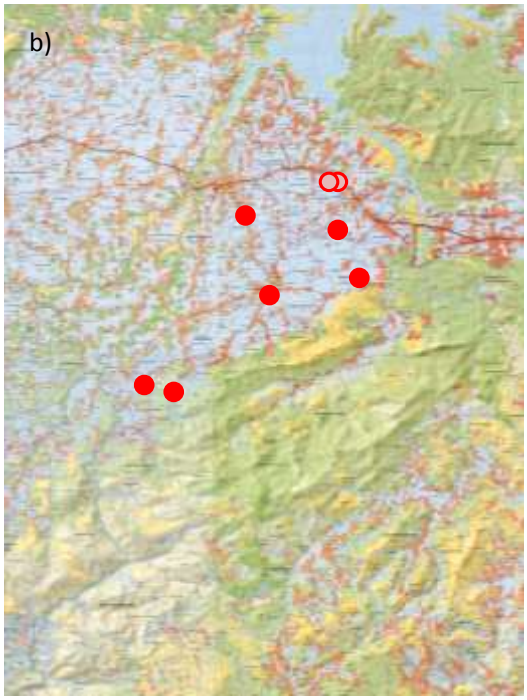
Overview of research area and activities

The research area is in the hydrographic basin of the Citarum River in West Java, Indonesia. The basin includes Bandung, the third largest city in Indonesia, and a total of 5 million people live in the area (Fig. 1a). Because the river is heavily polluted by human sewage and agriculture, the Asian Development Bank has called it the world's dirtiest river and approved a loan for clean-up in 2008. The river has 3 dams and one of 3 dams directly supplies water for Jakarta; thus clean-up of the river is of urgent concern.

Field observations have been conducted in the Bojongpicung and Ciranjang districts, Cianjur regency, West Java. The area is located between the Saguling and Cirata reservoirs and is downstream of Bandung. We selected 40 paddy fields, of which 30 were planted with rice and 10 were planted with soybeans in August 2011 (Fig. 1b). In addition to the chemical properties of the soil, the nitrogen and phosphate concentrations of soil solutions are periodically monitored. Because the nutrient concentrations in irrigation water are also measured along 3 points in the major channels, the nutritional environment for crop growth can be assessed in terms of nitrogen and phosphate levels.

Plant growth is nondestructively evaluated in terms of plant height and canopy cover using a digital camera. The spectral reflectance of the canopy is measured once a week with a reflectance-measuring instrument (MS-720, Eiko seiki Co. Ltd.). The plants are also destructively sampled once per month to determine the leaf area index (LAI), above-ground dry matter and root density. The grain yield and above-ground biomass will be measured at maturity.





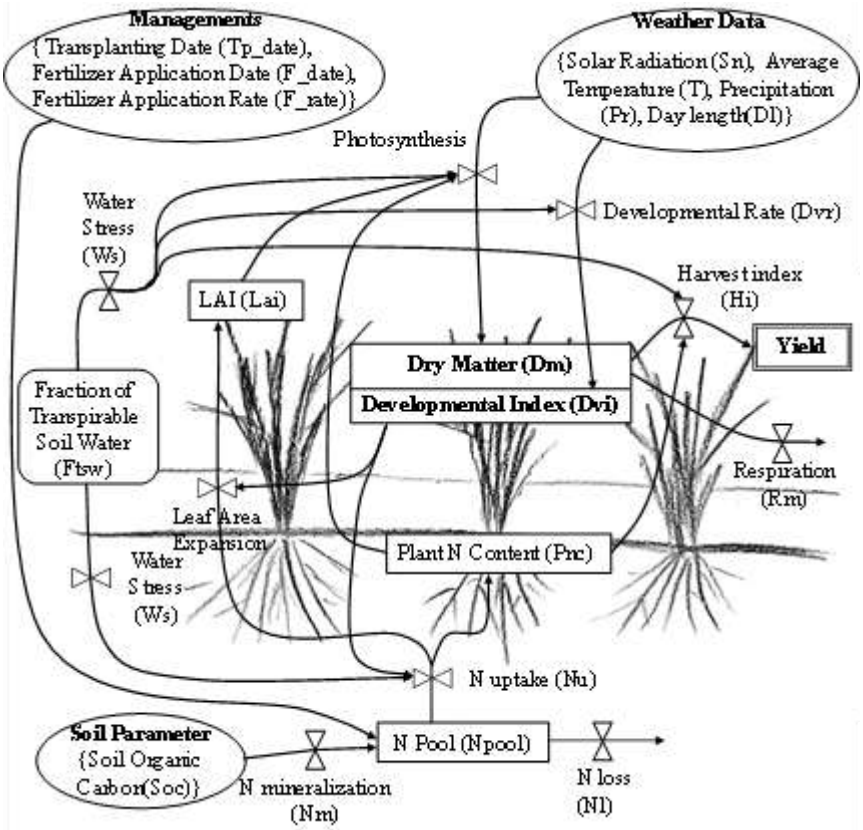
We selected 30 rice fields from 6 locations (●) and 10 soybean fields from 2 locations (o). Irrigation water is periodically monitored at 6 locations for rice fields.

Figure 1. Maps of the hydrographic basin of the Citarum River (a) and the field observation site (b).

Strategies for analysis and evaluation

To develop strategies for managing crop production in the hydrographic basin, the geophysical distribution of environmental resources and crop growth must be evaluated. However, the data we obtain are point-based. Accordingly, a method that scales up from point to area is needed. Satellite-based remote-sensing can be used for this purpose, and one of the co-authors of this paper had already developed a method that has been adapted in an agricultural area of Japan (Nuarsa *et al.* 2011). However, obtaining satellite images for analysis is difficult because the area often obscured by clouds.

To compensate for the missing data, we employed a simulation model. The authors developed the simulation model to predict rice growth and yield under rainfed conditions based on a field survey in Northeast Thailand, where the water and nutrients are quite limited (Fig. 2; Homma and Horie, 2008). Because the model was modified from SImulation Model for RIce Weather relations (SIMRIW), which has a good reputation for predicting the effects of climate change on rice production (Horie *et al.* 1995), the modified model may be able to handle climate change issues for the advanced basin model. We are now developing a simulation model to use in combination with remote-sensing (Fig. 3; Maki and Homma, 2011). The combined model will be used to evaluate the nutritional environment and predict rice yield by correcting the simulated data with satellite-based remote-sensing data. The combined model may also help to analyze the relationship between the remote-sensing data and field observations. The analysis will first be used for rice production and may be expanded to examine green soybean (edamame) production.



The basic concept of the model is derived from SIMRIW (SImulation Model for RIce Weather relations, Horie *et al.* 1995), and modified for rainfed rice production in Northeast Thailand (Homma and Horie, 2008).

Figure 2. Schematic illustration of the rice growth simulation model.

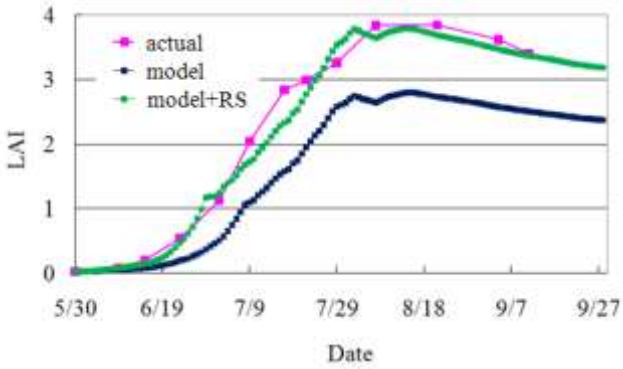


Figure 3. Examples of leaf area index (LAI) simulated by the rice growth simulation model and corrected by remote-sensing (Maki and Homma, 2011).

Conclusions

Here, we describe one of our activities in Cianjur, Indonesia. To develop better management practices for overcoming environmental problems and adapting to climate change, evaluation of the nutritional environment and rice growth are necessary. For this purpose, we have begun to evaluate the nutrient concentrations of irrigation water, soil solutions and plants. The relationship between these nutrient concentration and rice growth will be analyzed using a rice growth simulation model. The simulation model and remote sensing technologies will be used in combination to evaluate the geographic distribution of nutritional environment and rice growth.

Acknowledgement

The project is supported by the Environment Research and Technology Development Fund, Ministry of the Environment, Japan (E1104).

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The Nutritional Environment in Nonfertilized Rice Production and Its Effect on the Nutritional Quality of Brown Rice

Ritsuko Hara [a], Koki Homma [a *], Yoshihiro Hirooka [a], Mitsuo Kuwada[ab] and Tatsuhiko Shiraiwa[a]

[a] *Graduate School of Agriculture, Kyoto University, Kyoto, Japan.*
[b] *NPO Nonorganic, Nonchemical Crop Production Research Group, Kyoto, Japan.*
** Corresponding author: Graduate School of Agriculture, Kyoto University, Sakyo-ku, 6068502, Kyoto, Japan.*
Tel.: +81 75 753 6042; fax: +81 75 753 6065.
homma@kais.kyoto-u.ac.jp

Abstract

The nonfertilized-nonchemical crop production (so-called nature farming) proposed by Mr. Mokichi Okada is a popular farming method in both Japan and worldwide. The farming method uses neither fertilizers nor chemicals but obtains comparable yields to those produced by conventional farming. Although the method was designed to utilize natural resources, the details of the mechanism are still unknown. This study was aimed to evaluate the nutritional environment on the basis of the measurement of the nutrients in the soil solution and to analyze the effect on the nutrient concentrations of brown rice. For this purpose, we selected 2 nonfertilized-nonchemical paddy fields (NN fields) and 1 experimental paddy field (Expt field) as a reference. One of the NN fields was cultivated for 6 years without fertilizer (6-year NN), and another was cultivated in this way for 59 years (59-year NN). In the Expt field, 3 types of fertilizer treatments (None, Basal and Conventional) were conducted. The NH_4^+ concentrations in the 6-year NN fields was lower than those in the fertilizer-applied plots (Basal and Conventional), but they were comparable to that in the None treatment. Conversely, NH_4^+ was not detectable in the 59-year NN field. Other nutrients were extremely different between the NN fields and Expt field, namely, the fertilizer treatments (Basal and Conventional) had little effect on the nutrient concentration of the soil solution. The P_2O_5 and K^+ levels in the Expt fields were notably higher than those in the NN field, whereas Ca^{2+} and Mg^{2+} showed an opposite trend. Significant interactions of the genotype by environment were observed for the nutrient concentrations in brown rice. The relatively low NH_4^+ and K^+ level in the soil solution in the NN fields tended to result for brown rice in low concentrations of those nutrients, which Japanese people tend to prefer. The relationship between the nutritional environment and the uptake and concentration of nutrients in brown rice should be studied further.

Keywords: rice, nonfertilized-nonchemical crop production, soil solution, nutritional quality of rice

Introduction

Agrochemicals such as fertilizers and pesticides largely increase agricultural production and support the huge population of the world. However, excess inputs of agrochemicals leak into the environment and cause various kinds of problems that pose threats to human life (Reichenberger et al. 2007). Organic farming or nature farming is derived from the reactions against such agrochemical agriculture.

The nonfertilized-nonchemical crop production proposed by Mr. Mokichi Okada is popular in both Japan and worldwide. This method of farming uses neither fertilizers nor chemicals but obtains comparable yields to those produced by conventional farming (Okumura, 2002). Okumura reported that the sources of some of the nitrogen were irrigation water and biological fixation but that the majority comes from soil. Although the method of farming was designed to utilize natural resources, the details of the mechanism are still unknown.

Because crop productivity is restricted by nutrients supplied through soil, an evaluation of the nutritional environment in the soil is quite important when discussing productivity. In general, the availability of nutrients for plants is evaluated by chemical analyses. However, the nutritional

environment is quite influenced by the temperature, soil moisture and plant uptake and exudates, which suggests that periodic monitoring of the nutritional environment is required to analyze the plant response to nutrition and the nutrient availability (Boivin et al., 2002).

When plants take up nutrients from the soil, the procurement does not occur on the surface of the soil particles but through the liquid phase of the soil (soil solution). Nutrients in their ion forms, such as ammonium and potassium, move with the soil solution (mass flow) or by diffusion in the solution. Accordingly, nutritional analyses of the soil solution are suggested to evaluate the nutritional environment (Smethurst, 2000).

This study investigated nonfertilized-nonchemical fields and an experimental field as a reference. The soil solutions were sampled once every 2 weeks for the nutritional evaluation. Six representative rice cultivars were planted to evaluate any cultivar differences in nutrient uptake, dry matter production, yield and grain quality. This report focused on the changes in the nutrients in soil solution and the effect on the nutrient content of brown rice.

Materials and Methods

The experiment was conducted in 2010. We used two types of paddy fields: one was operated under nonfertilized-nonchemical management by NPO Nonorganic, Nonchemical Crop Production Research Group (NN fields; Ogura, Uji city, Kyoto Prefecture, 34° 54' N, 135° 46' E), and the other was an experimental field in the Graduate School of Agriculture, Kyoto University (Expt field; Sakyo, Kyoto city, Kyoto Prefecture, 35° 02' N, 135° 47' E). The NN fields included 2 fields: one was cultivated for 6 years without fertilizer or agrochemicals (6-year NN), and the other was cultivated for 3 years without fertilizer or agrochemicals after the top-soil was converted using the soil of a field where nonfertilized-nonchemical management was conducted for 56 years. Thus, the soil of the converted field was used for a total of 59 years under nonfertilized-nonchemical management (59-year NN). The experimental year was the 7th and 60th year of cultivation, respectively. In the Expt field, 3 kinds of fertilizer treatments (None, Basal and Conventional) were conducted under pesticide application. The none treatment was conducted without fertilizer, whereas the Conventional and Basal treatments included the application of chemical fertilizer at a rate of N-P_2O_5-K_2O = 5-5-5 g m^{-2} as basal, and the Conventional treatment was top-dressed with fertilizer on the 22th of July and the 7th of August. The rate of each top-dress application was N-P_2O_5-K_2O = 2.5-2.5-2.5 g m^{-2}. Thus, the total application rate for the Conventional treatment was N-P_2O_5-K_2O = 10-10-10 g m^{-2}. The Basal treatment was designed to starve nutrients around the heading period.

Based on the results of a study conducted in the same field (Matsuyama et al., 2010), six representative rice cultivars were selected. Beniasahi (Japan) is a traditional japonica cultivar and has been self-seed-produced for the 59 years under the nonfertilized-nonchemical management, suggesting that the cultivar has adapted to these conditions (Okumura, 2002). Nipponbare (Japan) and Kasalath (India) are standard cultivars of japonica and indica, respectively (Kojima et al., 2005). Takanari (Japan) is a high-yielding indica cultivar and is promising for the production of rice flour and as a feed crop. B6144F-MR-6-0-0 (B6144F) was bred for upland cultivation in Indonesia (Atlin et al., 2006). Bei Khe (Cambodia) is a cultivar that produced the largest amount of dry matter in the experiment conducted by Matsuyama et al. (2010). The cultivars were sown on the 10th of May and transplanted to the Expt field on the 3rd of June and to the NN fields on the 8th of June. The transplanting density was 22.2 hill m^{-2}, with 1 plant $hill^{-1}$. The experiment was arranged in a randomized block design for each field.

A soil solution sampler (DIK-301B, Daiki Rika Kogyo Co., Ltd.) was placed at a 10 cm-depth in every Nipponbare and B6144F plot. The soil solution was sampled by a syringe aspiration method once every 2 weeks and the concentrations of NH_4^+, P, K, Mg and Ca in the solution were measured. The soil chemical properties were measured by a standard method. The brown rice

grains at maturity and the above-ground biomass and leaves at heading were sampled, and its N, K and Mg concentrations were determined.

Results and Discussion

Evaluation of the nutritional environment determined on the basis of the soil solution

The three major soil solution nutrients, NH_4^+, P and K, were quite different among the fields and treatments (Fig. 1). The NH_4^+ concentration under the Basal and Conventional treatments in the Expt field was initially quite high due to the effect of the basal fertilizer, but it markedly decreased by 30 days after transplanting. The conventional treatment maintained a relatively higher NH_4^+ concentration due to the top-dress application of fertilizer, whereas the NH_4^+ concentration under the Basal treatment exhibited a lower level (almost 0 ppm) than the None treatment. The Basal treatment resulted in nitrogen starvation conditions, as was intended by the experimental design. In the NN fields, the NH_4^+ concentration in the 6-year NN field peaked at 60 days after transplanting and maintained levels of approximately 0.70 ppm and 0.25 ppm before and after the peak at 60 days, respectively. The NH_4^+ concentration in the 59-year NN field maintained levels of approximately 0.2 ppm and 0 ppm for 30 days after transplanting and thereafter, respectively.

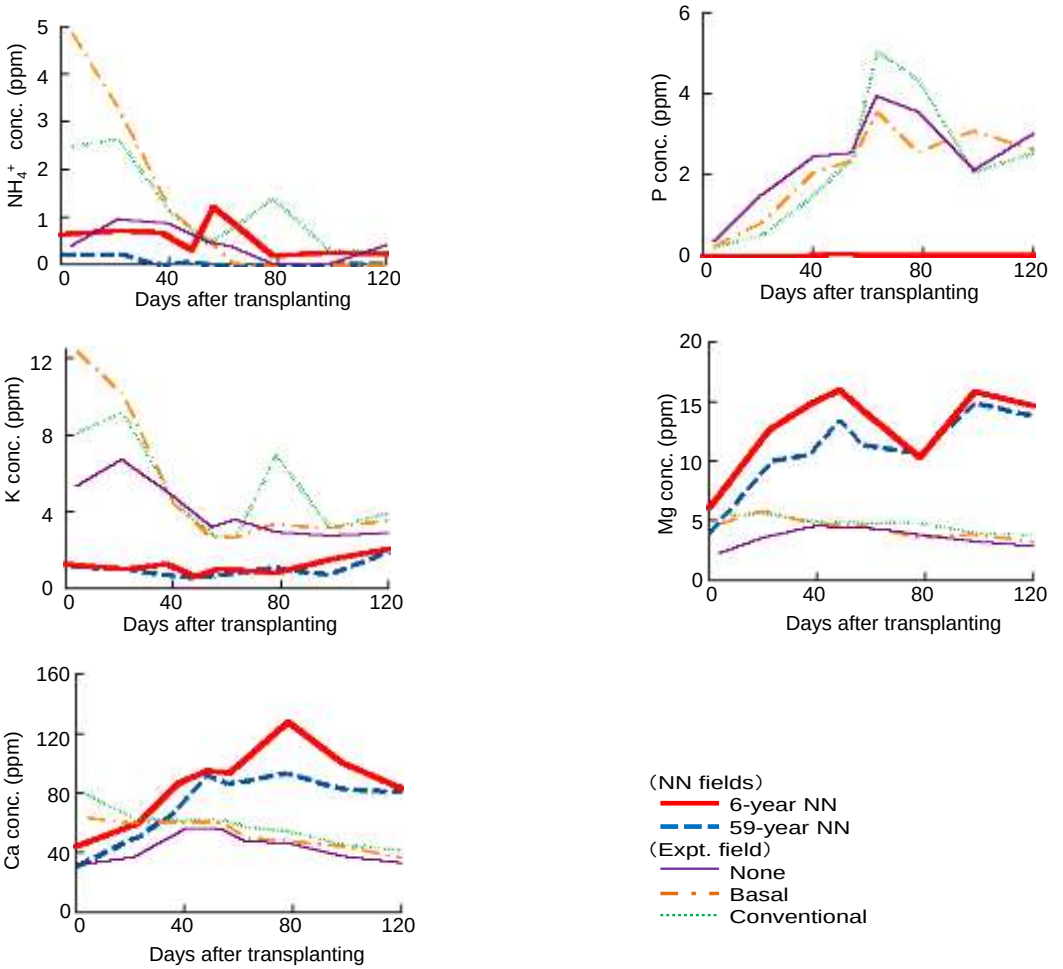


Figure 1. Change of nutrients concentration in soil solution.

The P concentration in the Expt field increased from the transplanting up to 70 days after transplanting, and differences among the fertilizer treatments were not obvious, suggesting that the annual P application accumulated P in the soil. Conversely, P was barely detectable in the NN fields. The difference in the P concentrations between the Expt and NN fields was corresponded to the soil chemical analysis (data not shown). Although the P concentration in the soil solution was negligible, the plants took up P from the soil (data not shown). Moreover, although the P concentration in the soil solution in the 6-year NN field was as low as that in the 59-year NN field, the P uptake by the plants in the 6 year-NN field was 1.6 times as large as that in the 59-year NN field. These data suggest that another indicator is required to evaluate the availability of P.

The K concentration was affected by the fertilizer application in the Expt field, but the effect was short-lived: the baseline concentrations under the Basal and Conventional treatments were similar to that under the None treatment. The K concentration in NN fields was one half of the basal concentration in the Expt field, and apparent differences were not observed between the 6-year and 59-year NN fields.

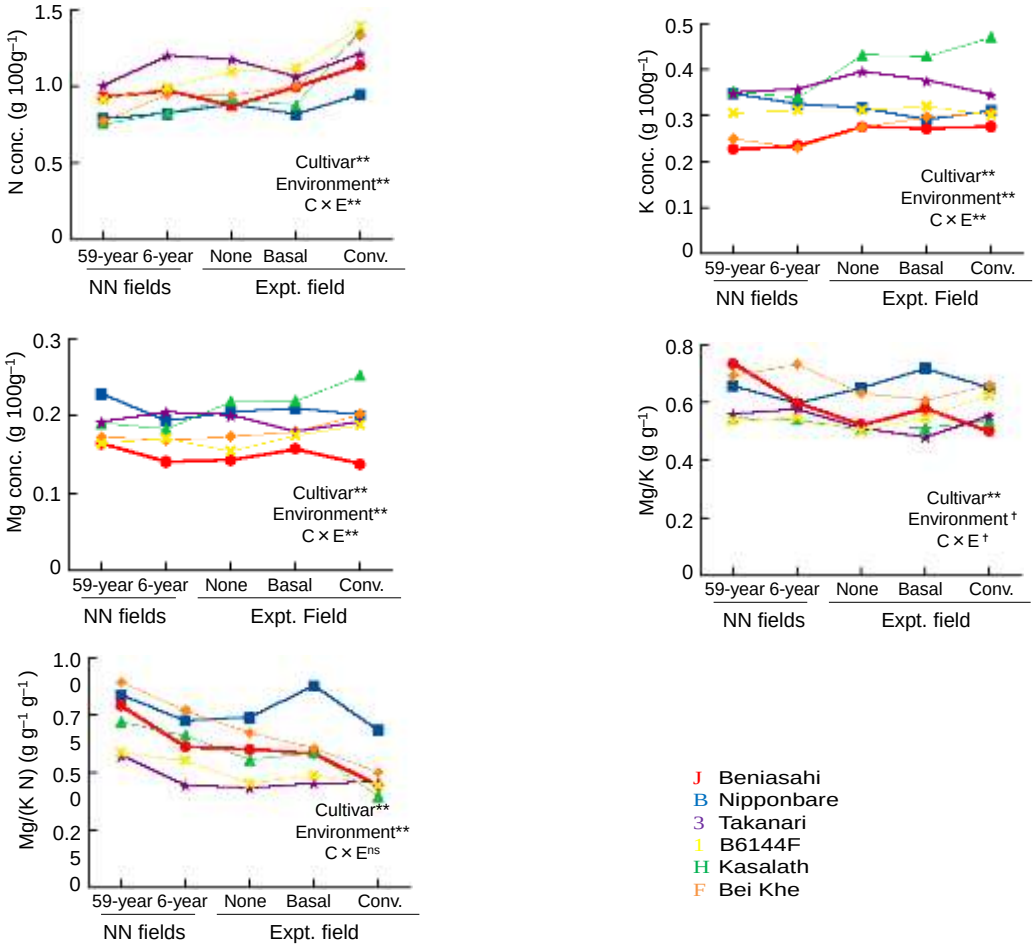
The Ca and Mg concentrations were different at transplanting among the fertilizer treatments in the Expt field, but the difference disappeared by 30 days after transplanting. The concentration gradually decreased with time, whereas the concentrations increased until 50 days after transplanting in the NN fields. The increase was repeated in a flood incubation using the soil sampled before transplanting (data not shown), suggesting that the trend shown in Fig. 1 is derived from the characteristics of the soil or the forms of Ca and Mg in the soil.

The effect of nutrients in the soil solution on nutrient concentrations of brown rice

The N and K concentrations in brown rice almost reflected those in the soil solution: the concentrations of the brown rice in the NN fields tended to be lower than those in the Expt field. However, the Mg concentration of the brown rice in the 6-year NN field was lower than that under the Conventional treatment in the Expt field; thus, the concentrations of the brown rice did not reflect those in the soil solution. The interaction effects of the cultivar and the environment were significant for all of the nutrients, indicating that the plant responses to the nutritional environment were different among the cultivars.

Previous studies report that the N concentration in brown rice is affected by that in the flag leaves at heading (Taira, 1997). The relationship in this study suggests that the indica cultivars are more affected than the japonica cultivars (Fig. 3). As available data atre inadequate for K and Mg, the relationship of the K and Mg concentrations of the brown rice was compared with that in the above-ground biomass at the heading stage. The relationships of the K concentration between the brown rice and the above-ground biomass were significant for the traditional varieties but not significant for the improved varieties. The relationships of the Mg concentration between the brown rice and the above-ground biomass were significant for the traditional indica cultivars but not significant for the other cultivars (data not shown). As shown in Fig. 3 these cultivar differences may be one of the causes of the significant interaction of the cultivar and the environment on the nutrient concentration in brown rice.

In terms of the nutrient concentration of brown rice, 1/N, Mg/K or Mg / (K N) were proposed as grain palatability indexes for Japan (Nakagawa et al., 2000). As mentioned above, the N and K concentrations of the brown rice in the NN fields tended to be lower than those in the Expt field, increasing the indexes in NN fields higher than those in the Expt field. However, as nitrogen starvation under the Basal treatment apparently decreased the N and K concentrations of the Nipponbare brown rice, the index for Nipponbare was the highest under the Basal treatment.



Results of ANOVA are shown in the figure: †, *, ** and ns indicates 10, 5, 1 % and non- significance, respectively.

Figure 2. Effect of cultivar and environment on nutrition concentration of brown rice (a, b and c), and grain palatability index (d and e).

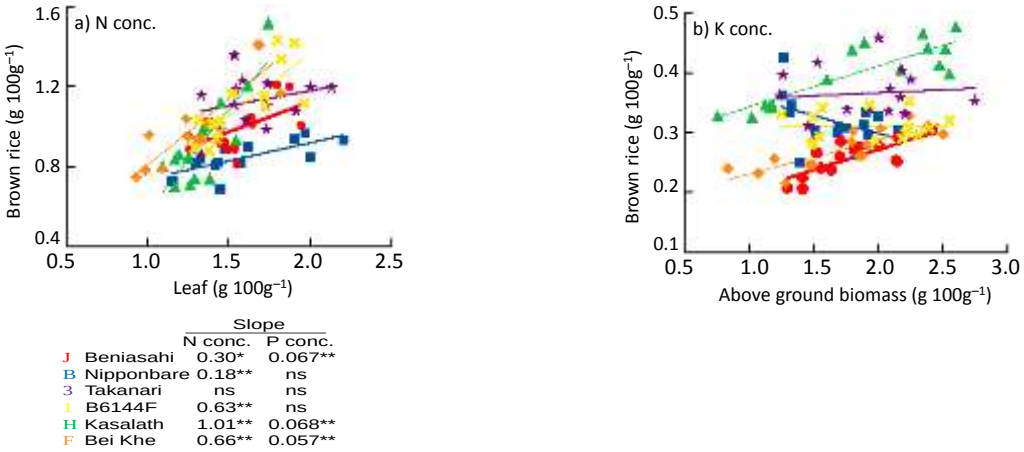


Figure 3. (a) Relationship between N concentration in leaf at heading and that in brown rice at maturity. (b) Relationship between K concentration in above-ground biomass at heading and that in brown rice at maturity.

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Effect of Slow Release Fertilizer on Yield and Yield Components in Chinese High-Yielding Rice Cultivars

Toshiaki Kokubo [a], Akira Miyazaki [b*], Tetsushi Yoshida [a], Yoshinori Yamamoto[b], Jing Ju [c] and Yulong Wang [c]

[a] *The United Graduate School of Agricultural Sciences, Ehime University, Japan*
[b] *Faculty of Agriculture, Kochi University, Japan*
[c] *Yangzhou University, China*
** Corresponding author: Fac. of Agr, Kochi Univ., 200 Monobe-Otsu, Nankoku-shi, Kochi, 7838502 Japan.*
Tel & FAX.: +81 88 864 5123.
miyazaki@kochi-u.ac.jp

Abstract

The effect of slow release fertilizer on yield and yield components in Chinese high-yielding rice cultivars were evaluated and analyzed to avoid over-fertilization in China. Two different types, linear (L) and sigmoid (S) fertilizer as a nitrogen (N) source were applied at basal dressing, in comparison with split applications of ammonium chloride (C type). The application of the slow release fertilizer increased the brown rice yield significantly in Chinese high-yielding cultivars, Yangdao 4 (YD) and Wuyugen 3 (WY), and was more effective at low N conditions than at high N conditions. Maximum yield was achieved in YD grown at 21 g N m^{-2} by S type, and in HH grown at 21 g N m^{-2} by L type. This indicates that about 30 % of N fertilizer could be saved by the application of the slow release fertilizer. These effects on yield resulted from the increase in sink size (the number of spikelets m^{-2} multiply one grain weight) due to the increase in the number of spikelets m^{-2}. The effect of the S type slow release fertilizer was more effective on the increase in sink size and the number of spikelets $panicle^{-1}$ than that of L type. Grain filling percentage in WY and HH decreased with the increase in sink size, while it was slight in YD. Therefore, the increase in sink size was important for YD to improve the yield production. These responses to the N supply were different between YD and WY, suggesting that the optimum amount of N fertilizer applied should be varied with these cultivars.

Keywords: Chinese high-yielding cultivar, Nitrogen fertilizer application, rice, slow release fertilizer, yield

Introduction

In Jiangsu province in China, one of the representative rice-producing areas, high-yielding rice cultivars are widely used for breeding and cultivation with applying a large amount of nitrogen (N) fertilizer more than 30 g m^{-2} to achieve high-yield (Peng *et al.* 2006). These cultivars have large yield potentials (Amano *et al.* 1993; Wang *et al.* 1995; Yao *et al.* 2000) and require the application of large amounts of nutrients, especially nitrogen (N). However, an excess fertilizer application causes not only an increase of costs but also an outflow of nutrients with an environmental burden. This requires an appropriate management of the fertilizer application and an improvement of fertilizer use efficiency (brown rice yield / the amount of N fertilizer application) in the rice cultivation. The method of fertilizer application has been improved by changing the amounts and timings of fertilizer application (Peng *et al.* 2006), and by using farmyard manure (Liu *et al.* 2008) and supplemental nitrification inhibitor (Huang *et al.* 1996) to raise the fertilizer use efficiency. As one of the effective solutions for high fertilizer recovery rate, a slow release fertilizer has been commonly used in Japan for the rice cultivation. However, there are few studies in Chinese high-yielding cultivars (Ju *et al.* 2006). As recent high-yielding cultivars can achieve high yield even in a small quantity of fertilizer application (Hasegawa 2003; Taylaran *et al.* 2009), the optimum fertilizer condition should be examined by using the slow release fertilizer. In this study, effects of different

types of the slow release fertilizer on yield in Chinese high-yielding rice cultivars were evaluated and analyzed to determine effective methods and amounts of N fertilizer application.

Materials and Methods

Experiments were conducted in 2006 to 2009 (Exp.1) and 2010 (Exp.2) in a paddy field of Faculty of Agriculture, Kochi University, Japan. Three treatments were designed by types of N fertilizer applied. Ammonium chloride was applied as conventional N fertilizer (C type) for basal and top-dressing at each growth stage (basal:20 days after transplanting : 20 days before heading : 10 days before heading : heading = 2 :1:1:1:1). Two types of the slow release fertilizer, linear type (LP coat 100, L type) and sigmoid type (LP coat SS 100, S type) were applied as basal dressing all at once, except for the S type in 2006 and 2007, in which both 6 g m^{-2} of sigmoid type and 4 g m^{-2} of ammonium chloride were mixed at basal dressing and 2 g m^{-2} of ammonium chloride was additionally applied at 20 days after transplanting to promote early growth. In Exp.1, Chinese high-yielding Indica cultivar, Yangdao 4 (YD), Chinese high-yielding Japonica cultivar, Wuyugen 3 (WY) and conventional Japonica cultivar, Hinohikari (HH) were grown at the rate of 12 g N m^{-2} for each types of N fertilizer. In Exp.2, three different amounts of N, 12, 21, 30 g m^{-2} for each type of fertilizer were applied in YD and HH. The same amounts of P_2O_5 and K_2O as N in both experiments were applied at basal dressing and at each growth stage (basal : 20 days before heading : 10 days before heading =4:1:1), respectively. All treatments were arranged with 2 replications by a randomized block design. Yield and yield components were determined by using 20 hills sampled at maturity.

Results and Discussion

The effect of the slow release fertilizer on brown rice yield

Brown rice yield significantly increased with the application of slow release fertilizer in Exp.1 (Table 1). The effect of the slow release fertilizer on yield did not differ significantly among cultivars, but the average increasing percentage in YD and WY (6 to 15%) was higher than that in HH (0 to 5%). This result agreed with reports by Sato *et al.* (1993; 1997) in which the yield increased by 33 to 55% and 12 to 14% respectively.

The effect of the slow release fertilizer on yield was not significant in Exp.2 (Table 2). However, the increasing percentage at 12 g N m^{-2} was higher than that at 30 g N m^{-2} in both cultivars, showing that the slow release fertilizer was more effective on yield in low N fertilizer conditions than in high N fertilizer conditions. In addition, this effect was significantly different between cultivars, and the increasing percentage in YD was higher than that in HH. Maximum yield was obtained in YD grown at 21 g N by S type (781 g m^{-2}), and in HH grown at 21 g N by L type (539 g m^{-2}), which were higher than those grown at 30 g N by C type. This result indicates that the slow release fertilizer saves about 30% of N fertilizer applied, compared with the conventional fertilizer.

Table 1. Yield and yield components of rice cultivars in Exp.1 (the average value of 2005-2009)

Cultivar	Types of N fertilizer	Grain yield ($g\ m^{-2}$)			Sink size ($g\ m^{-2}$)			Spikelet number (m^{-2})			Panicle number (m^{-2})			Spikelet number ($panicle^{-1}$)			Grain filling percentage (%)			1000 grain weight (g)		
YD	C	692	100	ab	931	100	abc	29115	100	c	188	100	d	153	100	b	83.2	100	a	28.6	100	a
	L	731	106	a	A	988	107	ab	A	32051	110	bc	B	195	104	d	C	161	105	ab	A	82.2	99	ab	A	27.8	97	ab	A
	S	732	106	a		915	119	a		34044	117	ab		198	104	d		172	113	a		79.9	96	ab		27.0	94	bc	
WY	C	600	100	bc		762	100	cd		28696	100	c		332	100	c		87	100	e		79.2	100	ab		26.6	100	c	
	L	646	108	abc	B	856	112	abc	A	34251	119	ab	AB	369	111	ab	B	93	107	de	B	75.1	95	bc	B	25.1	94	d	B
	S	687	115	ab		915	120	a		36778	128	a		355	107	bc		104	120	c		75.2	95	bc		25.0	94	d	
HH	C	563	100	c		830	100	d		29295	100	c		352	100	bc		83	100	e		81.5	100	ab		23.6	100	e	
	L	589	105	c	C	774	112	cd	B	35179	120	ab	A	400	113	a	A	88	106	e	B	76.1	93	bc	B	22.0	93	f	C
	S	563	100	c		808	117	bc		37343	127	a		376	107	ab		100	120	cd		69.6	85	c		21.7	92	f	

ANOVA																							
	Cultivar (C)	***			***			*			***			***			***			***		
	Type (T)	*			***			***			***			***			***			***		
	Year (Y)	***			***			***			n.s.			***			n.s.			**		
	C*T	n.s.			n.s.			n.s.			n.s.			n.s.			*			n.s.		
	C*Y	n.s.			n.s.			n.s.			n.s.			***			***			**		
	T*Y	n.s.			n.s.			n.s.			n.s.			***			n.s.			n.s.		
	C*T*Y	n.s.			n.s.			n.s.			n.s.			n.s.			n.s.			n.s.		

Note: Italic values indicate the increasing percentage by the slow release fertilizers.
Values with different small letters indicate significant differences at the 5% level among methods of the fertilizer application and cultivars.
Values with different capital letters indicate significant differences at the 5% level among varieties.
*, **, ***; Significant at 5, 1, 0.1% levels by ANOVA, respectively; ns is Not significant.

Analysis of yield components

These effects of the slow release fertilizer on yield resulted from the increase in sink size (the number of spikelets m^{-2} multiply one grain weight, Venkateswarlu and Visperas, 1987) due to the increase in the number of spikelets m^{-2} in both experiments, although 1000 grain weight significantly decreased with the increase in the number of spikelets m^{-2} (Table 1, 2). The increase in the number of spikelets m^{-2} was attributed to the increase in both the number of panicles m^{-2} and the number of spikelets $panicle^{-1}$. The effect of the S type slow release fertilizer was more effective on the increase in sink size and the number of spikelets $panicle^{-1}$ than that of L type. Kamekawa (1990) reported that sink size increased with the application of the slow release fertilizer of linear type mainly due to the increase in the number of panicles m^{-2} in Japanese cultivars. These results show that there is a difference of the effect on the yield components between L and S types.

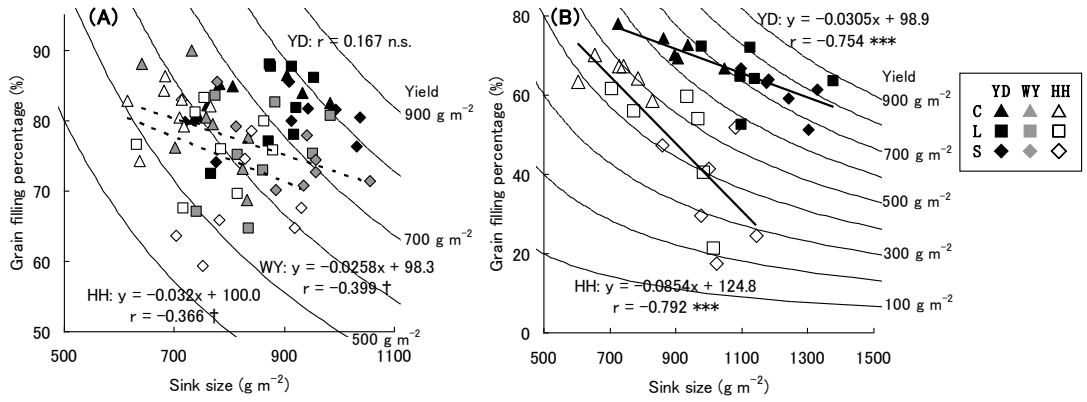
The increase in sink size resulted in the decrease in grain filling percentage in both experiments. However, Tanaka (1988) and Nakanishi *et al.* (1990) reported that grain filling percentage increased despite the increase in the number of spikelets m^{-2} with applying the slow release fertilizer at panicle neck-node differentiation stage. This result may be because of late fertilizer application in comparison with our study.

A negative relationship between sink size and grain filling percentage was observed in WY and HH ($P<0.10$), but not observed in YD in Exp.1 (Figure 1A). A similar trend was observed in Exp.2; the decrease in grain filling percentage in YD was less than that in HH when grown with high N fertilizer conditions (Figure 1B). Yang *et al.* (2002) reported that grain filling percentage in recent Chinese cultivars did not always decrease with the increase of the number of spikelets $panicle^{-1}$. These results show that YD maintains high grain filling percentage with high sink size in response to high N conditions, resulting in high fertilizer use efficiency. However, Chinese high-yielding Japonica cultivar, WY had a different trend with YD, and showed the significant decrease ($P<0.10$) in grain filling percentage with the increase in sink size. This indicates that the optimum amount of fertilizer application should be different between YD and WY.

Table 2. Yield and yield components of rice cultivars in Exp.2 (2010)

Cultivar	Types and amounts of N fertilizer	Grain yield (g m^{-2})		Sink size (g m^{-2})		Spikelet number (m^{-2})		Panicle number (m^{-2})		Spikelet number (panicle^{-1})		Grain filling percentage (%)		1000 grain weight (g)	
YD	C12	599 *100* abcd		794 *100* defg		28412 *100* e		178 *100* e		160 *100* cd		76.2 *100* a		27.9 *100* a	
	L12	759 *127* ab		1054 *133* abcdef		38221 *135* abcde		207 *117* e		186 *116* abc		72.0 *95* a		27.6 *99* a	
	S12	737 *123* abc		1136 *143* abc		41958 *148* abcd		211 *119* e		199 *125* a		64.9 *85* abc		27.1 *97* a	
	C21	653 *109* abc		921 *100* cdefg		32360 *100* de		211 *100* e		154 *100* d		71.0 *100* a		28.5 *100* a	
	L21	719 *119* abc	A	1118 *121* abcd	A	40907 *126* abcd	A	222 *105* de	B	185 *120* abc	A	64.3 *91* abc	A	27.3 *96* a	A
	S21	781 *130* a		1255 *136* ab		46898 *145* a		237 *112* de		198 *129* a		62.6 *88* abc		26.7 *94* a	
	C30	664 *100* abc		971 *100* abcdef		34440 *100* bcde		207 *100* e		166 *100* bcd		68.5 *100* ab		28.2 *100* a	
	L30	725 *109* abc		1239 *128* abc		44718 *130* abc		233 *113* de		192 *116* ab		58.0 *85* abc		27.7 *98* a	
	S30	696 *105* abc		1273 *131* a		46824 *136* a		252 *121* de		186 *112* abc		55.2 *81* abcd		27.2 *96* a	
HH	C12	422 *100* cde		629 *100* g		27158 *100* e		307 *100* cd		89 *100* ef		66.7 *100* ab		23.2 *100* b	
	L12	435 *103* bcde		741 *118* fg		33478 *123* bcde		374 *122* abc		90 *101* ef		58.7 *88* abc		22.1 *96* b	
	S12	407 *97* cde		930 *148* bcdefg		42587 *157* abcd		385 *125* abc		111 *125* ef		44.3 *66* bcde		21.8 *94* b	
	C21	487 *100* abcde		779 *100* efg		33470 *100* bcde		352 *100* bc		95 *100* ef		63.0 *100* abc		23.3 *100* b	
	L21	539 *111* abcde	B	953 *122* abcdefg	B	42313 *126* abcd	A	433 *123* ab	A	98 *103* ef	B	56.7 *90* abc	B	22.6 *97* b	B
	S21	423 *87* cde		1029 *132* abcdef		47101 *141* a		429 *122* ab		110 *116* ef		40.7 *65* cde		21.8 *94* b	
	C30	502 *100* abcde		765 *100* efg		32712 *100* cde		392 *100* abc		84 *100* f		65.7 *100* ab		23.4 *100* b	
	L30	302 *60* de		1001 *131* abcdef		45270 *138* ab		444 *113* a		102 *122* ef		30.7 *47* de		22.1 *95* b	
	S30	229 *46* e		1085 *142* abcde		50302 *154* a		440 *112* ab		114 *137* e		21.0 *32* e		21.6 *92* b	
ANOVA	Cultivar (C)	***		***		n.s.		***		***		***		***	
	Type (T)	n.s.		***		***		***		***		***		***	
	Amount (A)	n.s.		***		***		***		n.s.		***		n.s.	
	C*T	*		n.s.		n.s.		n.s.		***		**		n.s.	
	C*A	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
	T*A	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
	C*T*A	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	

Note: Italic values indicate the increasing percentage by the slow release fertilizers.
Values with different small letters indicate significant differences at the 5% level among methods of the fertilizer application and cultivars.
Values with different capital letters indicate significant differences at the 5% level among varieties.
*, **, ***; Significant at 5, 1, 0.1% levels by ANOVA, respectively; ns is Not significant.



***, †; Significant at 0.1 % and 10 % levels, respectively. n.s.; Not significant.

Figure 1. Relationship between sink size and grain filling percentage in Exp.1 (A) and Exp.2 (B).

Yearly differences

The effect of the slow release fertilizer on yield was minimum in 2006 (-1%) and maximum in 2009 (12%) on average of cultivars in Exp.1. This yearly differences of the slow release fertilizer was because of the decreasing rate in grain filling percentage and the increasing rate in sink size due to the increase in the number of spikelets panicle^{-1}. That is, the remarkable decrease in grain filling percentage and the slight increase in sink size were observed in 2006, while the opposite trend was observed in 2009.

Conclusion

From the above results, the application of the slow release fertilizer increased the brown rice yield significantly, especially in YD and WY, and was more effective at low N fertilizer conditions than at high N fertilizer conditions. Maximum yield was achieved in YD grown at 21 g N m^{-2} by S type, and in HH grown at 21 g N m^{-2} by L type. Therefore, about 30% of N fertilizer could be saved by the application of the slow release fertilizer without any decrease in yield, in comparison with the conventional fertilizer. This resulted from the significant increase in sink size due to the increase in the number of spikelets m^{-2}. The slow release fertilizer of S type was more effective on increases in sink size and the number of spikelets panicle^{-1} than that of L type. The increase in sink size resulted in the decrease in grain filling percentage in WY and HH, while it was negligible or slight in YD, which contributed to the increase in the yield production. These responses to the N supply were different between YD and WY, both Chinese high-yielding cultivars. Therefore, the amount of N fertilizer applied should be varied with these cultivars.

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Drought Resistance of NERICA Compared with Asian Rice, African Rice and Millets in the Field with Different Fertilization Levels

Michihiko Fujii[a,*], Seiji Ishihara[a], Ryohta Shinohara[a]

[a]*Faculty of Education, Shizuoka University, Shizuoka, Japan*
** Corresponding author: Faculty of Education, Shizuoka University, 836, Ohya, Shizuoka, 422-8529, Japan.*
Tel.: +81 54 238 4680; fax: +81 54 238 4680.
eimfuji@ipc.shizuoka.ac.jp

Abstract

Recently NERICA (New Rice for Africa) was developed by a crossing between African rice (*Oryza glaberrima* Steud.) with Asian rice (*Oryza sativa* L.) in West Africa, and is considered to be drought resistant, but it is not clarified enough about the difference in reactions under dry condition. In this research, NERICA, Asian rice, African rice and millets were cultivated in the field under drought condition with different fertilization levels to compare dry matter production, stomatal and leaf characteristics and water absorbing characteristics to comfirm the characteristics of NERICA under drought condition. Fertilization levels were 6gN/m^2 in standard fertilization level and 2gN/m^2 in low fertilization level. Stomatal conductance was measured by porometer and soil water contents at individual depths (0-60cm) were measured by TDR method. Leaf thickness was measured by micrometer and and SPAD value was determined with SPAD meter. In standard fertilization level under drought condition, top dry weight at harvest was high in Dular, drought resistant Asian rice cultivar. Dry weight at harvest in low fertilization level tended to be higher than that grown in standard fertilization level. In low fertilization level one of NERICA showed high dry weight. Dry weight of g*laberrima* parent of NERICA was high in both standard and low fertilization levels, but proportion of dead leaf was high. In standard fertilization level, Dular showed highest stomatal conductance followed by *sativa* parent of NERICA and one of NERICA. In low fertilization level *sativa* parent of NERICA, Dular and one of NERICA showed high stomatal conductance and it tended to be higher than in standard fertilization level. *Glaberrima* parent of NERICA showed low stomatal conductance in both fertilization levels. Average soil water content at 0-60cm depth before harvest in standard fertilization level was low in Dular followed by g*laberrima* parent of NERICA. In low fertilization level soil water content was maintained highest in one of NERICA. Soil water content at 0-20 cm depth in standard fertilization level was low in g*laberrima* parent of NERICA. There was large cultivar differences in soil water content at 40-60 cm depth and soil water decreased significantly in Dular in standard fertilization level. On the contrary it was maintained highest in one of NERICA in low fertilization level. Dry weight was high in Dular, but soil water content decreased especially in the standard fertilization level. On the contrary, dry weight was high in one of NERICA and soil water content was maintained high especially in low fertilization level and at deep soil layer (40-60 cm depth). There was a significant correlation (r=0.907**) between average stomatal conductance and dry weight at harvest except g*laberrima* parent of NERICA. In low fertilization level stomatal conductance and dry weight at harvest tended to be larger than in standard fertilization level. There was a significant correlation (r=0.892**) between average leaf thickness and dry weight at harvest except g*laberrima* parent of NERICA and cultivar with thick leaf tended to maintain high dry matter production. There was a significant correlation (r=0.828**) between average leaf thickness and SPAD value in rice. In one of NERICA leaf thickness and SPAD value were higher in low fertilization level than in standard fertilization level. SPAD value tended to be low in Dular. High leaf thickness and SPAD values seem to be effective to maintain dry matter production under limited water condition with high water use efficiency. NERICA seems to be appropriate for the cultivation under drought condition with the effective use of limited water for the sustainable crop production especially in low fertilization level.

Keywords: drought resistance, fertilization levels, NERICA, soil water content, stomatal conductance

Introduction

Global water shortages are getting worse and drought is the major constraint to the crop production (Blum, 2009, Boyer, 2010, Serraj *et al*., 2011). Recently NERICA (New Rice for Africa)

was developed by a crossing between African rice (*Oryza glaberrima* Steud.) with Asian rice (*Oryza sativa* L.) in West Africa, and is considered to be drought resistant, but it is not clarified enough about the difference in reactions under dry condition. In this research, NERICA, Asian rice, African rice and millets were cultivated in the field under drought condition with different fertilization levels to compare dry matter production, stomatal and leaf characteristics and water absorbing characteristics to confirm the characteristics of NERICA under drought condition.

Materials and Methods

Plant Materials and Cultivation

In this study two NERICA lines which showed superior drought resistant in our previous reports (Fujii *et.al*, 2004, 2008) and their two parent cultivars were used. Cultivars WAB450-24-3-P3-1-HB, WAB450-I-B-P-82-2-1, *sativa* parent: WAB56-104, *glaberrima* parent: CG14. Rice cultivars of *O. sativa* L. (Koshihikari (japonica, lowland, Japan), Dular (indica, lowland-upland, India) and IRAT13 (japonica, upland, Cote d'Ivoire)) and common millet (*Panicum miliaceum* L.) were also used. Plants were seeded in paper pots on May 31, 2007 and seedlings were planted at upland field in the vinyl house of Shizuoka University on June 21. Sides of vinyl house were kept open. Irrigation was applied on June 21, 27 and July 10, and after that no irrigation was applied. Plots were fertilized by compound fertilizer at the rate of 6 g/m^2 as nitrogen in standard fertilization plots and 2 g/m^2 in low fertilization plots individually.

Measurements

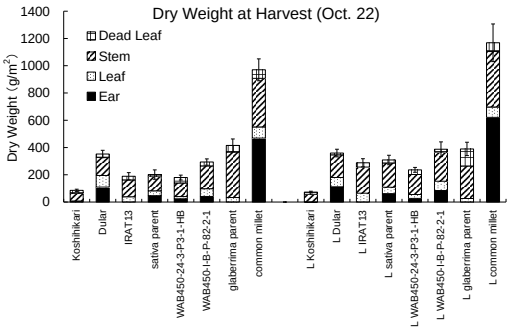
Plants were sampled on June 25, July 31, September 13 and October 22 at the harvesting time. After dividing into organs, dry weight was measured after desiccating in the drying oven. Stomatal conductance was measured at the center of abaxial side of topmost three leaves by dynamic diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, UK) on sunny days during the daytime. Measurements were made on August 7, 13 and 20. Soil water contents at individual depths were measured by TRIME-T3 tube access probe system (IMKO micromodultechnik, Ettlingen, Germany) by TDR method. Measurements were made at intervals of 10cm depth on August 6, 17, 30 and September 11.

Leaf thickness was measured by micrometer (No.193-111, Mitutoyo, Kawasaki, Japan) at the middle length of topmost fully extended leaves avoiding midrib on August 10, 17, 22, 30 and September 12. SPAD value was measured by SPAD meter (SPAD502, Konica Minolta, Tokyo, Japan) on August 10, 17, 22, 30 and September 10. Both measurements were replicated 10 times in each plot.

Results and Discussion

Top Dry Weight

In standard fertilization level under drought condition, top dry weight at harvest was high in Dular, drought resistant cultivar in Asian rice (Figure1). Dry weight at harvest in low fertilization level tended to be higher than in standard fertilization level. In low fertilization level one of NERICA showed high dry weight. Dry weight in g*laberrima* parent of NERICA was high in both standard and low fertilization levels, but proportion of dead leaf was high and heading was not observed.



Bars show standard errors.

Figure1. Top dry weight of individual organs at harvest, Oct. 22.

Stomatal Conductance

In standard fertilization level Dular showed highest stomatal conductance followed by *sativa* parent of NERICA and one of NERICA (Figure 2). In low fertilization level *sativa* parent of NERICA, Dular and one of NERICA showed high stomatal conductance and it tended to be higher than in standard fertilization level. *Glaberrima* parent of NERICA showed low stomatal conductance in both fertilization levels.

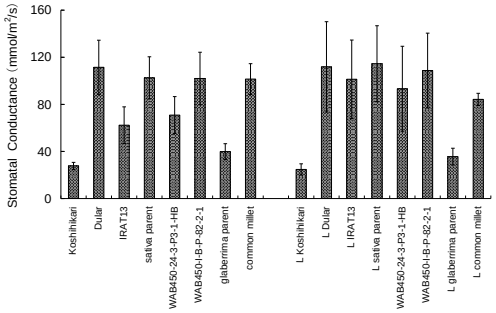


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Figure 2. Average stomatal conductance from August 7 to 20.

Soil water content

Average soil water content at 0-60 cm depth before harvest in standard fertilization level was low in Dular followed by g*laberrima* parent of NERICA. In low fertilization level soil water content was maintained highest in one of NERICA. Soil water content at 0-20 cm depth in standard fertilization level was low in g*laberrima* parent of NERICA (Figure 3). There was large cultivar differences in soil water content at 40-60 cm depth and soil water decreased significantly in Dular in standard fertilization level (Figure 4). On the contrary it was maintained highest in one of NERICA in low fertilization level.

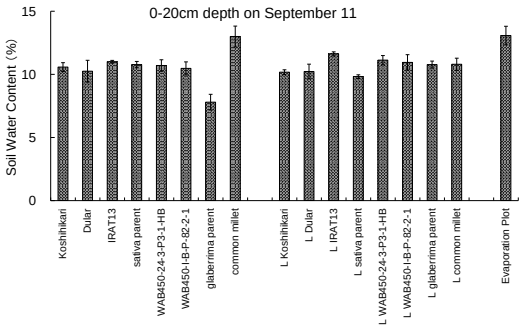

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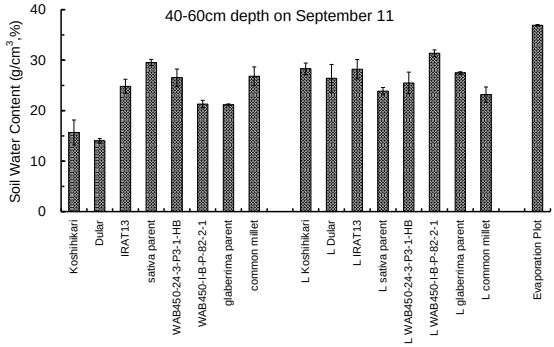
Figure 3. Soil water content at 0-20 cm depth on September 11.

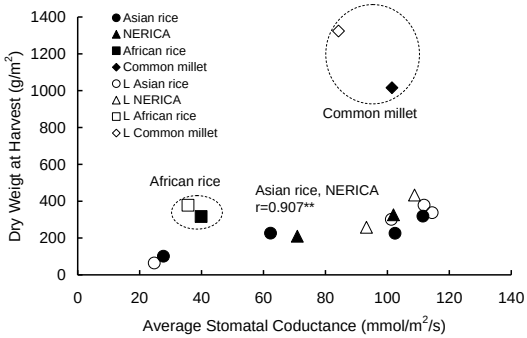


Bars show standard errors

Figure 4. Soil water content at 40-60cm depth on September 11.

Relationship between stomatal conductance and dry weight

There was a significant correlation (r=0.907**) between average stomatal conductance and dry weight at harvest except g*laberrima* parent of NERICA (Figure 5). In low fertilization level stomatal conductance and dry weight at harvest tended to be larger than in standard fertilization level.



**: significant at 1%

Figure 5. Relationship between average stomatal conductance from August 7 to 20 and top dry weight at harvest on Oct. 22.

Relationship between leaf thickness and dry weight

There was a significant correlation (r=0.892**) between average leaf thickness and dry weight at harvest except g*laberrima* parent of NERICA and cultivar with thick leaf tended to maintain high dry matter production (Figure 6).

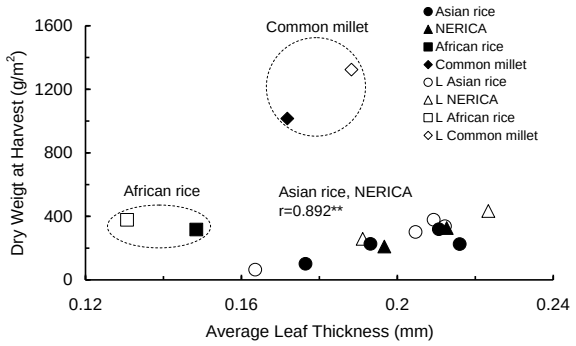


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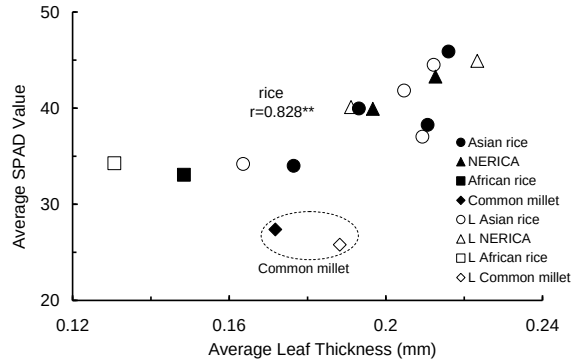
Figure 6. Relationship between average leaf thickness from August 10 to September 12 and top dry weight at harvest on Oct. 22.

Relationship between leaf thickness and SPAD value

There was a significant correlation (r=0.828**) between average leaf thickness and SPAD value in rice (Figure 7). In one of NERICA leaf thickness and SPAD value were higher in low fertilization level than in standard fertilization level. SPAD value tended to be low in Dular.



**: significant at 1%

Figure 7. Relationship between average leaf thickness from August 10 to September 12 and average SPAD value from August 10 to September 10.

Conclusions

Dry weight was high in Dular, but soil water content decreased especially in the standard fertilization level. On the contrary dry weight was high in one of NERICA and soil water content was maintained high especially in low fertilization level and at deep soil layer. High leaf thickness and

SPAD values seem to be effective to maintain dry matter production under limited water condition with high water use efficiency. Condon *et al.* (2004) showed cultivar differences in water use efficiency in wheat. In rice, NERICA seems to be appropriate for the cultivation under drought condition with the effective use of limited water for the sustainable crop production especially in low fertilization level.

Acknowledgements

We thank Emeritus Professor Horie and Professor Shiraiwa, Kyoto University, for kindly giving an opportunity to use seeds of NERICA lines in this study.

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Social Experiment of Volumetric Irrigation Fee Scheme: Case of Gravity Irrigation System in Bohol, the Philippines

Shigeki Yokoyama[a*], Kei Kajisa[b], Tatsuro Miyazaki[c]

[a]*Japan International ResearchCenter for Agricultural Sceinces, Tsukuba, Japan*
[b] *International Rice Research Institute, Los Banos, the Philippines*
[c] *Tsukuba University, Tsukuba, Japan*
** Corresponding author: Japan International ResearchCenter for Agricultural Sceinces, Ohwashi 1-1, Tsukuba, 305-8686, Japan.*
Tel.: +81 29 838 6343; fax: +81 29 838 6342.
syokoyam@affrc.go.jp

Abstract

Increasing in water productivity in rice is crucial as irrigated rice production consume a substantial part of the total world's fresh water. To promote the adoption of water saving technologies, farmers are provided with a tangible incentive to save water is necessary. The social experiment survey of volumetric pricing had been conducted in Bohol, the Philippines, where double rice cropping has been started since 2008 under gravity irrigation. The preliminary results showed that both economic incentive and technical training promoted the efficient water use.

Keywords: social experiment, volumetric pricing, AWD, gravity irrigation, the Philippines

Introduction

The current scheme of irrigation fee, in most of Asia, is charged according to the area irrigated. This scheme may induce overuse of irrigation water as the payment amount is fixed regardless of the actual water use. Area basis charge scheme has long been practiced due to technical difficulty of measuring water intake at farm level, for gravity irrigation system in particular. Fresh water has become increasingly scarce resource worldwide due to increasing in demand of urban and industrial use. Global warming and climate change may worsen this situation.

Considering that irrigated rice production consume a substantial part of the total world's fresh water use, increasing in water productivity in rice is crucial (Shivakoti *et al* 2005). To promote the adoption of water saving technologies, providing farmers with a tangible incentive to save water is necessary. Charging irrigation fee according to the volume of actual water use is such an incentive. The social experiment survey of volumetric pricing had been conducted in Bohol, the Philippines, where double rice cropping has been started since 2008 under gravity irrigation. Research questions were: 1) Whether volumetric incentive effectively induces water saving efforts of water users group, and 2) Whether training of water saving technology (Alternate Wetting and Drying, AWD) contributes efficient water use.

Materials and Methods

Bayongan Dam System of Bohol Integrated Irrigation System, Stage II (BIS-II), was selected as the study site (Map) (Figure 1). The system was constructed with the financial and technical support of JICA (Japan International Cooperation Agency). The system started operation in May 2008 covering 4,000 ha (designed area).The main canal of 6,662 m and 15 laterals, Lateral-A to O, are fully lined, and each TSA (Turnout Service Area, unit of water users) is equipped with

spindle gate, by which farmers can control water intake by themselves, and a level gauge by which the volume of water is measured by the third party (Nippon Koei *et al.* 2007).

The experiment design was as follows. The volume of water intake was estimated based on the gauge reading three times a day (morning, noon and afternoon) at head gate of each TSA. Irrigation fee was firstly collected by area basis as usual, then to be paid back to TSA according to the percentage of saved water based on the required water volume for conventional continuous flooding practice.

Randomized field experiment was applied to 67 TSAs of upper portion of the system among total 147 TSAs. The data collections were conducted for five crop seasons from November 2008 to May 2011. No intervention was applied in the season 0 for all samples as the base line. Volumetric incentives had been applied to the half of TSAs randomly selected for four seasons from the season 1 to the season 4. Technical training of AWD was provided to the half of experimental TSAs and the half of control TSAs before the season 3 (May-Sep 2010). These two interventions resulted in the four groups (Table 1).

Figure 1. Map of the Philippines.

Table 1. Number of sample TSAs by treatment and season

Season 0 Nov08-Mar09	1 May-Sep09	2 Dec09-Apr10	3 Jun-Nov10	4 Dec10- May11	Treatment
67	34	34	16	16	CC
			18	18	CA
	33	33	20	20	VC
			13	13	VA

CC: No intervention
CA: AWD training only
VC: Volumetric only
VA: Volumetric and AWD training

Results and Discussion

In this analysis only the data of the season 0 to 3 were used, as the data of season 4 was still being processed. Profile of the sample TSAs is presented in Table 2. The average size of TSA was around 15 ha of irrigated paddy field with 25 members of farmers. Double rice cropping was practiced in irrigated paddy, while rice-upland crop in rainfed paddy and cassava in upland were common cropping systems. As the designed irrigation area was larger than the actual one, land leveling was still in process and irrigated area was gradually expanding. Main Farm Ditch (MFD) was the soil canal draws water from a lateral into TSA area. National Irrigation Administration (NIA) was responsible to design and construct MFD, while after completion its maintenance was assigned to each TSA. Efficient water use and equity distribution among the members were highly dependent on operation and maintenance of MFD. Almost all of the TSAs organize a regular monthly meeting and collective maintenance work as members obligation. In average, water intake reduced by 30% from Season 0 as baseline to Season 3, the third session of the experiment. Though other factors affecting water intake such as rainfall should be carefully took into account, the experiment seems successful as a whole.

Table 2. Profile of sample TSAs

n=67	Average	STDV
Number of members/TSA		
Season 0 (Nov08-Mar09)	24.7	14.4
Length of Main Farm Ditch (m)		
Season 0 (Nov08-Mar09)	650.5	495.2
Area irrigated (ha)		
Season 0 (Nov08-Mar09)	14.36	9.32
Season 3 (Jun10-Nov10)	16.44	9.02
Water intake (m^3/ha)		
Season 0 (Nov08-Mar09)	10,600	5,493
Season 3 (Jun10-Nov10)	7,308	4,964

Source: TSA interview survey, 2009.

Table 3. Rice yield under rainfed and irrigation by Lateral

Lateral	Rainfed (2007)			Irrigated (2009)			Irrg/Rain
	n	Average (t/ha)	STDV	n	Average (t/ha)	STDV	
A	28	2.39	0.67	84	2.79	1.43	1.17
B	5	2.46	0.59	12	2.22	0.86	0.90
C	NA	NA	NA	10	2.62	0.72	NA
D	11	2.68	0.83	29	2.92	1.59	1.09
E	5	2.04	0.55	14	2.87	1.6	1.41
F	13	2.02	1.14	33	2.42	1.39	1.20
G	5	1.86	0.9	14	2.67	1.45	1.44

Note: Lateral, A to G, aligns from up to down along the main canal.

Before evaluating the impacts of the experiment in detail, the effects of irrigation construction were briefly examined. The rice yield increased by 20 to 40% after irrigation, with some exception (Table 3). It should be noted that the effect was more obvious in down portion, suggesting

irrigation contributed to not only production increase as a whole but also to mitigation of spatial disequilibrium.

Change in Locational Water Distribution

The distribution of water intake by TSA in Season 0 and 3 are shown in Figure 2. As expected, there was a tendency that TSAs in upper portion used more water than those in lower portion both at lateral level and within a lateral. This finding was consistent with the frequently cited "Upstream vs Downstream problem." Taking the advantage of position in the system, farmers in upstream likely to take water excessively under the condition of area basis water fee. This caused water shortage in the downstream, often resulting in conflict between the up- and downstream farmers. It is worth to point out that additional water use did not necessary resulted in higher yield (Table 3). This suggested that saving water in upper portion could contribute to increase in total rice production of the system level, provided saved water is to be effectively distributed down portion which suffers insufficient and unstable water supply.

Comparing the two seasons, a down ward slope from right to left became slightly flattened after experiencing three experiments. The upper portion (Lateral A-D) saved more water than the lower E-F, suggesting saved water was transferred to down portion (Lateral G and after), resulted in more equitable water distribution in the system-wide.

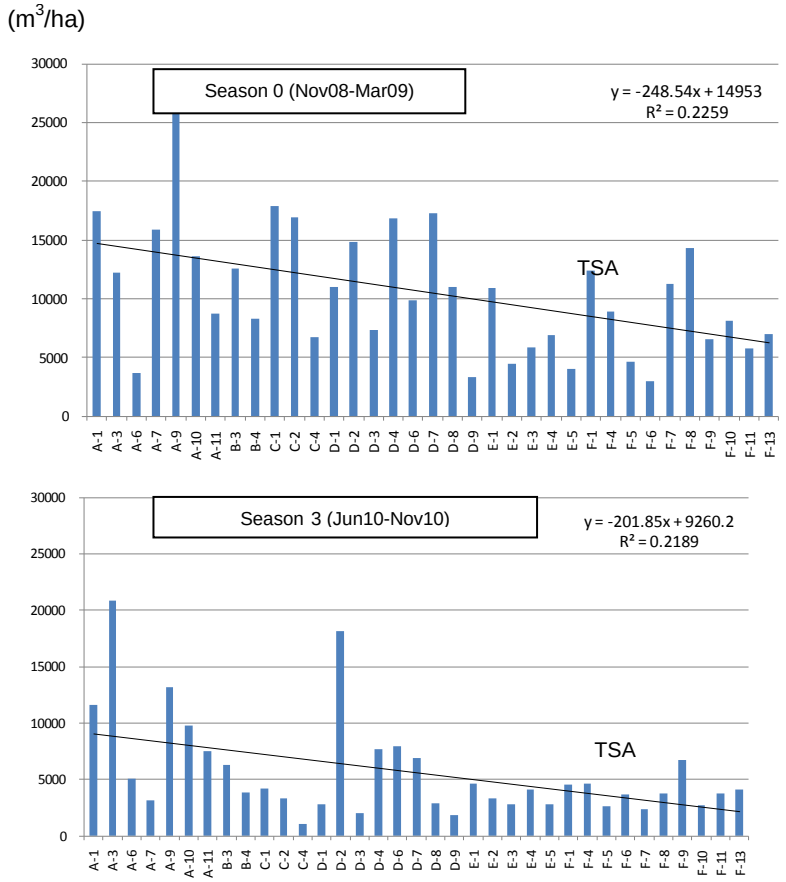
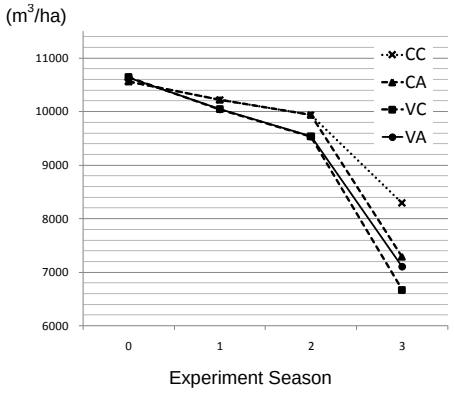


Figure 2. Spatial distribution of water intake by TSA.

Effects of Experimental Intervention

For further examining farmers' response to experimental interventions, changes in water intake by experimental group were compared. Average of water intake by experimental group is presented in Figure 3. Corresponding to the difference of experimental interventions, CC and CA were treated as one group, same as VC and VA, from Season 0 to 2. As the samples were selected randomly, water intakes of the two groups were mostly identical at the baseline (Season 0). The volume has been consistently declined including the control group (CC). The factors affecting overall water intake other than the experiment are rainfall, amount of water release from the dam, NIA's promotion of water saving technology. To evaluate the experiment effects separately from these factors, deviation of experimental groups from the control group should be focused. Economic incentive for water saving was effective, and efficacy of water saving efforts seem to be intensified as time goes. It seems that once farmers are convinced of no adverse effects on crop production (Season 1), they continue water saving efforts and learned effective ways by themselves (Season 2-3).

Providing technical training also showed its effectiveness, as the group of AWD training only (CA) substantially declined their water use compared with the control group (Season 3). There was anecdotal evidence from farmer interview, indicating ADW or intermittent irrigation may enhance yield by increased number of tillers and mitigating damage of golden apple snail. During the experiment period, farmers had chance to learn these information from their neighbors. Part of the sharp decline from Season 2 to 3 might be explained by this spillover effect. In addition, farmers had also chance to attend trainings by NIA and other institutions such as extension offices and NGOs. The synergy effect of economic incentive and training was not obvious. The performance of the group of volumetric and training (VA) is not so striking compared with the group of volumetric only (VC) and training only (CA) (Season 3).



CC (n=16): No intervention; CA (n=18): AWD training only
VC (n=20): Volumetric only; VA (n=13): Volumetric and AWD training

Figure 3. Change in water intake by experimental group.

Conclusions

The preliminary results of social experiment for volumetric water pricing clearly show 1) farmers well respond to economic incentive to save irrigation water use, 2) technical training was also effective to reduce water intake independently of economic incentive, 3) saved water was efficiently redistributed to down portion, enhancing system-wide equity. In this preliminary study social factors, which are highly expected to influence collective actions like irrigation management,

were not considered. Reduced water may entail the risk of yield loss, while technical training and collective actions for water saving may contribute to yield increase and stabilization. These were the remaining issues for further analyses.

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Ultraviolet-induced Fluorescence of Rice Leaf as Influenced by Nitrogen Application and Cultivars

Mikio Sekinuma[a,*], Takehiro Natori[b], Futoshi Sasaki[a], Futoshi Kato[b], Naoto Inoue[a,b]

[a] *Interdisciplinary Graduate school of Science and technorogy, Shinshu University, Japan*
[b] *Faculty of Agriculture, Shinshu University, Japan*
** Corresponding author: Shinshu University, 8304 Minamiminowa, 399-4598 Nagano, Japan.*
Tel.: +81 265 77 1409; fax: +81 265 77 1409.
sekinuma.cultivator@gmail.com

Abstract

New measurement system of ultraviolet-induced fluorescence was developed for detecting the secondary metabolic materials and chlorophyll fluorescence in order to evaluate the photosynthetic activity of crops. In this study, 4 rice cultivars; Koshihikari, IR72, Banten (traditional javanica variety), and CH86 (traditional indica variety), were grown in pots and nitrogen fertilizers were added in 5 different levels. Fluorescence of leaves was measured before and after the heading stage. Leaf blades were excited by light of 370 nm wavelength, which was derived by passing light from xenon lamp through a band-pass filter. The results showed that the fluorescence peaks were located in the region of 400-650 nm, 685 nm and 740 nm, and the spectrum patterns varied among cultivars and different nitrogen levels. Fluorescence intensity (400-650 nm wavelength) observed under high nitrogen condition were relatively low as compared with observed under low nitrogen conditions. These results indicated that the fluorescence pattern of the leaf blade was affected from plant growth and nitrogen level of soil. Our findings show that spectrum analysis of ultraviolet-induced fluorescence is useful for evaluation of rice plant nutrition.

Keywords: ultraviolet-induced fluorescence, nondestructive, rice

Introduction

Development of plant nutrition diagnosis systems is required for sustainable food production in order to conserve resources. Diagnosis of crop is able to provide good information about plant conditions, especially the nutrition status of a plant. Moderate amounts of fertilization make it possible to conserve resource, and cost and to half nutrient enrichment of the soil.

In rice cultivation, increase of yield was established by restricting nitrogen supply in the middle of the growth stage (Matsushima *et al*., 1964; Matsushima *et al*., 1966), as excess nitrogen caused lodging. Nitrogen restriction was determined with basis on leaf color (Matsushima *et al*., 1970; Matsuzaki *et al*., 1974). This cultivation method was termed the V-shaped rice cultivation. Using leaf color to enable diagnosis is delaminated with information only of the leaf surface. However, recent studies have attempted express to express the condition of a plant through analysis of internal leaf conditions; many methods have succeeded more easily with indirect estimation. Direct detection will be expected to estimate photosynthetic ability and function in the future of diagnosis.

A useful diagnosis method is light detection by chlorophyll fluorescence. Fluorescence detection has been used as a nondestructive, noncontact, and continuous detection method to evaluate photosynthetic ability (Zoran *et al*., 1999). Chlorophyll concentration and water stress are monitored by calculating the ratio of chlorophyll fluorescence (Saito, 2005).

The choice of an excitation light source is important in chlorophyll fluorescence because it determines the fluorescence information that can be obtained. Visible (blue) excitation for plants emits both red (RF, 630–700 nm) and far-red fluorescence (FRF, 700–800 nm). Ultraviolet (UV) excitation emits RF, FRF, and blue-green fluorescence (BGF, 400–630 nm). The BGF spectrum

includes information on leaf materials and structure, and this information reveals plant cultivation conditions that are related to chlorophyll a information.

In this study, we developed a new UV-induced fluorescence measurement system to detect the fluorescence of secondary metabolic materials and of chlorophyll in order to evaluate the photosynthetic activity of crops. To achieve this purpose, we investigated whether our system was able to distinguish rice cultivar and nitrogen fertilizer levels.

Materials and Methods

Plant material

Four rice cultivars, Koshihikari (Japonica), IR72, Banten (traditional javanica variety), and CH86 (traditional indica variety), were grown in pots and nitrogen fertilizers were added in 5 different amounts (Table 1). The rice was grown in the Shinshu University greenhouse in Nagano Prefecture (35°51′N, 137°56′E, 740 m above sea level). Measurement of leaf fluorescence was carried out twice, before and after the heading stage, in 2010. The fluorescence of the cut leaves was measured after dark adaption for 30 min at 25°C. Room temperature was maintained at 25°C with air conditioning.

Table 1. Plant material

Cultivar	Ecotype	Plant type	Notes
Koshihikari	Japonica	Standard	Japanese commercial var.
IR72	Indica	Multi tillering	
BANTEN	Javanica	Large panicle	
CH86	Indica	Grassy	Yellow leaf

Fluorescence measurements

Figure. 1 illustrates the new fluorescence measurement system (FMS) for detection of the fluorescence spectrum. In this system, leaf blades were excited by a 370 nm-wavelength light derived by passing light from a xenon lamp (LAX101, Asahi Spectra Co., Ltd., Japan) through a band-pass filter at 370 nm (XBPA370, Asahi Spectra Co., Ltd., Japan). UV light was directed to the target leaf through a quartz fiber. The band-pass filter had a center wavelength of 370 nm with a 10 nm half-bandwidth. The detector unit was a Photonic Multichannel Analyzer (PMA-11, Hamamatsu Photonics, Japan). A long-pass filter (XUL0400, Asahi Spectra Co., Ltd. Japan) was positioned in front of the detection fiber and the reflection of light below a 400 nm wavelength was ensured. The detected fluorescence wavelength was 400–850 nm. The distance between the edge of the fiber and the leaf sample was adjusted to approximately 5 mm. The PMA was operated in 50 msec durations for each leaf area (point) and this was repeated 1000 times in the same area to determine fluorescence excitation. Each leaf sample consisted of 5 areas (point). The acquisition spectra were determined as the highest peak of the spectrum that had a maximum intensity at 680 nm and 740 nm in the 1000 runs. The acquisition spectra were identical to the mean spectra of peak timing in the Kautsky curve (Govindjee, 1995).

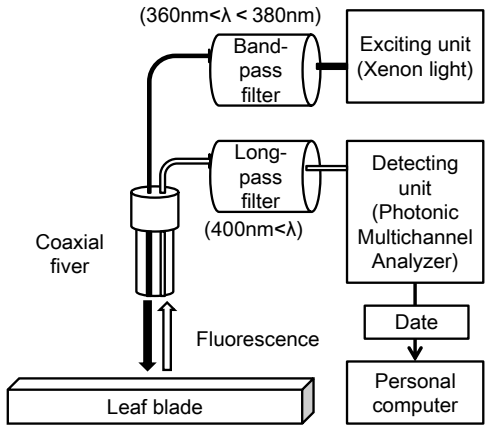
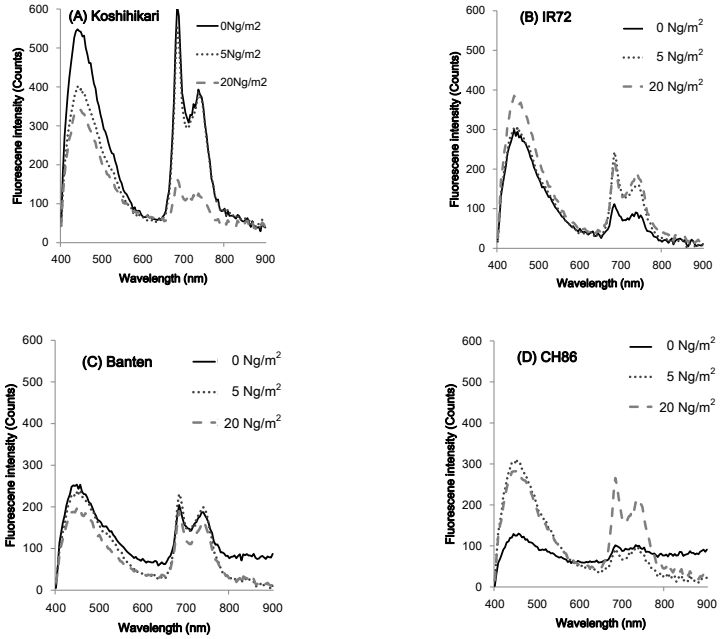


Figure 1. Schematic diagram of fluorescence measurement system. Detected bandwidth of spectrum had wavelengths of 400–850 nm.

Results and Discussion

Figure 2 indicates the fluorescence patterns of leaf blades at various nitrogen levels. The fluorescence peaks were in the region of 400–650 nm, 685 nm, and 740 nm, and the spectrum patterns varied with the different nitrogen levels. The intensity of BGF observed under high-nitrogen conditions was low compared with BGF intensity observed under low-nitrogen conditions.



(A) Koshihikari, (B) IR72, (C) BANTEN, (D) CH86.

Figure 2. Fluorescence spectra of rice leaf. The spectra by excitation at 370 nm were measured at the leaf apex prior to the heading stage.

Figure 3 illustrates the fluorescence spectrum of each cultivar. Fluorescence intensity varied with each cultivar, and high BGF intensity was obtained from the Koshihikari spectrum. These results might describe the fluorescence information containing the condition of plant metabolic material affected by nitrogen levels. The effect of nitrogen treatment was related to BGF intensity. BGF is caused by excitation of ferulic acid derivatives, other phenylpropanoids, and NAD(P)H; RF and FRF are caused by excitation of chlorophyll a (Cerovic *et al*., 1999). BGF enables monitoring of the influence of nitrogen on the degree of growth. We believe that this system can function as a nitrogen level monitor at the same growth stages in rice.

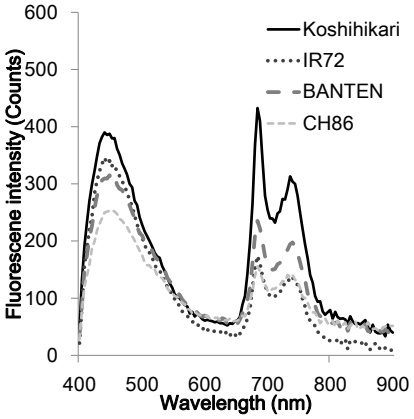


Figure 3. Fluorescence spectra for the cultivars. The spectra by excitation at 370 nm were measured at the leaf apex prior to the heading stage. All cultivars were grown at 5 Ng/ m^2.

The fluorescence spectrum of each cultivar demonstrated different patterns. However, we also need to consider leaf structure, rate of development, and the response to nutrients. In fluorescence detection, it has been suggested that a balance of FRF and BGF enables estimation of wheat lamina growth (Meyer, 2003). In this study, the balance of FRF and BGF may have enabled monitoring of the degree of growth for the cultivars. This result suggests that the detection for cultivars requires further testing and analysis in combination with their growth rates and conditions.

In conclusion, the differences in these results indicate that soil nitrogen levels and different cultivars influence the fluorescence patterns of leaf blades. Our findings show that spectrum analysis of UV-induced fluorescence is useful for evaluation of rice plant nutrition. In the future, UV laser-induced fluorescence methods will be developed if a new diagnosis system becomes necessary.

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Locus for Malate Secretion in Rice Chromosome 3

Miftahudin[1]*, Irfan Martiansyah[1], **Dewi Indriyani Roslim**[2], Tatik Chikmawati[1]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Kampus IPB Darmaga, Bogor-Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universiy of Riau Pekanbaru, Riau*
**Corresponding author: miftahudinm@yahoo.com*

Abstract

Aluminum (Al) toxicity is a major limiting factor of rice production in acid soil. One of the tolerance mechanisms of plant to Al stress is the secretion of malate from plant root. The objective of this research is to identifiy quantitative triat loci (QTL) in chromosome 3 that controls malate secretion from rice root during the period of Al stress. The research was conducted in laboratory and green house. An F2 population derived from the cross between rice genotype Hawara Bunar and cultivar IR64 was used in this experiment. Analysis malate secretion was carried out based on enzymatic method. Rice simple sequence repeats from chromosome 3 were as moelcular marker. The mapping and QTL anaysis was performed using Mapmaker 3 and Mapmaker/QTL. The result showed that the malate secretion trait was normally distributed in the rice F2 population indicating that the trait is polygenic trait. A QTL for malate secretion was identified in the short arm of rice chromosome 3 located in between markers RM545 and RM517.

Keywords: *Aluminum stress, Chromosome 3, Rice, QTL*

Introduction

In general, aluminum tolerance in plant is genetically controlled, and the diversity of this trait can be found inter and intra plant species, including the member of Gramineae (Aniol and Gustafson 1990). Aluminum tolerance mechanism in plant can be divided into two models (Kochian 1995, Matsumoto 2000). First, internal detoxification mechanism, which is the mechanism of plant cells that are able to detoxify Al in the cell through organic acid chelation (Ma et 1998), vacuolar accumulation, protein detoxification, or reactive oxigen species reduction in the root cells (Ezaki et al 2000). Internal detoxification mechanism can also be achieved through activation of *calmodulin-independent NAD$^+$ kinase* (Aniol, 1991).

In the second model, Al is excluded from root tip cells through excluding Al accors plasma membrane, increasing rhizosfeer pH, producing exudate or secreting organic acid (Taylor 1991, Kochian 1995). Among those external mechanisms, organic acid secretion from root cells, such malate, citrate and oxalate, has been the most accepted mechanism. Organic acid secretion has important role in Al tolerance mechanism in wheat, rye and maize (Ryan et al 1995, Li et al 2002, Pellet et al 1995), however the similar role has not been elucidated in rice.

Several researchers have shown that organic acid secretion closely related to Al tolerance mechanis. However, the main factor that controlled organic acid secretion from root cells during Al stress still unclear. The recent finding showed that over expression of malate transporter gene that isolate from wheat could increase Al tolerance in transgenic tobacco, but the gene could not increase Al tolerance in rice (Sasaki et al 2004).

This paper reported our research in identifying locus for malate secretion trait in rice using an F2 population derived from a cross between an Indonesian local rice genotype that tolerant to Al and a rice variety IR64 that sensitive to Al. It is expected that the locus can be used to isolate the gene controlling malate secretion in rice.

Materials and Method

Plant Materials

Rice genotype Hawara Bunar (Al-tolerant parent) and cultivar IR64 (Al-sensitive parent), F1 plants and F2 population derived from a cross between Hawara Bunar and IR64 were used in this research.

Nutrient culture technique for alumininum stress treatment

A simple hydrophonic technique was used to grow rice seedlings. Seeds were sterilized and soak in destilate water for 24 hours, and followed by germination for 48 hours. The seedlings with homogenous root length were put in sterofoamfloated on sterile minimal nutrient solution (Miftahudin et al. 2004) at pH 4.0 in 15 ml tube. A 15 ppm of Al in the form of $AlCl_3.6H_2O$ was adminitered fro 24 hours. The seedlings were grown at room temperature in growth chamber with 12 hours light photoperiod.

Determination of malate secretion

Malate secretion was determined from F1 plants, 400 individual F2 population and both parents. Determination of malate secretion followed procedure as described by Delhaize et al. (1993). A 1.35 ml nutrient solution was sampled from each tube and was added with 1.5 ml buffer solution (0.4 M hydrazine and 0.5 M glycine, pH 9) and 0.1 ml 40 mM NAD. The reaction mixtures were thenincubated at room temperaturefor 30-60 min. The absorbance of the mixture was measured using spectrophotometer at λ =340 nm (absorbance 1). The mixture was then added with 5 µL Malate Dehidrogenase (5 mg/mL, Sigma, USA), and the absorbance 2 was then measured.The difference between absorbance 1 and 2 indicated the amount of NADH produced and was used to calculate malate content of the solution as the following equation:

$$C = \frac{V \times M}{\varepsilon \times d \times v \times 1000} \times \blacktriangle A$$

Notes: C = Malate concentration (mg/l)
V = Volume Total (ml)
v = Sample Volume (ml)
M = Molecular Weight of Malate (g/mol)
d = Cuvette width (cm)
ε = Absorption coefficientof NADH at λ 340 nm = 6.3 (l/mmol.cm)
▲A = the difference between absorbance 1 dan 2

Moelcular marker analysis

The polymorphic SSR markers from rice chromosome 3 were applied to 300 individuals F2 population. The primers from those markers were used to amplify DNA. A 50 ul PCR reaction mixture consist of100 ng DNA, 100 mM Tris-Cl pH 8.0, 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.3-0.5 uM tiap primer, and 1 U Taq DNA polimerase (NEB, USA) was made for each individual plant. PCR process were performed using thermocycler with the following condition: 1 cycle of 94^{o}C for 5 minutes followed by temperature cycles of94^{o}C (35 second), 55^{o}C (35 second), and 72^{o}C (1 minutes, 45 second) for 35 cycles and finally one cycle of 72^{o}C for 10 minutes . PCR products were analyzed using 2.5 - 3.0% superfine agarose gel electrophoresis in 0.5 x TBE (Tris-Borate-EDTA) buffer.

Genetic mapping and QTL identification

Segregation analysis of molecular marker in the F2 population was performed using *Chi Square*test pada α = 0.05. Genetic linkage and QTL analyses usedMAPMAKERS/EXP ver 3.0 andMAPMAKERS/QTL ver 1.1, respectively.

Results and Discussion

Malate secretion in parent plants under Al stress

Malate secretion was analyzed from nutrient culture media that has bee used for growing F1, F2 and both parent seedling under 72 hours Al stress. The result showed that the malate secretion was higher when the seedlings were Al stressed and the secretion increse as the period of stress increase (Figure 1 and 2). The Al stress at thelevel of 15 ppm could differentiate malate secretion level from both parents. In rice cv IR64, although malate secretion increase as the increase of stress period, there was no significant difference between malate scretion at 0 and 15 ppm of Al stress along the 72 hour period of stress. Conversely, there wassignificant difference of malate secretion along the 72 hours stress period between 0 and 15 ppm Al stressin Hawara Bunar.The significant increase of malate secretion occurred when the seedlings were Al-stressed at 15 ppm at 72 hours. Therefore, the Al stress level of 15 ppm for 72 hour stress duration was used for phenotyping the F2 population. The result also showed that the average malate secretion from IR64, Hawara Buanr, F1 and F2 plants were 2.2, 3.8, 3.1 and 4.1 ppm, respectively.

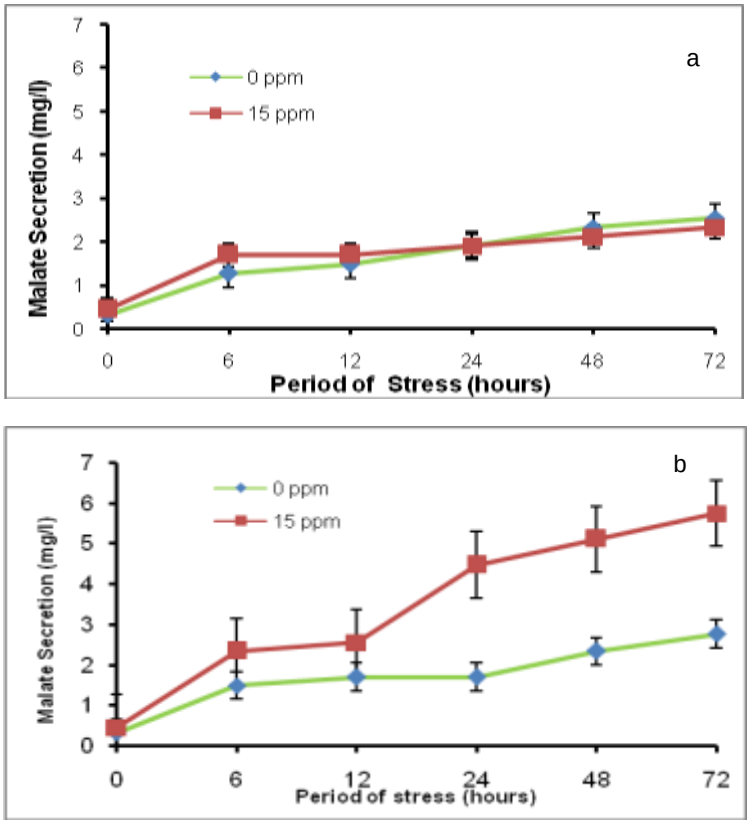

Figure 1. Malate secretion from, rice root cv IR64 (a) and Hawara Bunar (b) along the 72 hour period of Al stress.

Distribution of Malate Secretion in F2 Population

Analysis of malate secretion in 400 individual F2 population showed normal distribution (Figure 2), which indicated that malate secretion character was multigenic controlled. It can be seen from the Figure 2 that the distribution curve rather skewed to the right and the secretion class falled to higher value than that both parents. This is suggested transgresive segregation phenomenon in this population. The average malate secretion of F2 population was also higher than that of Hawara Bunar.

When the malate secretion was grouped into two class of secretion based on the range of each parent malate secretion, malate scretion in F2 population followed monogenic inharitance. For individual F2 that secreted malate < 3 ppm, it was grouped into Al-sensitive plants, otherwise the plants were grouped into Al-tolerant plants. The Chi square test showed that the segregation fit to 3:1 ratio for Al-tolerant to Al-sensitive plants (Table1).

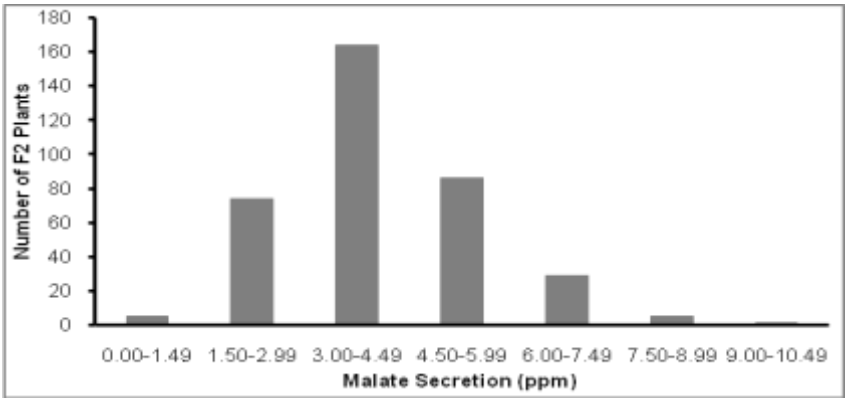

Figure 2. Distribution of malate secretion among individual F2 population.

Table 1. Chi square test for monogenic inharitance pattern of malate secretion in F2 population

Malate Secretion Class (ppm)	Number of Observed Plants	Number of Expected Plants (3:1)	Chi test
< 3.0	79	91	0.15
≥ 3.0	285	273	

Locus for malate secretion in rice chromosome 3

Analysis of genetic factor controlling malate secretion trait in rice was performed into two approaches. First, malate secretion trait was treated as polygenic trait based on the normal distribution of malate secretion in the F2 population, and second, malate secretion trait was treated as monogenic trait. The result showed that among the three chromosome that habe been analyzed, which were chromosome 1, 2 and 3, the locus for malate secretion trait was only possibly found om chromosome 3. QTL analysis based on poygenic trait indicated the presence of the QTL in the short arm of chromosome 3 in the region between markers RM%$% and RM517. However, the LOD score in that region maximum only 0.98, which was not enough to conclude that the QTL present in that region.

When analysis was performed based on monogenic trait, it was found that a locus for malate secretion trait present in the region with LOD 3.02. Genetic map of the rice chromosome 3 that harbored the locus for malate secretion trait contained five markers i.e. RM569, RM545, RM517, RM251, and RM232 with the total distance 131.8 cM (Figure 3). The locus for malate secretion trait was located in between marker RM545 and RM517 with the exact position at 4 cM

from RM517 toward RM545. The result of this research was similar to Nguyen et al (2001) who found QTL position for Al tolerance loci in the short arm of rice chromosome 3, but the position is rather shifted to other region of the short arm. This difference was due to the different rice background used in both experiments.

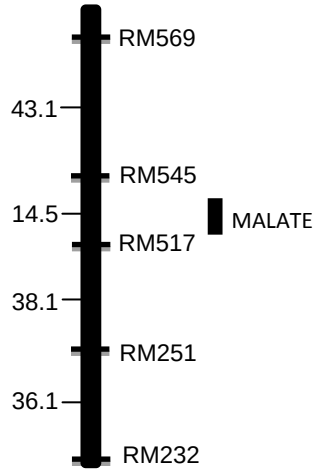

Figure 3. Genetic map of malate secretion locus in rice chromosome 3.

Conclusion

Rice secreted malate during the period of aluminum stress. Al tolerant rice genotype secreted malate more than that of Al-sensitive rice genotype. The secretion increased as the duration of stress increase. There was a locus controlling malate secretion located in the short arm of rice chromosome 3.

Acknowledgement

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Responses of Nutrient Efficient Maize Genotypes to Bio-fertilizer at Low Chemical Fertilizer Doses

Marlin Sefrila[a], Munandar[a], **Renih Hayati**[a,*] , Sabaruddin[a]

[a] *Plant Science Department, Graduate School, Sriwijaya University, Jl Padang Selasa No 524, Bukit Besar, Palembang, 30139, Indonesia*
** Corresponding author: Tel.: +62 711 354222; fax: +62 711 317202*
renih_hayati@yahoo.com

Abstract

High dose of chemical fertilizer is usually required to obtain high maize (*Zea mays* L.) yield. Bio-fertilizer application in combination with nutrient efficient genotype was expected to reduce the chemical fertilizer need without reducing the yield. The objective of the research was to evaluate the responses of nutrient efficient genotypes to bio-fertilizer at low chemical fertilizer doses. The field experiment was conducted at Agro Techno Park (ATP), South Sumatra. The treatments were arranged in Split-Split-Plot Design with three replications. The main plots were chemical fertilizer doses, which were 25, 50, and 100 % of standard dose at ATP (400, 100, and 50 kg ha^{-1} Urea, SP-36, and KCl, respectively). The sub-plots were nutrient efficient genotypes (B41, L167, and S219), and hybrid B 816 as comparison. The sub-sub-plots consisted of treatments with or without bio-fertilizers. The bio-fertilizer (10 ml L^{-1}) was sprayed to the soil around the plants at 2, 4, 6, and 8 weeks after sowing. The yields of nutrient efficient genotypes (5.23 to 7.89 ton ha^{-1}) were comparable to the yield of B 816 (6.58 to 7.62 ton ha^{-1}) at all chemical fertilizer doses without bio-fertilizer. The genotypes responded differently to the bio-fertilizer application. Supply of bio-fertilizer at chemical fertilizer doses of 50 % tended to increase the yield of B 41 up to 16 % of its yield at 100 % chemical fertilizer dose without bio–fertilizer. This suggested that the chemical fertilizer dose can be reduced by 50 % only for B 41.

Key words: bio-fertilizer, low input, maize, nutrient efficiency

Introduction

High dose of chemical fertilizer is usually required to obtain high maize yield, especially for South Sumatra soil with deficiency of macro elements problem due to low soil pH (< 5.6). However, poor farmers often cannot afford it. According to Djafar and Halimi (1998), the farmers in South Sumatra only apply the chemical fertilizer with sub optimum rate for maize production (75, 50, and 50 kg ha^{-1} Urea, SP 36, and KCl, respectively). An excessive use of chemical fertilizer can also cause an environmental problem. Nutrient efficient maize genotype in combination with bio-fertilizer may offer an alternative to overcome the problem.

Nutrient efficient genotype has the ability to produce a higher yield than other genotypes under low nutrient supply (Presterl *et al*., 2003; Worku *et al.*, 2007). Department Agronomy, Sriwijaya University is now in the process of developing the nutrient efficient open pollinated maize genotypes and B 41, S 219, and L 164 used in this experiment are some of them (Munandar *et al.*, 2010).

Bio-fertilizer usually contains microorganisms having specific function such as *Azospirillum* to fix N2 and P solubilizing bacteria to solubilize P from the soil and fertilizer to be available to the plants (Saraswati & Sumarno, 2008). Several researchers had conducted the experiments to evaluate the responses of various plants such as young Robusta coffee (Junaedi et al., 1999), soybean (Noor, 2003; Totok & Rahayu, 2007), and turfgrass (Guntoro et al., 2007) to the bio-fertilizer application, but the results were still inconsistent. Further research is still needed in this area.

Bio-fertilizer application in combination with nutrient efficient genotypes in this research was expected to reduce the chemical fertilizer supply up to 25% of the standard dose without reducing yield. The objective was to evaluate the responses of several nutrient efficient genotypes compared to the hybrid to bio-fertilizer application especially at low chemical fertilizer rate.

Materials and Methods

The experiment was conducted at Agro Techno Park (ATP), South Sumatra in January to April 2011. The soil was dominated by sand (69.91%) with 4.4 pH, 28.3 g kg^{-1} C organic matter, 2.1 g kg^{-1} N, 59.1 mg kg^{-1} P-Bray, and 0.19 c mol kg^{-1} exchangeable K. The experimental design was Split-split-Plot with three replications. The main plot, the chemical fertilizer doses, consisted of 25, 50, and 100 % of the standard rate at ATP (400, 100, and 50 kg ha^{-1} Urea, SP 36, and KCl, respectively). The split plot was the genotypes which consisted of B 41, S 219, and L164 as the nutrient efficient genotypes and Hybrid B 816 as comparison. The split-split plots were treatments with and without bio fertilizers which consisted of two types, Subur (bio fertilizer 1), and EM 4 (bio fertilizer 2). Seeds were sown manually with 70 cm x 25 cm row spacing and two seeds per hole. Plants were thinned into one plant per hole at one week after sowing (WAS), so in each 2.8 m x 2.5 m sub-sub plot contained 40 plants. Chemical fertilizers (1/3 of Urea, SP 36, and KCl) were applied at sowing and the rest of Urea was applied at 4 WAS. Organic fertilizer (10 ton ha^{-1}) was applied at 2 WAS. Bio-fertilizer (10 ml L^{-1}) was sprayed to the soil at 2, 4, 6, and 8 WAS as recommended application time. Water was sprayed to the soil around the plants for treatment without bio- fertilizer.

All ears of each sub-sub-plot were harvested at harvest maturity, counted, and air dried in the drying room for two weeks, weighted .and converted into yield (ton ha^{-1}). Ears from three sample plants (randomly chosen) per plot were weighted and the average was used to get the ear weight per plant data.

Results and Discussion

The yield of nutrient efficient genotypes (B 41, S 219, and L 164) at all chemical fertilizer doses without bio-fertilizer (5.23 to 7.89 ton ha^{-1}) was comparable to the yield of hybrid B 816 (6.58 to 7.62 ton ha^{-1}). Among the nutrient efficient genotypes, S 219 had the lowest yield followed by B 41 and L 164 genotypes, especially at 25 and 50% chemical doses (Table 1). This suggested that L 164 genotype in this experiment had the highest nutrient use efficiency compared to B 41 and S 219 genotypes. The result was consistent with the previous experiment (Munandar et al., 2010). The yield or ear weight (ton ha^{-1}) significantly correlated with ear weight $plant^{-1}$ ($r = 0.54^*$).

Bio-fertilizer application had no significant effect on yield (data not presented), but tended to increase yield only for B 41 and S 219 genotypes especially for Bio-fertilizer 1 (Table 1). The yield of B 41 at 50 % chemical fertilizer dose was 94 % of its own yield at 100 % chemical fertilizer rate without bio-fertilizer. The yield of B 41 genotype increased only 16 % by the application of Bio-fertilizer 1 or 9 % by bio-fertilizer 2 (Table 1). The yield of S 219 genotype increased 8 to 11 % at 25 and 50 % chemical fertilizer dose, respectively, but the values after the addition of bio-fertilizers were still lower than the yield at 100 % chemical fertilizer dose without bio-fertilizer. The data suggests that the chemical fertilizer dose can be reduced by 50 % with the application of bio-fertilizer only for B 41 genotype. The effect of genotype on yield was higher than the bio-fertilizer (data not presented).

The L 164 genotype and hybrid B 816 did not give positive responses to both bio-fertilizers probably because their yields at all chemical fertilizer doses without bio-fertilizer were higher than the yields of B 41 and S 219. It indicated that L 164 genotype and hybrid B 816 had an ability to

take up or use nutrient more efficient than that of the B 41 and S 219 genotypes as suggested by Worku *et al*.(2007).

The bio-fertilizers used in this experiment contained microorganisms to fix N_2 and to solubilize P, but the P Bray of the soil was high. Therefore, small increase in yield of B 41 and S 219 genotypes by application of bio-fertilizer probably related to a better N use efficiency than P efficiency of the genotypes.

In conclusion, genotypes responded differently to the bio-fertilizers. Supply of bio-fertilizer at 50% of chemical fertilizer doses tended to increase the yield of B 41 genotype up to 16% of 100% chemical fertilizer dose without bio-fertilizer. This suggested that the chemical fertilizer dose can be reduced by 50% without reducing yield only for B 41 genotype.

Table 1. Yield (ear weight) of the nutrient efficient and hybrid genotypes as affected by bio and chemical fertilizers

Genotypes	Chemical Fertilizer (% of standard rate) ton ha^{-1}			mean
	25	50	100	
B 41 without biofertilizer	6.35 (101)	5.94 (94)	6.32 (100)	6.20
+ Bio fertilizer 1	5.84 (92)	6.96 (110)	6.89 (109)	6.56
+ Bio fertilizer 2	6.40 (101)	6.48 (103)	6.66 (105)	6.51
S 219 without biofertilizer	5.23 (79)	5.49 (83)	6.61 (100)	5.78
+ Bio fertilizer 1	5.74 (87)	6.22 (94)	6.58 (100)	6.18
+ Bio fertilizer 2	4.48 (68)	5.92 (90)	6.60 (100)	5.67
L 164 without biofertilizer	7.12	6.83	7.89	7.28
+ Bio fertilizer 1	6.37	6.23	7.44	6.68
+ Bio fertilizer 2	5.94	6.53	7.06	6.51
Hybrid B 816 without bioertilizer	6.58	7.62	7.19	7.13
+ Bio fertilizer 1	6.25	6.36	6.38	6.33
+ Bio fertilizer 2	6.03	7.05	6.83	6.64

Note: Number in parentheses is the relative to its yield at 100 % without bio-fertilizer

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Kinetin and Calcium Pantothenate Effects on Shoot Multiplication in *In Vitro* Cultured Cassava Var. Adira 2 and Adira 4

Nurul Khumaida [a,*], Sintho Wahyuning Ardie [a], Candra Catur Nugroho [a], Suwarto [a]

[a] *Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
** Corresponding author: Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University,*
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia.
Tel.: +62 251 862 9353; fax: +62 251 862 9353.
nkhumaida@yahoo.com

Abstract

Cassava (*Manihot esculenta* Crantz.) is one of largest carbohydrate sources in the world which also potentially developed as the source of bio-energy and raw materials for severals industries. As the demand for cassava is increasing, it is very important to supply the true-to-type cassava seedling continuously. *In vitro* propagation is one advanced technique that can be applied to meet the increasing demand of cassava seedling. The objective of the research was to study the effect of kinetin and Calcium Pantothenate (CaP) concentration on shoot multiplication of *in vitro* cultured cassava var. Adira 2 and Adira 4. This research was conducted in a completely randomized design with two factors. The first factor was kinetin concentration (0; 1; 1.5; and 3 ppm) and the second factor was concentration of CaP (0; 1; and 2 ppm) used in combination. The results showed that MS medium supplemented with 1 ppm CaP and 1.5 ppm kinetin promoted the growth of cassava explants of Adira 2 variety until 4 Weeks After Treatment (WAT). However, for longer culture period MS medium containing 3 ppm of BAP was better to support the explants growth. MS Medium containing 1 ppm of CaP and 3 ppm of kinetin promoted the growth of Adira 4 variety until 4 WAT. However, for longer culture period MS medium containing 2 ppm of CaP and 3 ppm of kinetin was better to support the explants growth. The highest shoot multiplication rate for 20 weeks old plantlets of Adira 2 and Adira 4 varieties was obtained at MS medium containing 3 ppm of BAP.

Keywords: calcium pantothenate, cassava, kinetin, shoot multiplication

Introduction

Cassava (*Manihot esculenta* Crantz) is the third largest source of carbohydrate for human consumption in the world. It is the principal carbohydrate source for more than 500 million people in the tropical world. Cassava plays a famine prevention role wherever it is cultivated widely. Cassava has adaptability to a range of climatic and edaphic conditions including tolerance to drought, to some pests and diseases relative to other crops, and confers a comparative advantage on cassava under conditions of famine against alternative crops.

More recently, cassava has gained importance as a possible fuel commodity not only in Indonesia but also in the Philippines, China, Thailand, and other countries which have more advanced national bio-fuel programs. In situations where water availability is limited (i.e. not enough for the cultivation of sugar cane), cassava is the preferred feedstock for ethanol production. However, cassava-based industries are facing the main problem of cassava availability in amount and continuity (Suryana, 2009). Therefore, in order to meet the large demand of cassava, farmers need large amount of good quality cassava seedling in a relaitvely short time.

In general, cassava is propagated by stem cutting. Eventhough stem cutting is easy to be done and relatively cheap, it is not always available when needed and it is difficult to assure to have the true to type seedling. *In vitro* technique is one method for mass propagation can be applied for rapid mass propagation of cassava seedlings. Research in cassava shoot multiplication and the induction of embryogenic callus had been done recently (Guohua, 1998; Sudarmonowati *et al*.,

2002; Onuoch and Onwubiku, 2007). Previous results showed that cassava 'Mentega' formed the highest shoot formation when cultured in MS medium containing 2 ppm of BAP (Sudarmonowati *et al*., 2002). In other varieties, Fauzi (2010) found that standard MS medium containing 1.5 ppm of BAP was the most effective medium for shoot multiplication. The objective of this research was to study the effect of Kinetin and Calcium panthothenate (Ca-P) addition in the shoot multiplication of Adira 2 and Adira 4 cassava varieties.

Materials and Methods

The research was conducted from January 2010 to January 2011 at Plant Biotechnology Laboratory, Department of Agronomy and Horticulture, Agriculture Faculty, Bogor Agricultural University. This research was consisted of two separated experiments differed in the cassava variety used as explants (Adira 2 and Adira 4 varieties). The experiment was arranged in Randomized Block Design with two factors and ten replicates. The two factors were kinetin and Calcium panthothenate (Ca-P) concentrations. There were four levels of kinetin concentrations (0; 1; 1.5; and 3 ppm), and three levels of Ca-P concentrations (0; 1; and 2 ppm). A control experiment was made using standard MS medium with an addition of 3 ppm BAP. Each replicate consisted of one bottle culture with one explants.

Plant material used was one node of axillary shoot from *in vitro* culture of Adira 2 and Adira 4 cassava varieties provided by Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development (ICABIOGRD). MS medium was used as basic medium of all treatments. Surface sterilization was conducted by dipping the axillary shoots in Agrept (2 g L^{-1}) and Dithane (2 g L^{-1}) solution for 2 h, and consecutively into Chloramfenicol (2 g L^{-1}) solution overnight. Sterilization by dipping in 10% NaClO solution for 5 min, then into 5% NaClO for 2 min was conducted in the laminar air flow cabinet. Sterilized shoots were planted in the precondition medium with 4 -5 explants in each bottle for 4 weeks. Explants used for experiment were micro cutting with single node of cassava (approximately 1-3 mm in length) cultured in the precondition medium. Those explants were transferred into MS medium containing kinetin or Ca-P according the treatments.

Planted explants were cultured in the dark for one week to induce shoot initiation. After that, cultures were transferred into 24 h light condition with 21^{o}C for 9 weeks. Observations were conducted on *in vitro* culture and acclimatized plants. On the *in vitro* culture, the first shoot formed and the time of callus formation were observed daily. Percentage of callus formation (%), number and percentage explants with shoot (%), number of shoot per explants, callus diameter (mm), callus color, structure of callus, length of shoot (mm), shoot morphology, number of leaves, number of explants with root, number of roots, and number of nodes were observed weekly until 10 Weeks After Planting (WAP). Data was test for normality then tested with F-test at α = 5%. Data with significant difference was tested with Duncan' Multiple Range Test.

Results and Discussion

The use of Ca-P in several concentrations only affected the callus diameter of Adira 2 variety, while kinetin addition significantly affected number of shoot and callus diameter of Adira 2 and Adira 4 varieties. Interaction between Ca-P and kinetin concentration only affected the callus diameter of Adira 4 variety. The contamination rate in this research was relatively low for Adira 2 variety (2.12%) and Adira4 variety (1.77%), respectively. Most of the contamination in the culture was caused by bacteria. Fungi contaminated the culture media in a lower level than bacteria.

As shown in Table 1, percentage of explants with shoots of Adira2 variety were higher except for three combination showing lower percentage (less than 50%) that were 1.5 ppm of

kinetin, 1 ppm of CaP, and 2 ppm CaP. All treatment combination did not significantly affect the shoot formation. In the other hand, medium containing 3 ppm of BAP resulted in 100% callus formation. Some treatments that failed to induce callus formation were in medium without kinetin or low kinetin concentration (less than 1.5 ppm). For Adira 4 variety, the highest percentage of explants with shoots was resulted in medium containing 0.5 ppm of kinetin. In general, percentage of explants with shoot was relatively high (more than 50 %) for all treatments, except for medium containing 3 ppm of kinetin (44.44 %). The highest number of callus formed was achieved in medium containing high level of cytokinin (3 ppm BAP and 3 ppm kinetin). In contrary, the root formation was inhibited with the presence of high level of cytokinin. Root formation was induced in medium containing low concentration of cytokinin.

Table 1. Percentage of shoot forming explants, callused explants, and rooted explants of Cassava Adira2 and Adira 4 variety at 10 WAP

Treatment		Adira 2			Adira 4		
		Shoot forming explants	Callused explants	Rooted explants	Shoot forming explants	Callused explants	Rooted explants
Ca-P (ppm)	Kinetin (ppm)	……%.....			……%.....		
0	0	75.00	0.00	75.00	66.67	11.11	44.44
	1	50.00	0.00	50.00	57.14	0.00	42.86
	1.5	33.33	0.00	0.00	80.00	0.00	40.00
	3	100.00	33.33	0.00	44.44	44.44	33.33
1	0	25.00	0.00	75.00	50.00	16.67	50.00
	1	50.00	25.00	25.00	77.78	22.22	44.44
	1.5	100.00	33.33	33.33	66.67	22.22	22.22
	3	75.00	25.00	25.00	77.78	66.67	10.00
2	0	33.33	0.00	33.33	70.00	40.00	50.00
	1	75.00	0.00	25.00	71.43	0.00	57.14
	1.5	100.00	50.00	100.00	66.67	11.11	33.33
	3	100.00	33.33	33.33	60.00	60.00	20.00
3 ppm BAP		100.00	100.00	0.00	75.00	50.00	0.00

At the 1 WAP, shoot of cassava was initiated at the nodes. Number of shoot formed in every treatment was compared to number of shoot formed in the control treatment (medium containing 3 ppm BAP, B3) and tested by Dunnet test as shown in Table 2.

Table 2 indicates that every treatments affected number of node when they were compared to the control medium B3, except for C0K3−B3 (9 WAP), C0K3−B3 and C1K3−B3 (10 WAP). In contrast, for Adira 4 variety none of the treatment affected number of node when compared to the control medium. Based on the regression analysis, kinetin addition could be increased above 3 ppm. For Adira 2 variety, the regression equation for shoot formation and kinetin concentration was linear ($y = 0.401x – 0.095$, $R^2 = 0.866$). This equation means that every 1 ppm increase of kinetin concentration would increase shoot formation 0.401 per explants. For Adira4 variety, the regression equation for shoot formation and kinetin concentration was also linear ($y = 0.278x + 0.224$, $R^2 = 0.613$). This equation means that every 1 ppm increase of kinetin concentration would increase shoot formation 0.278 per explants.

Table 2. Number of cassava shoot of Adira2 and Adira4 variety in the in vitro culture at 9 and 10 WAP

Treatment combination	Adira 2		Adira 4	
	Age (WAP)		Age (WAP)	
	9	10	9	10
C0K0-B3	−3.75*	−3.75*	−0.71 ns	−0.71 ns
C0K1-B3	−4.00*	−4.00*	−0.88 ns	−0.80 ns
C0K1.5-B3	−3.00*	−4.17*	−0.58 ns	−0.58 ns
C0K3-B3	−2.17 ns	−2.17 ns	−0.93 ns	−0.93 ns
C1K0-B3	−4.25*	−4.25*	−0.75 ns	−0.88 ns
C1K1-B3	−4.00*	−4.00*	−0.60 ns	−0.60 ns
C1K1.5-B3	−4.17*	−3.17*	−0.71 ns	−0.71 ns
C1K3-B3	−3.00*	−2.75 ns	0.53 ns	0.29 ns
C2K0-B3	−4.17*	−4.17*	−0.48 ns	−0.58 ns
C2K1-B3	−3.75*	−3.75*	−0.60 ns	−0.66 ns
C2K1.5-B3	−3.50*	−3.50*	−0.71 ns	−0.71 ns
C2K3-B3	−2.50*	−3.50*	1.33 ns	1.23 ns
F test	*	*	*	*

Note : Variety was not compared to each other; C0, C1, C2 = 0, 1, 2 ppm CaP, respectively; K0, K1, K1.5, K3 = 0, 1, 1.5, 3 ppm kinetin, respectively; B3 = MS + 3 ppm BAP; ns = not significant at P = 0.05; * = significantly different (P<0.05)

Table 3. Number of leaf of cassava Adira2 and Adira4 variety at 3, 7, and 10 WAP

Treatment		Adira 2			Adira 4		
		Age (WAP)			Age (WAP)		
CaP (ppm)	Kinetin (ppm)	3	7	10	3	7	10
0	0	1.25	2.00 b	2.75	0.80 jkl	0.30	0.33
	1	1.00	0.33 b	0.00	0.70 jkl	0.33	0.43
	1.5	0.50	0.25 b	0.00	0.60 jkl	0.14	0.20
	3	1.25	1.33 b	1.33	0.40 jkl	0.22	0.11
1	0	0.25	0.25 b	0.25	1.10 j	1.00	0.67
	1	0.50	0.50 b	1.25	1.00 jk	0.67	0.33
	1.5	2.00	2.00 b	1.00	1.20 j	0.33	0.44
	3	0.50	0.50 b	0.25	1.20 j	1.10	0.56
2	0	0.00	0.00 b	0.00	1.10 j	0.30	0.40
	1	1.00	1.25 b	1.25	0.90 jk	0.33	0.57
	1.5	1.00	1.67 b	2.50	0.60 jkl	0.20	0.22
	3	0.75	1.33 b	1.67	0.00 l	0.70	2.00
3 ppm BAP		1.00	5.50 b	7.50	0.11 kl	0.50	1.00
F test		ns	*	ns	*	ns	ns

Note: Variety was not compared to each other; Numbers followed by the same letter in the same columns are not significantly different based on DMRT at level α = 5%.

Leaf formation of cassava explants started at the 2 WAP. The leaf formation rate was slower than the leaf senescence rate at the 4 WAP and resulted in the decreased of leaf number in every observation point. The leaf senescence might be caused by the production of ethylene gas, nutrient deficiency, and toxicity. Magdalita *et al*. (1997) found that the accumulation of ethylene fasten the senescence of leaves in *in vitro* culture. Nutrient deficiency might caused by longer

culture, thus periodically subculture might be needed at 4 WAP. Leaf senescence might be also caused by endogen auxin-cytokinin imbalance in the plants tissues (Lizawati *et al*., 2009). As shown in Table 3, the highest number of leaf for Adira 2 variety at 10 WAP resulted in medium containing 3 ppm of BAP. In contrast, medium containing 1 ppm of kinetin, 1.5 ppm of kinetin, and 2 ppm of CaP resulted in lowest leaf number (0). The highest number of leaf for Adira 2 variety at 10 WAP resulted in medium containing 2 ppm of CaP in combination with 3 ppm of kinetin, while medium containing 1.5 ppm of kinetin resulted in the lowest number of leaf formed.

Shoot multiplication rate could be increased by periodically subculture (Hartmann and Kester, 1983). In this research, the subculture was done twice. The first subculture was done at 10 WAP, while the second subculture was done 4 weeks after the first subculture. The multiplication rate for Adira 4 variety was observed only at the first subculture. As shown in Table 4, the highest multiplication rate was achieved in the medium containing 3 ppm of BAP for both varieties.

Table 4. Shoot multiplication rate of in vitro grown cassava Adira 2 and Adira 4 varieties

Variety	Treatment		Replication	∑ Initial explants	∑ Initial shoots	∑ Shoot first sub-culture	∑ Shoot second sub-culture	Total number of Shoot
	Kinetin (ppm)	CaP (ppm)						
Adira 2	0	0	1	1	1	5	2a	8
	3	0	1	1	1	3	3b	7
	3	0	3	1	5	3	–	8
	Means ± Standard deviation							7.50 ± 0.71
	1	1	1	1	1	5	3bc	9
	1.5	1	1	1	1	2	–	3
	3	2	1	1	1	1	2	4
	3 ppm BAP		1	1	7	17	34	58
	3 ppm BAP		2	1	2	2	–	4
	Means ± Standard deviation							31.00 ± 38.18
Adira 4	1	0	4	1	1	2	–	3
	3	1	7	1	3	4	–	7
	3	1	8	1	2	1	–	3
	Means ± Standard deviation							5.00 ± 2.83
	0	2	1	1	2	1	–	3
	3	2	3	1	6	8	–	14
	3	2	6	1	6	11	–	17
	3	2	7	1	5	1	–	6
	3	2	9	1	8	1	–	9
	Means ± Standard deviation							11.50 ± 4.93
	3 ppm BAP		10	1	6	27	–	33

Note : Variety was not compared to each other; a = sub-cultured to MS0 + 3 ppm kinetin; b = sub-cultured to MS0 + 2 ppm CaP + 3 ppm BAP; c = sub-cultured to MS0 + 2 ppm CaP + 3 ppm kinetin

Conclusions

The use of several kinetin concentrations (0; 1; 1.5; dan 3 ppm) gave a positive linear response to number of shoots for both varieties (Adira 2 and Adira 4). In contrast, concentration of kinetin had negative linear response to number of roots. Media composition with 1 ppm of CaP in combination with 3 ppm of kinetin promoted the growth of cassava explants of Adira 4 variety until 4

WAT, but for longer culture period MS medium containing 2 ppm of CaP and 3 ppm of kinetin was better to support the explants growth. The highest shoot multiplication rate was achieved in the medium containing 3 ppm of BAP for both varieties.

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Wet Injury of Wheat in Upland Field Converted from Paddy Field in Japan

Kentaro Kawaguchi*

Wheat and Barley Research Divison, NARO Institute of Crop Science (NICS),
National Agriculture and Food Research Organization (NARO), Japan
**Corresponding author: Wheat and Barley Research Divison, NARO Institute of Crop Science (NICS),*
National Agriculture and Food Research Organization (NARO).
2-1-18, Kannondai, Tsukuba, Ibaraki 305-8518, Japan.
Tel. +81 29 838 8694; Fax. +81 29 838 8694
kentaro@affrc.go.jp

Abstract

Wheat and barley are very important crops used as staple food and feed. Growth delay or injury of wheat and barley under waterlogged soils is one of agricultural constraints to be solved. The common symptoms are germination failure, leaf color degradation, and wilting. Since there are different types of waterlogging conditions, the mechanisms of injury also varied. However, root sensitivity to low-oxygen atmosphere in soil is critical. Rice plant has been widely cultivated in Japan. In recent years the agricultural policy enhanced rice-wheat-soybean rotation using paddy fields for economic reasons. Crop production using paddy fields is thought to be an important key for sustainable agriculture in eastern Asia but wet injury of wheat usually occurs in the upland fields converted from paddy fields because of the poor drainage. Under a particular case, a phenomenon called ground subsidence due to the earthquake induced wet injury in this year. The most popular measure to mitigate the waterlogging damage on farm level is the field management to facilitate drainage. In addition, we have great hopes for establishing tolerant varieties; however, such commercial one is not yet obtained by traditional breeding. We are now engaged on an innovative research project about root aerenchyma which is thought to facilitate gas exchange between root and aerial parts of plants. We hypothesized its modification could change the sensitivity of root to low-oxygen. The project plan was; i) to investigate anatomical and physiological basis of aerenchyma formation in the recipient wheat cv. Bobwhite. ii) to establish transgenic wheat lines using several candidate genes for root aerenchyma formation. iii) to analyze function of the genes using the transgenic Bobwhite lines.

Keywords: aerenchyma, barley, subsidence, waterlogging, wheat

Introduction

The main production areas of wheat in Japan are in Hokkaido, Kanto and Kyushu district. Except in Hokkaido, they have relatively high precipitation. It is over one thousand mm a year. Sowing and harvest of wheat often coincide with the two rain periods, we call them "*Akisame*" (autumnal rain in September and November) and "*Baiu*" (rainy season in June). Moreover, wheat is widely grown in upland fields converted from rice paddy fields in Japan. The main reason to cultivate wheat instead of rice is due to decline of the amount of rice consumption. Full efficient utilization of rice paddy fields is one of the main policies of the Japanese government. Average of wheat acreage in recent 10 years is 210 thousand ha and the over half of the acreage is the rice fields. Oyanagi (2008) reported that about 20% of the wheat acreage is affected by waterlogging.

Wet injury usually occurs in the converted fields because of the poor drainage and it causes severe damage on the grain yields as well as their quality. One of the causes of the wet injury is shortage of oxygen in the low layer, where the wheat roots develope and obtain oxygen to grow. The most effective way to prevent wet injury is the field management for well drainage, for example, ditch making and raised bed farming, etc., however, the economic cost for such field management is relatively high in the farm level. Therefore, we develop new lines of wheat having high tolerance to wet soil.

To overcome the agricultural constraint, understanding of the mechanisms of wet injury and tolerance in wheat is essential. In our study, first, we characterized wheat growth under wet conditions in farmer's fields and in our experimental paddy fields. The results showed that i) there were high correlations between yield and soil moisture in the farm level and ii) wheat growth suffered from wet stress from the early development under the condition of paddy field. Second, we focused on root aerenchyma. Aerenchyma is thought to be enhancing gas exchange between subterranean part (root) and aerial part (shoot) of plant tissues. Therefore, root aerenchyma is one of the important traits to contribute wet tolerance of plants. To investigate the basic nature of root aerenchyma formation during early development of wheat, we examined when and where root aerenchyma were formed in the wheat seminal root under waterlogging condition using a plant pot system.Third, we recently studied the strategy to improve wheat using modern biotechnology.

Materials and Methods

Field experiment

Wheat (*Triticum aestivum* L.) cultivars. Bobwhite (Mexican cultivar), Norin 61 (Japanese cultivar), Iskra and NS-302 (Yugoslavian cultivars) were used in this experiment. The latter two were selected as candidates for high tolerant cultivars (Dr Sato, unpublished data). Norin 61 is the major cultivar in Japan except in Hokkaido region. Bobwhite is often used as a host for establishing fertile transgenic by means of particle bombardment in wheat. Experimental fields were located at Yawara, Tsukuba-mirai city, Ibaraki, Japan. Five are area each of contiguous two upland fields converted from paddy fields were used. One was used under non-tilling cultivation as control plot and the other was used after soil puddling as wet treatment plot. Wheat seeds were sown with 70 cm rows on 30th October, 2009 and 10th November, 2010. Fertilizers (6, 9 and 6 kg/10a each as N, P_2O_5 and K_2O) was applied at ten days before sowing. Growth characters were monitored in 3 weeks interval. The soil water contents were measured by TDR-method (Time Domain Feflectometry) using a HydroSense CD620 display and CS620 sensor with a 12-cm probe (Campbell Scientific Australia Pty. Ltd., Thuringowa Central, Australia) in the top 12 cm of the soil. The soil's redox potentials at 10cm depth were recorded using an Eh meter (PRN-41, Fujiwara Scientific Co.LTD., Tokyo, Japan) with a reference electrode.

Pot experiment for aerenchyma study

Wheat cv. Bobwhite line SH 98 26 was used. Three seeds were sown at a 3-cm depth in each of 27 soil-filled pots (Fujiwara Scientific, Tokyo, Japan; 1/5000a deep type Wagner pot, 30 cm height x 15.9 cm inner diameter). The wheat plants were grown in a greenhouse maintained at a temperature of approximately 20°C day/night, with natural light. Water treatments were imposed to 5 d old seedlings: (i) control, with a well-drained; (ii) T-15 treatment, the water level was maintained at 15 cm below the soil surface, and (iii) T+3 treatment, the water level was maintained at 3 cm above the soil surface. The O_2 concentrations were recorded at a 14 cm depth in the soil using an OXY-10 O_2-sensor (PreSens Precision Sensing GmbH, Regensburg, Germany). Transverse cross-sections were obtained from primary seminal roots using a D.S.K. Microslicer (DTK-1000, Dosaka EM Co. Ltd., Kyoto, Japan). The sections were examined and photographed with a light microscope equipped with a camera (BX51 microscope and DP72 camera, Olympus Co. Ltd., Tokyo, Japan) at 10 magnification.

Results and Discussion

Growth of 4 cultivars of wheat in the experimental fields

The soil conditions in the experimental fields were measured. Ground water levels were almost less than 75 cm below the soil surface during the entire experiment period. Soil water contents were higher in puddling plot than that in control plot until April 19. Redox potentials in the soil were constantly over +500 mV and showed no significant difference between the plots. The plant height and dry weight were significantly affected by puddling (wet) treatment (Figures 1 and 2 show the results of 2009 – 2010 season). Similar tendency was observed in the repeated testing in the next cropping season of wheat. Puddling/Control ratio in dry weight was very low (20%) at young (on 17 February) and was gradually recovered (80%) at ripening (on 13 May). These results indicated that wheat suffered from wet stress since their early stage of development and suggested that high tolerance to wet conditions at the seedling stage is important for wheat production in paddy fields in Japan.

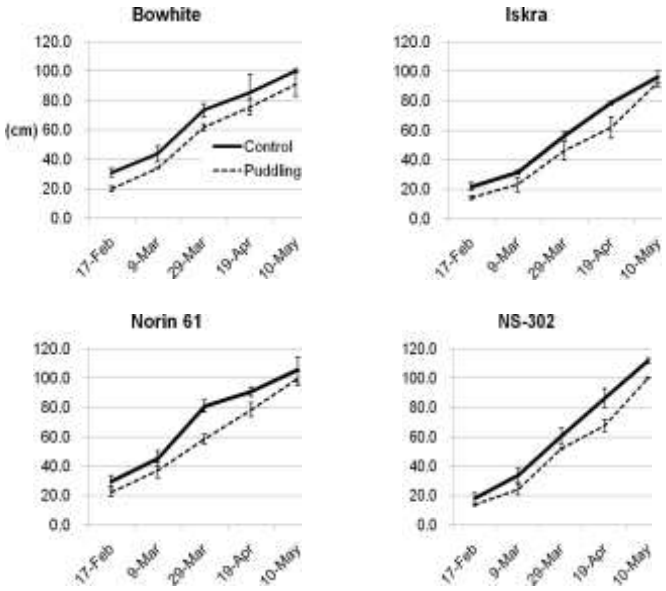


Figure 1. Changes in the plant height of 4 cultivars of wheat under control condition (solid line) and puddling (wet) condition (dashed line) in 2009 – 2010 season.

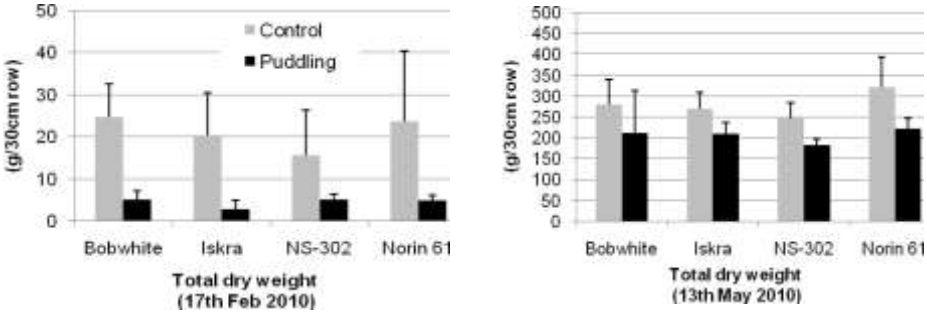
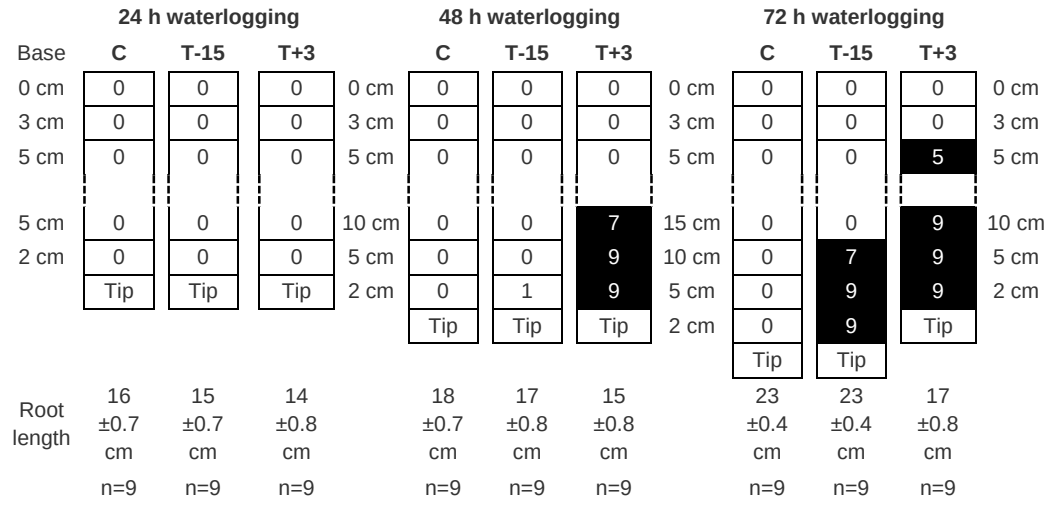


Figure 2. Dry weight of wheat plants (aerial part only) under control condition (gray bar) and puddling (wet) condition (black bar) in 2009 -2010 season.

Growth and root aerenchyma formation of wheat in pot system

The soil conditions in the plant pot were measured by TDR-method. Under the control, T-15, and T+3, the soil water contents were 12-14; 23-27; and 74-82%, respectively. The O_2 concentrations after 72 h in T-15 soil decreased from the control soil 18.7 to 10.1%, whereas that in T+3 soil decreased to 4.9%. While the redox potential in the control soil remained around +400 mV, the values in the T-15 and T+3 soils were about +360 and +290 mV, respectively, after 72 h treatment. These results showed that the soil conditions in T-15 and T+3 became hypoxic in order of water depth during waterlogging treatment.

The length of primary seminal roots and the position of aerenchyma were schematically drawn in Figure 3. The root length was reduced by 25% in the T+3 plants. The reduction of seminal root dry mass was 12.5% in T-15 and 31% in T+3 plants, respectively. For the aerial parts, the plants did not show significant difference (data not shown). Wheat primary seminal roots showed no aerenchyma in our control condition. Aerenchyma was appeared after 48 h under T+3 and after 72 h under T-15 waterlogging conditions, respectively, at 2 to 5 cm behind the root tip. The aerenchyma in T+3 plants extended to 2-10 cm behind the tip at 72 h waterlogging.



Numbers of root sections with developing aerenchyma are indicated at the sampling position. Roots (n=9) were tested. Black background indicates aerenchyma formation in more than half number of the nine sections.

Figure 3. Aerenchyma formation in the seminal root of wheat seedlings under waterlogging conditions.

Our results provide basic information on the aerenchyma formation process in seminal roots of wheat. To our knowledge, this is the first report to describe the time of the aerenchyma formation in wheat seminal roots under hypoxic conditions (Haque *et al.* 2010). The development nature of aerenchyma, such as the formation timing, might be similar in part among several upland crops. Root aerenchyma can be seen within 48 h after the initiation of hypoxia in the seminal (our study) and adventitious roots (Malik *et al.* 2003) in wheat. In maize, aerenchyma formation in the seminal (Gunawardena *et al.* 2001) and adventitious roots (Drew *et al.* 1981) is induced by hypoxia within a few days. Thus, wheat requires several days to form aerenchyma by waterlogging stress, while rice plant constitutively forms well developed aerenchyma in their roots. Such different ability to form aerenchyma among plant species may be one of the important keys to determine their adaptive nature to water.

Wheat plants grown in our pot system can be used for analyzing the molecular and physiological mechanisms of aerenchyma formation. Proteome analysis using the wheat seminal root showed that 10 candidates of proteins would be associated with the metabolism during root

development under hypoxia (Haque *et al.* 2011). Elucidation of the protein function during aerenchyma formation is a next step of this study.

Future direction

Wheat cv. Bobwhite line SH 98 26 is known to be one of the most useful recipients for producing transgenic wheat lines (Pellegrineschi *et al.* 2002). Therefore, the information obtained from this study will be useful not only for understanding the basic nature of aerenchyma formation, but also for efforts to improve aerenchyma formation, including the creation of transgenic plants. In fact, the transgene approach in our experiment showed the ability satisfactorily on the investigation of seed dormancy of wheat and the related gene (Nakamura *et al.* 2011). Introducing genes can be widely selected from various plant species, including rice and teosinte, etc., which have high capacities to form aerenchyma (Nakazono *et al.* 2011 in this session, Mano and Omori 2009).

Acknowledgements

The author wish to thank the International Maize and Wheat Improvement Center (CIMMYT), Mexico for providing seeds of wheat cv. Bobwhite SH 98 26 and the National Institute of Agrobiological Sciences (NIAS) Genebank for supplying seeds of wheat cv. Iskra and NS-302. I would like to express my thanks to Drs. M. Nakazono, Y. Mano and S. Shimamura for their valuable suggestions and critical comments to this study. I also thank Mr. S Hamada for his skillful management of the field experiment. All of the achievements reported here were produced through the collaborative investigation with my colleagues, Drs A. Oyanagi, F. Abe, Md Emdadul Haque and M. Mori in NICS. The research was supported by a grant-in-aid from the Bio-oriented Technology Research Advancement Institution (Promotion of Basic Research Activities for Innovative Biosciences, No. H20 / seeds-01-01).

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Roles of Root System Development and Function in the Growth and Yield under Waterlogged Condition in Common Wheat

Tomohito Hayashi[a], Tomofumi Yoshida[b], Kiyoshi Fujii[bc], Takako Tsuji[b], Yurie Okada[a], Eriko Hayashi[a], Akira Yamauchi[a]*

[a]*Graduate School of Bioagricultural Sciences, Nagoya University, Japan*
[b]*Aichi Prefecture Agricultural Research Center, Aichi, Japan*
[c]*Agricultural Production Division, Aichi Prefectural Government, Aichi, Japan*
**Corresponding author: Graduate School of Bioagricultural Science, Nagoya University, Nagoya 464-8601, Japan*
ayama@agr.nagoya-u.ac.jp

Abstract

Waterlogging stress substantially reduces the productivity of common wheat. Unavailability of oxygen to the roots is the major growth-limiting factor for plants exposed to waterlogging stress. The development and function of roots play significant roles in expression of waterlogging tolerance of wheat under such conditions. However, the mechanism of root function in the waterlogging tolerance is not clear yet. In this study, we, therefore, aimed to evaluate the roles of root system development and function in the growth and yield of wheat plants that were exposed to waterlogged condition at jointing stage. This study was conducted in 2009 at Aichi Agricultural Research Center experimental field. Plants were grown under two different conditions; control for rainfed upland conditions while in waterlogging treatment (WL), water level was maintained around 5 cm below the soil surface from jointing stage till maturity. Soil type of this field was andsol. We previously examined a set of germplasm (144 cultivars) collection, and selected Nishikazekomugi (high waterlogging tolerance), Iwainodaichi (moderate) and UNICULM (low). Grain yield of Nishikazekomugi in WL was 64% of control, while that of Iwainodaichi and UNICULM was 38 and 4%, respectively. The cultivars of greater watelogging tolerance further showed the ability to maintain higher stomatal conductance in WL, which should reflect higher root ability of soil water uptake. In fact, they showed higher root development as evaluated in total root length. Increase in nodal root porosity of Nishikazekomugi and Iwainodaichi in response to exposure to WL then contributed to the significant increase in total root length. Higher root porosity indicates greater root aerenchyma formation in response to waterlogging stress. These results indicated that the tolerant cultivars had higher ability of aerenchyma formation, which promoted root system development, and eventually contributed to the increase in shoot dry matter and grain yield under waterlogged conditions.

Keywords: aerenchyma, common wheat, hydraulic conductivity, porosity, waterlogging tolerance

Introduction

Common wheat is known to be susceptible to waterlogging, and the grain yield substantially decreases by excess moisture (Oyanagi *et al*, 2001) in most of the wheat producing countries such as UK (Belford and Cannel, 1979), Australia (Setter *et al*., 2009). In Japan, 27% of wheat-planted field (approximately 57,000 ha) is affected by excess moisture stress (Sakagami *et al*., 2010).

When the water table rises to or above the soil surface, roots surrounded by the waterlogged soil suffer from O_2 deficiency (Jackson *et al*., 1999; Malik *et al*., 2003). As one of mechanisms for waterlogging tolerance, aerenchyma in root provides a low-resistance internal diffusion pathway to supply O_2 to the root apex (Arikado, 1975; Colmer, 2003; Armstrong and Armstrong, 2005). Therefore, root aerenchyma formation under waterlogged condition may play important roles in waterlogging tolerance in wheat (Setter and Waters, 2003). Although wheat plants generally have low efficiency of root aerenchyma formation (Watkin *et al*., 1998), genotypic

variations in its functional roles in the maintenance of waterlogging tolerance under waterlogged condition is not yet clear.

In this study, we examined if genotypes with high ability of root aerenchyma formation and root system development under waterlogging produce high dry matter and yield by evaluating the impacts of root aerenchyma formation on root development, stomatal conductance, photosynthetic rate and grain yield under waterlogged conditions, and compared with those under rainfed upland conditions in the field. Such hypothesis of this study was summarized in Figure 1. The objective of this study was, therefore, to examine the roles of root aerenchyma formation in the maintenance of waterlogging tolerance under waterlogged condition in wheat.

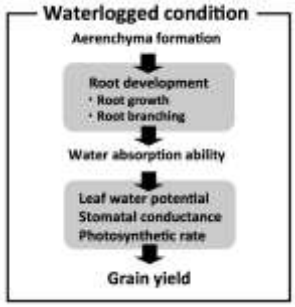


Figure 1. Schematic diagram of hypothetical mechanism of waterlogging tolerance in wheat.

Materials and Methods

Experiment design and Plant materials

A field experiment was conducted at Aichi Agricultural Research Center. Plants were grown under two different conditions e.i control for rainfed upland conditions while in waterlogging treatment (WL), water level was maintained around 5 cm below the soil surface from jointing stage till maturity. Soil type of this field was andsol.

The seeds were sown on December 3, 2008. The waterlogging treatment lasted for 50 days from April 13 to June 1, 2009. The plants were harvested on June 9, 2009 (188 days after sowing). Based on the results of our previous study (Hayashi *et al*., 2008) that examined a set of 144 cultivars collection, we selected three cultivars e.i. Nishikazekomugi (high waterlogging tolerance), Iwainodaichi (moderate) and UNICULM (low).

Grain Yield

Plants were harvested in six replicates for each cultivar. For shoots, after air-drying for about three weeks, leaves and stems were oven-dried and weighed, and the grains were threshed. After the grain moisture content was measured with a grain moisture tester, grains were weighed, and the yield components were determined.

Root Development

To determine total root length, after the harvest of shoots, roots were sampled in six replicates for each cultivar. At the end of waterlogging treatment, the roots were sampled with the round monolith method (Kang *et al*. 1994) by using stainless cylinder of 15 cm in diameter, and the soil cores of 20 cm in depth were taken. Soil samples were carefully washed to collect roots. After removing the debris, the roots were stained and arranged on water in a transparent plastic tray. They were then scanned to convert to a digitized image, and total root length was determined with the use of NIH Image ver. 1.62 and Root Length ver. 1.54 (Kimura, 1999).

Leaf Water Potential

We measured leaf water potential of the flag leaves in six replicates in each cultivar at -4, 4, 11, 16, 19, 28 and 34 days after the onset of waterlogging treatment. Leaf water potential was measured with thermocouple psychrometry using sample chambers (Model C-52, Wescor) and a microvoltmeter (Model HR-33T, Wescor). The sample chambers had been calibrated with NaCl solution of a known molarity in advance.

Stomatal Conductance and Photosynthetic Rate

We measured stomatal conductance and photosynthetic rate of the flag leaves in three replicates for each cultivar at -4, 4, 11, 16, 19, 28 and 34 days after the onset of waterlogging treatment. The measurements were conducted from 800 to 1300 on sunny days at PPFD of 1400 μmol m^{-2} s^{-1} with a portable type apparatus for photosynthesis and transpiration measurements (LI-6400, LI-COR, Lincoln, NE, USA). Leaf temperature and CO_2 concentrations surrounding leaf was adjusted to be 28±1.7℃ and 400 μmol mol^{-1}, respectively. Measurement duration average was about 40 to 60 s. The leaf areas enclosed by chamber were from 3.0 to 6.0 cm^2.

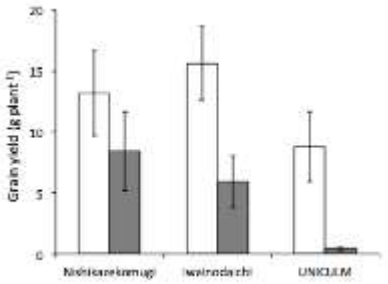
Root Porosity

We measured porosity of the nodal root in six replicates for each cultivar. Nodal root porosity was measured 50 days after the onset of waterlogging treatment.The nodal root axis was used for root porosity measurement following the microbalance method (Visser and Bögenmann, 2003; Suralta *et al*., 2010).

A 1-cm segment from the middle portion of each root was cut. From each plant (replicate), sixty 1-cm segments were further sampled. Each 1-cm root segment was cut with a sharp razor blade and gently blotted by rolling it with a small brush on tissue paper for about 2 s to remove adherent water. Then, to prevent weight loss by evaporation, the segments were transferred into a capsule with cover that had been tared on a microbalance. After closing the capsule, the segments were weighed (w1 in μg), transferred to a holder with small vials filled with water, and stored for 30 min. Up to 60 samples were weighed before they were infiltrated with tap water twice under vacuum for 30 min. After water infiltration, the root segments were blotted again on tissue paper for about 2 s and weighed in a capsule (w2 in μg). Using the specific weight (SW) obtained from larger samples, the porosity was calculated using the formula: Porosity (%; v : v) = 100 × (w2 – w1) × SW/w2

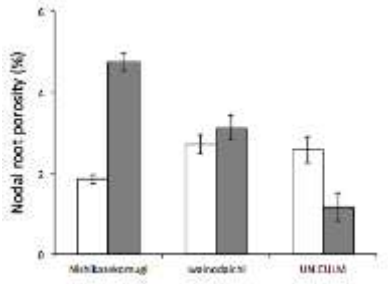
Results and Discussion

In this study, we evaluated the roles of root system development and function in the growth and yield of wheat plants that were exposed to waterlogged condition at jointing stage. Grain yield of Nishikazekomugi in WL was highest (64% of control), followed by Iwainodaichi (38%), and UNICULM (4%) (Figure 2). Nodal root porosity of Nishikazekomugi in WL was 284% of control, while that of Iwainodaichi and UNUCULM was 113% and 44%, respectively (Figure 3). Total root length of Nishikazekomugi in WL was 104% of control, while that of Iwainodaichi and UNICULM was 70 and 38%, respectively (Figure 4). These results showed that the cultivar with high porosity in response to exposure to WL tended to have greater total root length. Specifically, increase in nodal root porosity of Nishikazekomugi and Iwainodaichi contributed to the significant increase in total root length. Higher root porosity indicates greater root aerenchyma formation in response to waterlogging stress.



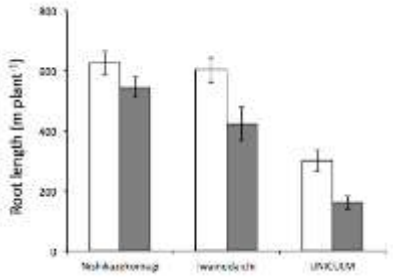
Data are means of 6 replicates. Vertical bar show standard errors (n=6). □ and ■ represents control and waterlogging conditions, respectively.

Figure 2. The effect of waterlogging stress on grain yield under rainfed upland (control) and waterlogged conditions.



Data are means of 60 replicates. Vertical bar show standard errors (n=60). □ and ■ represents control and waterlogging conditions, respectively.

Figure 3. The effect of waterlogging stress on nodal root porosity under rainfed upland (control) and waterlogged conditions.



Data are means of 6 replicates. Vertical bar show standard errors (n=6). □ and ■ represents control and waterlogging conditions, respectively.

Figure 4. The effect of waterlogging stress on root length under rainfed upland (control) and waterlogged conditions.

We also examined the effect of waterlogging stress on leaf water potential, stomatal conductance and photosynthetic rate. Leaf water potential of Nishikazekomugi maintained to the level of control for 16 days after waterlogging treatment onset, for 11 days in Iwainodaichi and for 4 days in UNICULM. Stomatal conductance of Nishikazekomugi maintained to the level of control for 16 days, for 16 days in Iwainodaichi and for 11 days in UNICULM. Likewise, photosynthetic rate of Nishikazekomugi maintained to the level of control for 16 days, for 11 days in Iwainodaichi and for 11 days in UNICULM. As stated above, the cultivars of greater root system development (greater total root length in WL) further showed the ability to maintain high leaf water potential and stomatal conductance, which maintained higher photosynthetic rate in WL.

These results indicated that the tolerant cultivars have higher ability of aerenchyma formation, which promotes root system development, and probably root hydraulic conductance, both of which, through high ability to provide water to shoot, eventually contributed to the increase in shoot dry matter and grain yield under waterlogged conditions.

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Growth and Physiological Responses of Sago Palm against Aluminum Stress in Acidic Conditions

Ornprapa Anugoolprasert[a], Hiroshi Ehara[a,*], Shina kinoshita[a], Hitoshi Naito[b], Somchai Chakhatrakan[c]

[a]*Graduate School of Bioresources, Mie University, Mie, Japan*
[b]*College of Life Science, Kurashiki University of Science and the Arts, Okayama, Japan*
[c]*Department of Agricultural and Technology, Thammasat University, Pathumthani, Thailand*
**Corresponding author: Graduate School of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan.*
Tel.: +81 59 231 9495; fax: +81 59 231 9495.
ehara@bio.mie-u.ac.jp

Abstract

Sago palm (*Metroxylon sagu* Rottb.) grows in natural peat swamps, which are poorly drained and has high acidity and generally contain highly exchangeable Al. It is, therefore, considered to be acid- and Al-resistant. In this study, the growth, physiological characteristics and nutrient concentrations in the plant tissues of sago palm grown under a hydroponic system were investigated for 4.5 months. When sago palm seedlings were cultured at pH 5.7, pH 4.5 and pH 3.6, the leaf morphogenesis, nutrient uptake and dry matter production were maintained regardless of a small decrease in the photosynthetic rate through the decrease of stomatal conductance. In the case in which seedlings were grown at pH 3.6 with different levels of $AlCl_3 \cdot 6H_2O$ corresponding to 0, 10, 20, 100 and 200 ppm Al, the plant length and dry matter production increased with a mild Al concentration in the growth media, such as 10 ppm Al. This result was attributed to the increase in the P and N uptake. In contrast, all the growth parameters significantly decreased under the 200 ppm Al treatment. The critical value to inhibit the growth of sago palm was considered to be around 200 ppm Al in the growth media. In addition, sago palm maintained a low Al^{3+} concentration in all of the plant parts. Therefore, it could be concluded that sago palm has high resistance to Al with mechanical restriction of the excess Al based on the Al exclusion ability under the acid condition.

Keywords: acid resistance, aluminum resistance, dry matter growth, photosynthetic rate, stomatal conductance

Introduction

Competition between biofuel production and food production has occurred in recent years in the context of the current social background regarding the exhaustion of fossil energy and the growing world population. Various plants are receiving attention as sustainable energy resources for the production of bioethanol and biodiesel. However, worldwide arable lands are limited. Thus, the development and/or improvement of new plant resources and their utilization are needed as a strategy to secure a sufficient amount of biomass for producing foods and biofuel sources (Ehara, 2009).

Sago palm (*Metroxylon sagu* Rottb.) stores large quantities of starch in its trunk. The total starch storage in one trunk is approximately 300 kg dry weight (Ehara, 2005). As a staple food, sago palm continues to be important in parts of Southeast Asia and in areas inhabited by the Melanesian people (Ehara *et al*., 2000). The carbohydrate or starch can be further processed into various basic raw materials for human and animal consumption as well as an industrial energy source, such as ethanol. In addition, sago palm grows in swampy, alluvial and peaty soils where almost no other major crops can grow without drainage or soil improvement (Sato *et al*., 1979; Jong & Flach, 1995). Nevertheless, the deep peat soils in swampy areas are usually characterized by low pH values, a deficiency in mineral elements and a high rate of exchangeable Al (Sato *et al*., 1979). Some former field studies on the growth of sago palm reported that sago palm grew under acid

conditions (Purwanto *et al*., 2002; Osaki *et al*., 2003). According to Foy & Fleming (1978), there was a possitive correlation between Al-resistant plants in nutrient solution and resistance to low pH conditions. It is, therefore, assumed that sago palm is resistant to acidic pH and Al. However, few studies have compared the growth characteristics of sago palm at widely different pH levels as well as the Al-induced changes on sago palm growth. Thus, the objective of the present study was to compare the physiological features and growth characteristics of young seedlings of sago palm at different levels of pH and Al concentration under low pH condition in a hydroponic system to elucidate the acid- and Al-resistance.

Materials and Methods

Plant materials and treatments

Sago palm fruits were collected in the swampy areas of Rattapum, Songkhla, Thailand, on 1 August, 2006. Fertilized and well-developed fruits were selected and treated physically to remove seed coat tissues. The cleaned seeds were placed in a plastic tray filled with tap water and then put them in a dark temperature-controlled room at 30ºC in Thammasat University, Thailand. The germinated seeds were brought to Mie University, Japan and each of them was transplanted to a 1/5000a Wagner pot filled with vermiculite and Kimura B culture solution containing (μM) 36.5 $(NH_4)_2SO_4$, 9.1 K_2SO_4, 54.7 $MgSO_4$, 18.3 KNO_3, 36.5 Ca $(NO_3)_2$, 18.2 KH_2PO_4 and 3.9 FeO_3 (Baba & Takahashi, 1958). The initial pH of the culture solution was adjusted to 5.5 using 1.0N HCl before irrigation into pots. The pots were placed in a greenhouse under natural sunlight and maintained at over 15ºC, even at night, at Mie University. Daily additions were made to the culture solution, according to the amount of solution consumed, and the culture solution was renewed twice weekly.

In Experiment 1, three seedlings at the 7^{th} leaf stage, with the mean plant length of all plant material at 39 cm, were selected and treated with Kimura B culture solution at three different levels of pH, 5.7, 4.5 and 3.6. In Experiment 2, three seedlings of the same size as those in Experiment 1 were treated with Kimura B culture solution without Al (referred to as control hereafter) or containing different levels of $AlCl_3 \cdot 6H_2O$ corresponding to 10, 20, 100 and 200 ppm Al at pH 3.6. The pH of the culture solution was adjusted with 1.0N HCl as required. The pots were placed in the same greenhouse under natural sunlight. An air pump was connected to the pots to provide air to the roots. The culture solutions in each pH and Al treatment were supplemented and renewed every other day from 23 May to 9 October, 2007.

Photosynthetic rate, transpiration rate, and stomatal conductance

The leaflets of the most active leaves or the 4^{th} leaf position from the top of the treated plants (18 weeks after the treatments) were selected for measuring the net photosynthetic rate, transpiration rate, and stomatal conductance using a potable photosynthetic meter (Analytical Development Co., Ltd., LCA-4, England) at saturation irradiance with incident photosynthetically active radiation (PAR) of 800-1,000 μmol $m^{-2}s^{-1}$.

Sampling and nutrient concentrations in plants

The treated plants were separated into three parts: leaflets, petioles including rachis, and roots. The leaflet areas were measured using an automatic area meter (Hayashi-Denko AAM-9, Japan). The separated samples were dried in an oven at 80ºC for 72 h to measure the dry weight and then ground into powder in order to analyze the ion concentrations. The ground samples were reduced to ash in a furnace and extracted with 1.0N HNO_3, and the K^+, Ca^{2+} and Mg^{2+} concentrations were determined using a high-performance liquid chromatography (HPLC) method with a conductivity detector (Shimadzu CDD-6A, IC-C3, Japan). The concentration of P was evaluated by atomic absorption spectrophotometry. The total N concentration was determined by

the semi-micro Kjeldahl method, while the Al^{3+} concentration was determined calorimetrically by the aluminon method.

The statistical difference of the data was determined using NCSS 2001 (Number Cruncher Statistical Systems). The effects of treatments were determined by one-way ANOVA (analysis of variance), and the differences among the mean values of treatment were determined using the Tukey-Kramer test.

Results and Discussion

During the experiment, approximately 7 leaves emerged in each pH treatment. The number of dead leaves during the experiment was 4, 4 and 3 at pH 5.7, 4.5 and 3.6, respectively. There were no significant differences in the number of emerged, dead and living green leaves among the three pH treatments, which means that the low pH conditions had no effect on new leaf emergence and senescence. Plant growth in weekly increment of length and total leaflet area did not change with the pH treatments. Although there was no significant difference in the total dry weight per plant among the pH treatments, the total dry weight in the pH 3.6 treatment was 8.7% smaller than that in the pH 5.7 treatment (Table 1). Similarly, the photosynthetic rate in the pH 3.6 treatment was 8.3% smaller than that in the pH 5.7 treatment. The difference in photosynthetic rates among the three pH treatments could be attributed to differences in the stomatal conductance; the stomatal conductance in the pH 3.6 treatment was 7.5% smaller than that in the pH 5.7 treatment.

Table 1. Effect of low pH on weekly increment of plant length, leaflet area per plant and dry matter weight (Experiment 1)

pH Treatment	Weekly increment of plant length (cm)	Leaflet area per plant (cm^2)	Dry matter weight per plant (g)			
			Leaflet	Petiole	Root	Whole
pH 5.7	2.0 a	2400.6 a	18.2 a	23.7 a	8.9 a	50.8 a
pH 4.5	2.0 a	2457.2 a	19.5 a	22.0 a	10.8 a	52.3 a
pH 3.6	2.0 a	2418.8 a	17.3 a	20.1 a	9.0 a	46.4 a

Means with the same letters in a given column are not significantly different at the 0.05 level by the Tukey-

Under the Al treatments, the total dry weight and total leaflet area in the 10 ppm Al treatment were slightly larger than those in the other treatments, while those in the 200 ppm Al treatment were significantly smaller than those in the control treatment (Table 2). Moreover, the roots of sago palm seedlings under the 200 ppm Al treatment were stunted, brownish and thick, and the root dry weight was 58% smaller than that in the control treatment, representing a significant difference (Table 2). Consequently, the critical value to inhibit the growth of sago palm was considered to be approximately 200 ppm Al in the growth media, which is much higher than the real concentrations in the natural soil conditions.

The concentrations of Al^{3+}, N, P, K^{+}, Ca^{2+} and Mg^{2+} in the leaflets, petioles, roots and whole plants under the Al treatments are shown in Table 3. The Al^{3+} concentration in all the plant parts increased with the rise of the Al concentrations. Moreover, the Al^{3+} concentration in the leaflet was lower than that in the petiole and tended to be significantly higher in the root than in the top parts (leaflets and petiole) in all the Al treatments. Our current results in sago palm strongly support the assumption that Al^{3+} has a high binding ability with cellular components of the root and usually shows slight translocation to the upper parts of the plant (Ma *et al*., 1997). The total N and P concentrations in the whole plants of the 10 ppm Al-treated plant were higher than those in the control and other Al-treated plants, which could lead to an increase in the growth of sago palm under a mild Al concentration in the growth media. These results indicated that Al was unlikely to have induced the P and N deficiency in plant tissues but the uptake of these nutrients was higher

under a lower Al condition, as such evidence was also found in rice (Fageria, 1985) and some native plants (Osaki *et al*., 1997). The K^+ concentration in all the plant parts was independent of the Al treatment. Whereas the Ca^{2+} and Mg^{2+} concentrations in whole plants decreased by the increase of the Al concentrations in the growth media, a significant difference was clearly observed in the 200 ppm Al-treated plants. One interesting feature of these results is that Al^{3+} inhibited Ca^{2+} and Mg^{2+} absorption more than K^+ absorption in all the plant parts under the higher Al treatment. The possible mechanism to explain the different effects on cations is that the Al^{3+} toxicity was ameliorated by cations in the following order, H^+ approximately = C^{3+} > C^{2+} > C^+, and the amelioration was due to their binding to or screening the negative charges on the plasma membrane (Kinraide *et al*.,1992).

Table 2. Effect of Al concentration on weekly increment of plant length, leaflet area per plant and dry matter weight (Experiment 2)

Al concentration (ppm)	Weekly increment of plant length (cm)	Leaflet area per plant (cm^2)	Dry matter weight per plant (g)			
			Leaflet	Petiole	Root	Whole
Control	2.0 a	2418.8 ab	17.3 ab	20.1 ab	9.0 ab	46.4 ab
10	2.0 a	3008.6 a	23.0 a	27.1 a	13.6 a	63.7 a
20	1.9 ab	2092.0 b	15.7 bc	16.3 b	6.7 b	38.7 bc
100	1.9 ab	2151.7 b	15.1 bc	18.0 b	6.6 b	39.7 bc
200	1.7 b	1153.4 c	8.0 c	11.2 c	3.8 c	22.9 c

Means followed by different letters within a column are significantly different at the 0.05 level by the Tukey-Kramer test (n=3).

According to Chenery (1948), thousands of the plant species are classified, according to their Al concentrations in plant tissues, as Al-accumulators (≥ 1,000 mg Al kg^{-1} dry weight) or Al excluders (< 1,000 mg Al kg^{-1} dry weight). Some plant species known as the Al accumulators may contain more than 10 times this Al level without any Al injury. However, most plants contain no more than 300 mg Al kg^{-1} dry weight. In the current study, the range of Al^{3+} concentrations in whole plants of sago palm was from 9.4 to 15.6 µmol g^{-1} (254 to 420 mg kg^{-1}) dry weight even under the 200 ppm Al treatment (Table 3). Considering the result of the Al^{3+} concentration in whole plant tissues, sago palm is considered to have high resistance to Al with mechanical restriction of the excess Al based on its Al exclusion ability under acid conditions.

Table 3. Effect of Al concentration on nutrient concentrations in the leaflets, petioles, roots and whole plants (Experiment 2)

Nutrient concentration	Plant part	Al concentration (ppm)				
		Control	10	20	100	200
Al^{3+} (μmol g^{-1})	Leaflet	8.7 cB	9.5 bcB	10.3 bB	10.2 bcB	14.3 aB
	Petiole	8.9 bB	10.7 abB	11.6 abB	13.1 abB	15.1 aB
	Root	12.1 cA	15.1 bcA	16.3 abA	17.7 abA	19.8 aA
	Whole	9.4 b	11.2 b	11.9 ab	12.7 ab	15.6 a
N (mg g^{-1})	Leaflet	20.9 aA	24.6 aA	23.0 aA	22.6 aA	21.0 aA
	Petiole	8.8 aB	9.1 aB	8.7 aB	7.7 aB	6.3 aB
	Root	10.8 aB	9.9 aB	9.8 aB	10.2 aB	11.3 aAB
	Whole	13.6 a	14.8 a	14.6 a	14.1 a	12.6 a
P (mg g^{-1})	Leaflet	1.8 aA	1.9 aA	1.8 aAB	1.7 aAB	1.6 aAB
	Petiole	2.2 aA	2.3 aA	2.2 aA	1.9 aA	1.4 bA
	Root	1.6 abA	1.8 aA	1.4 abB	1.1 bcB	0.9 cB
	Whole	1.9 ab	2.0 a	1.9 ab	1.7 ab	1.4 b
K^{+} (μmol g^{-1})	Leaflet	93.5 aB	92.6 aB	97.8 aB	93.4 aB	98.5 aB
	Petiole	219.6 bA	199.5 bA	215.4 bA	220.0 bA	250.7 aA
	Root	253.4 aA	209.3 bA	226.7 abA	250.7 aA	267.0 aA
	Whole	178.2 b	162.8 b	170.7 b	173.9 b	200.0 a
Ca^{2+} (μmol g^{-1})	Leaflet	42.4 aB	45.3 aB	42.6 aB	32.8 abB	25.5 bA
	Petiole	55.7 abA	64.4 aA	65.2 aA	47.7 bA	28.9 cA
	Root	28.8 abB	36.1 aB	36.8 aB	30.5 abB	21.7 bA
	Whole	45.7 ab	51.4 a	51.3 a	39.2 b	26.7 c
Mg^{2+} (μmol g^{-1})	Leaflet	41.1 aB	40.3 aB	40.9 aB	34.8 abC	29.0 bA
	Petiole	56.7 abAB	60.4 aA	63.0 aA	46.6 bB	29.5 cA
	Root	63.9 aA	65.1 aA	67.4 aA	66.8 aA	36.2 bA
	Whole	52.1 ab	54.1 a	55.1 a	45.5 b	30.5 c

Means followed by different letters within a row are significantly different at the 0.05 level by the Tukey-Kramer test (n=3). Lowercase letters indicate a comparison among the Al treatments in each part of a plant. Capital letters indicate a

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Transplanted Sucker Stem Growth in Sago Palm (*Metroxylon sagu* Rottb.) Before Trunk Formation

Satoshi Nakamura[a,*], Keita Nabeya[b], Mutsumi Akama[b], Teiji Nakamura[b], Youji Nitta[c], Manabu Watanabe[d], Yusuke Goto[b]

[a]*School of Food, Agricultural and Environmental Sciences, Miyagi University, Sendai, Miyagi, Japan*
[b]*Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan*
[c]*College of Agriculture, Ibaraki University, Ami, Ibaraki, Japan*
[d]*Field Science Center, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan*
**Corresponding author: School of Food Agricultural and Environmental Sciences, 2-2-1, Hatatate, Taihaku-ku, Sendai, Miyagi, 982-0215, Japan. Tel.: +81 22 245 1278; Fax: +81 22 245 1278. nakamurs@myu.ac.jp*

Abstract

Sago palm sucker stems transplanted in fields creep along the ground surface during a rosette stage, later forming a vertical trunk. Many suckers grow at the stem bottom before trunk formation. Thinning and pruning of suckers, known as sucker control, is extremely important to maintain an appropriate density of trunks and to produce much starch in sago plantations over time. Selection of suckers with consideration of the suckers' creeping growth is necessary to determine the appropriate spread of trunks in a field. Nevertheless, few reports describe the creeping growth of sucker stems. This study was undertaken to clarify the creeping growth of suckers transplanted in a field under sucker control before trunk formation. Suckers were transplanted in a field in Mukah, Sarawak, Malaysia on September 3, 2005. The plant density was 330 plants ha^{-1}. The growth of 12 suckers was monitored and the sucker stem lengths were measured every year during 2008–2011. The average stem lengths were 74.2 ± 4.7 cm and 155.3 ± 6.3 cm, respectively, at 1,201 days and 2,120 days after transplantation. The length increased linearly at 31.5 cm $year^{-1}$ ($Y = 0.0862X - 25.301$, $r = 0.993$, $P < 0.001$, where Y is the stem length and X is the days after transplanting). Each year, 10.5 leaves expanded. Although sucker growth differs among soil types, regions, and varieties, the information collected in this study is expected to be useful for predicting the trunk position.

Keywords: creeping growth, sago palm, stem, sucker, sucker control

Introduction

Sago palm sucker stems transplanted in fields creep along the ground surface during a rosette stage, later forming a vertical trunk. Many suckers grow at the stem bottom before trunk formation. These suckers derive carbohydrates from the mother palm, the transplanted sucker, until the photosynthetic apparatus is sufficiently developed and absorbs enough sunlight (Flack, 1977). Emergence and growth of many suckers at the stem bottom are expected to exacerbate competition between the suckers and their mother-palm for nutrition from soil because of their proximity. For that reason, the mother-palm growth might be suppressed. Reportedly, the rate of leaf emergence of non-controlled suckers is significantly lower than that of controlled suckers during the first five months of growth (Nakamura *et al*., 2009). Consequently, thinning and pruning of suckers, known as sucker control, is extremely important to promote the growth of mother palm, to maintain an appropriate density of trunks, and to maintain higher starch productivity in sago plantations over time. Selection of suckers with consideration of the suckers' creeping growth is therefore necessary to determine the appropriate spread of trunks in a field. Nevertheless, few reports describe the creeping growth of sucker stems. This study was undertaken to clarify the creeping growth of suckers that had been transplanted in a field, with sucker control applied before trunk formation.

Materials and Methods

Suckers were transplanted in a field in Mukah (N 02°56'31", E 112°18'26"), Sarawak, Malaysia on 3 September 2005. The plant density was 330 plants ha^{-1}; the plant mean area was 30.5 m^{-1}. The growth of 12 suckers was monitored: the sucker stem lengths were measured each year during 2008–2011. The transplanted sucker stem length was defined as the distance from its end to its shoot apex, which was covered with thick leaf sheaths. We were able to monitor their growth. Therefore, the shoot apex position was estimated from anatomical features of young sago palms sampled in the same field in 2008 (Figure1). The leaves of suckers were marked for monitoring using a marker pen. The number of expanded leaves was recorded. The number of their green leaves was also recorded each year.

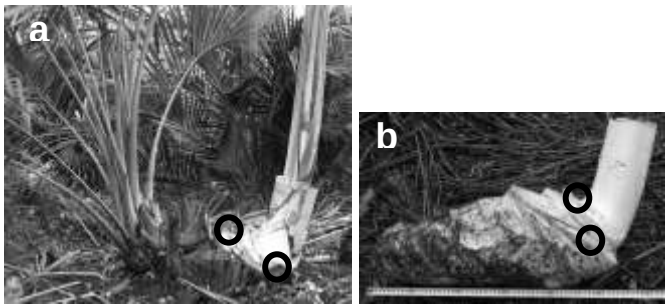

Figure 1. Mother palm sampled in the field in 2008 (a) and its stem, from which leaf sheaths were removed (b). Circles show the estimated position of the shoot apex.

Results and Discussion

All transplanted suckers grew well during 2005-2011 (Figure 2). The average stem lengths were 74.2 ± 4.7 cm and 155.3 ± 6.3 cm, respectively, at 1,201 days (17 December 2008) after transplanting (1,201 DAT) and 2,120 DAT (24 June 2011) (Figure 3), which suggests that the length increased linearly at 31.5 cm $year^{-1}$ ($Y = 0.0862X - 25.301$, $r = 0.993$, $P < 0.001$, where Y is the stem length and X is the DAT). The stem length was observed to increase gradually, probably increasing exponentially until the start of linearly incremental extension of the stem length (Goto *et al*., 2010). Because some suckers that start trunk formation were observed at 1,201 DAT, the rate of creeping growth of sucker is expected to decrease. The final stem length is reached soon thereafter.

Figure 2. Mother palm, of which the stem has been creeping, at 2,120 days (in 2011) after transplantation.

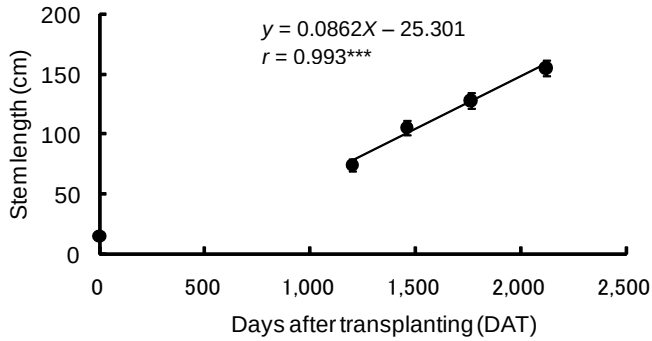

Figure 3. Stem length of the sucker after transplanting. Vertical bars represent standard errors.

The average numbers of living leaves of the suckers were 16.0 ± 0.6 and 11.2 ± 0.5, respectively, at 1,201 DAT and 2,120 DAT (Figure 4). The green leaf number tended to decrease, although the total and each area of leaves increased. It was observed that the size of leaves and the number of leaflets at 2,120 DAT was greater than those at 1,201 DAT. Results of leaf position data showed that 26.5 new leaves expanded from 1,201 DAT to 2,120 DAT, suggesting that 10.5 leaves expanded each year.

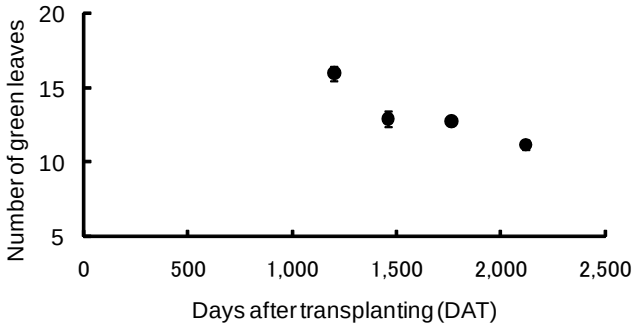

Figure 4. Number of green leaves of the sucker after transplanting. Vertical bars represent standard errors.

Conclusions

In conclusion, the rate of creeping growth of sucker stem in a field was specified after transplantation by monitoring the stem length. Although sucker growth is expected to differ among soil types, regions, and varieties, the information collected in this study will be useful for predicting the trunk position in a field.

Acknowledgements

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Secondary Aerenchyma Formation and Root Growth Response of Soybean (*Glycine max*) Seedlings under Flooded Conditions

Toshihiro Mochizuki [a,*], Satomi Sakazono [b], Sayuri Kajihara [c], Satoshi Shimamura [d]

[a] *Faculty of Agriculture, Kyushu University, Fukuoka, Japan*
[b] *Graduate School of Life Sciences, Tohoku University, Miyagi, Japan,*
[c] *School of Agriculture, Kyushu University,*
[d] *National Agricultural Research Center for Tohoku Region, Akita, Japan*
** Corresponding author: Experimental Farm, Kyushu University, Harumachi 111, Kasuya-machi, Kasuya-gun, Fukuoka 811-2307, Japan*
Tel.: +81 92 612 2865; fax: +81 92 612 2865.
mochizuki@farm.kyushu-u.ac.jp

Abstract

Most of wetland species can develop their roots into flooded soils because of the presence of longitudinal aerenchyma channels that facilitates oxygen diffusion from shoot to root tips. This tissue is called as primary aerenchyma because it is formed in fundamental tissues. It is also formed in rice root, consequently rice plants can grow well in paddy field. On the other hand, it is considered that most of mesophytes such as field crops cannot grow under flooded and excess moisture conditions because of their low ability to develop aerenchyma. However, we found that soybean plants could develop aerenchyma and grow well in flooding compared with other leguminous crops such as *Vigna* and *Phaseolus* species. This type of aerenchyma, which is consisted of white spongy tissue filled with gas space and is differentiated from secondary meristem (phellogen), is called assecondary aerenchyma. It plays a role in supplying oxygen from the aerial parts to the flooded roots and nodules. In our recent study, it was observed that there was a wide range of varietal differences on secondary aerenchyma formation and adventitious root development in soybean seedlings under flooding. Although the research for secondary aerenchyma in soybean plants is on the way, it may be able to breed soybean varieties with flooding tolerance.

Keywords: anaerobiosys, flooding, secondary aerenchyma, soybean, water logging

Introduction

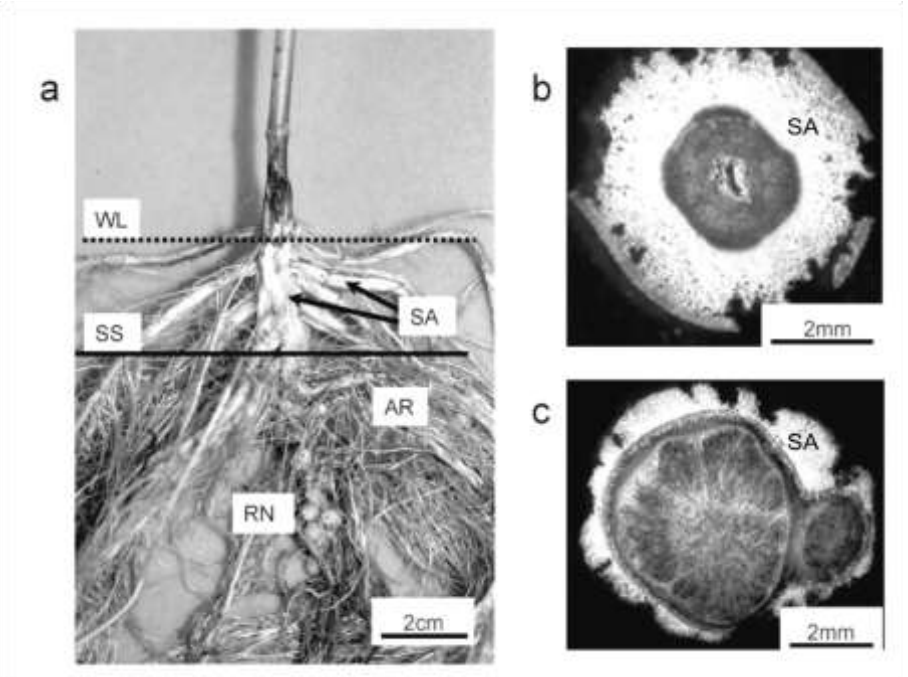
Flooding is a major problem in many areas of the world and most of mesophytes such as field crops are susceptible to the stress. Therefore, an understanding of the morphological mechanisms of flooding tolerance is important for developing flood-tolerant genotypes. Aerenchyma formation is one of the morphological changes that occurs in plants grown under flooded and hypoxic conditions and it is thought to enhance the internal diffusion of atmospheric and photosynthetic oxygen from the aerial parts to the flooded roots, allowing the roots to maintain aerobic respiration (Armstrong, 1979). The two types of aerenchyma are classified. One is cortical (lysigenous or schizogenous) aerenchyma formed in the roots of rice, maize, barley, wheat and some *Rumex* species. The other is white spongy tissue filled with gas spaces and is formed in the stem, hypocotyl, tap root, adventitious roots and root nodules of *Glycine soja*, *Sesbania rostrata, Lotus uliginosus* and *Viminaria juncea* grown under flooded conditions. It is differentiated from secondary meristem (phellogen) and is called secondary aerenchyma (Jackson and Armstrong, 1999). Secondary aerenchyma seems to play a role in supplying oxygen to roots and nodules. In

contrast to cortical aerenchyma, however, there is little information about the morphological and anatomical processes of secondary aerenchyma formation and its function.

Soybean is generally susceptible to flooding, and its growth and yield are negatively affected by the stress. Arikado (1954) suggested that soybean had low flooding tolerance because of its low ability to develop aerenchyma in the shoot and roots. However, some soybean genotypes can form lysigenous aerenchyma in the root cortex (Bacanamwo and Purcell, 1999) and secondary aerenchyma in the hypocotyl and tap root of seedlings grow under flooded conditions (Mochizuki *et al*., 2000). In this paper, we introduced our recent studies of the morphological and anatomical processes of secondary aerenchyma formation and its function as an oxygen pathway from the aerial parts to flooded roots, and we described here the relationship between aerenchyma formation and the flooding tolerance of soybean.

Secondary aerenchyma formation in flooded soybean

We investigated secondary aerenchyma formation in soybean seedlings grown under flooded conditions (Shimamura *et al*., 2003). As a result, within 3 weeks of flooding, large volumes of secondary aerenchyma developed in flooded hypocotyl, tap root, adventitious roots and nodules (Fig. 1). This tissue has large intercellular spaces and consists of living cells whose walls do not become suberized, whereas phellem has no intercellular spaces and consists of dead cells whose walls become suberized. It is differentiated from secondary meristem (phellogen) and is homologous with cork tissue (Fig. 2). Cells were exposed to the outside through lenticels in hypocotyl and roots where epidermis and cortex layers were collapsing. The outer part of the nodules was covered with a layer of secondary aerenchyma that was relatively thinner in the nodules than in the hypocotyl and roots. Nodules attached to the face of roots had pink infected tissues, which suggested the presence of leghemoglobin and the survival of nodules under flooded conditions.



a, root system; b, transverse section of an adventitious root; c, transverse section of a nodule. WL, water level; SS, soil surface; AR, adventitious roots; RN, root nodules; SA, secondary aerenchyma.

Figure 1. Root system and secondary aerenchyma development in soybean seedlings after 3 weeks of flooding.

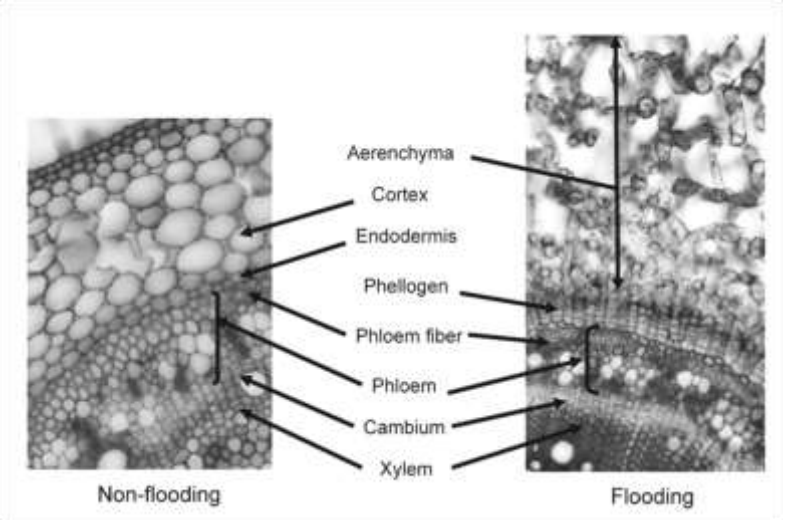
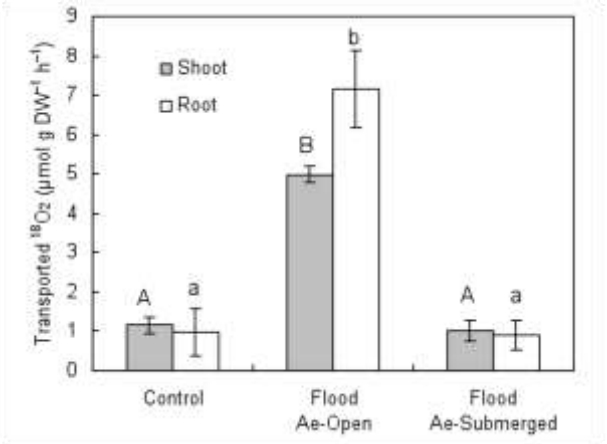


Figure 2. Cross sections of young hypocotyl in soybean.

Function of secondary aerenchyma in flooded soybean

Oxygen dynamics in aerenchymatous stems were investigated using Clark-type O_2 microelectrodes, and O_2 transport to roots was evaluated using stable-isotope $^{18}O_2$ as a tracer, for plants with shoots in air and roots in flooded sand or soil (Shimamura et al., 2010).

After introducing $^{18}O_2$ gas via the stem lenticels, significant $^{18}O_2$ enrichment in water extracted from roots after 3 h was confirmed, suggesting that transported O_2 sustained root respiration. In contrast, slight $^{18}O_2$ enrichment was detected 3 h after treatment of stems that lacked aerenchyma and lenticels. The results showed that hypertrophic lenticels in the lower stem of soybean, just above the water surface, are entry points for O_2, and these connect to aerenchyma and enable O_2 transport into roots in flooded soil.



Flood Ae-Open, aerenchyma and lenticels above the water; Flood Ae-Submerged, submerged aerenchyma and lenticeles. Values are the mean ± s.e. Means in the shoot followed by the same upper-case letter and means in the root followed by the same lower-case letter do not differ significantly (P<0.01, Tukey-Kramer's test).

Figure 3. Volume of $^{18}O_2$ transported from the stem to the roots with or without exposure of the aerenchyma to the $^{18}O_2$ gas in each treatment.

Interspecific difference in secondary aerenchyma formation of flooded leguminous crops

We investigated the secondary aerenchyma formation in hypocotyls just below the soil surface of young seedlings in wild soybean and six summer leguminous crops grown under upland and flooded conditions for 14 days (Mochizuki *et al*., 2000).

Under the upland conditions, secondary aerenchyma was scarcely observed in any species. Under the flooded conditions, however, there was an interspecific difference in the secondary aerenchyma formation. Secondary aerenchyma area per transverse section of hypocotyls under the flooded conditions was largest in soybean 'Asoaogari'; followed by soybean 'Akisengoku', wild soybean 'D5', cowpea 'Sanjakusasage', mung bean 'Bundomame', and mung bean 'Acc. 7703', and those in the other crops were less than 1 mm^2 (Table 1). Since a significant positive correlation was found between dry weight ratio (the ratio of dry weight of the aerial part under the flooded conditions to that under the upland conditions) and secondary aerenchyma area (r=0.738*), it is suggested that the ability of secondary aerenchyma formation is related to the flooding tolerance in leguminous crops.

Table 1. Secondary aerenchyma area of hypocotyl and dry weight ratio in summer leguminous crops

Scientific name	Variety	Secondary aerenchyma area (mm^2)	Ssecondary aerenchyma ratio (%)	Dry weight ratio (%)
Glycine soja Sieb. et Zucc.	D5	4.37±0.26b	173.01±15.88a	93±18.2a
G. max Merr.	Asoaogari	9.77±1.84a	146.19±35.76a	84±18.2ab
	Akisengoku	7.80±1.92a	71.14±16.40b	97±18.5a
Vigna angularis Ohwi et Ohashi	Acc.7703	1.52±0.92cd	59.45±40.50bc	52± 7.8bc
	Bundoumame	1.97±0.45cd	58.03± 2.16bc	51± 9.3bc
V. mungo Hepper	Sanjakusasage	2.63±1.09bc	27.06± 7.96cd	69±25.4abc
	Acc.3061	0.49±0.05cd	12.50± 0.99d	66± 6.1abc
V. radiata R. Wilczek	Acc.3083	0.31±0.21d	9.89± 7.50d	70± 6.5ab
	Hayateshouzu	0.48±0.18cd	10.73± 3.20d	53±12.6bc
V. sinensis Endle.	Erimoshouzu	0.49±0.27cd	8.50± 0.31d	49±17.0bc
Phaseolus vulgaris L.	Dover	0.85±0.14cd	7.36± 1.67d	35± 3.7c

Secondary aerenchyma ratio is the percentage of secondary aerenchyma area to steal area. Dry weight ratio is the percentage of dry weight of the aerial part under flooded conditions to that under upland conditions. Values followed by the same letter in each column are not significantly different at $P<0.05$ by Duncan's multiple test.

Effects of hypoxia on dry matter production and root development in soybean and wild soybean cultivars

Using 91 soybean and wild soybean cultivars, effects of hypoxia on dry matter production and root development were examined (Sakazono et al., 2011). Seedlings of eight days after sowing were grown in solution culture with (control) and without (hypoxia) O_2. Seven days after treatment, dry weight, root characters and hypocotyl diameter were measured. Root characters were measured with Win RHZO (Regent Instruments Inc., Quebec, Canada). In this experiment, we use the ratio of hypocotyl diameter (ratio of hypocotyl diameter in hypoxia to that of control) as an indicator of secondary aerenchyma development in hypoxia.

Effect of hypoxia was higher in root growth than shoot growth. Total root length, root surface area and root volume were decreased by hypoxia, whereas average root diameter and

hypocotyl diameter were increased. However, in every trait including hypocotyl diameter, there was wide variation among cultivars.

Table 2. Effects of hypoxia on dry matter production and root development in soybean and wild soybean cultivars

Trait	Treatment	Average (Max. - Min.)
Total dry weigt (mg)	Hypoxia	164.6 (262.8 - 19.5)
	Cont.	188.9 (359.3 - 19.8)
	Ratio (hypoxia / cont.)	0.89 (1.08 - 0.65)
Root dry weigt (mg)	Hypoxia	65.6 (101.1 - 8.5)
	Cont.	93.3 (162.7 - 9.8)
	Ratio (hypoxia / cont.)	0.73 (1.02 - 0.55)
Soot dry weigt (mg)	Hypoxia	99.1 (185.7 - 11.0)
	Cont.	95.8 (196.6 - 10.0)
	Ratio (hypoxia / cont.)	1.05 (1.29 - 0.81)
Total root length (cm)	Hypoxia	159.2 (405.6 - 58.2)
	Cont.	477.7 (916.1 - 71.5)
	Ratio (hypoxia / cont.)	0.37 (0.87 - 0.16)
Root surface area (cm^2)	Hypoxia	31.0 (56.9 - 7.3)
	Cont.	79.7 (136.8 - 9.2)
	Ratio (hypoxia / cont.)	0.43 (0.88 - 0.23)
Root volume (cm^3)	Hypoxia	0.50 (0.87 - 0.07)
	Cont.	1.08 (1.71 - 0.10)
	Ratio (hypoxia / cont.)	0.46 (0.93 - 0.30)
Average root diameter (mm)	Hypoxia	0.66 (1.62 - 0.39)
	Cont.	0.53 (0.69 - 0.38)
	Ratio (hypoxia / cont.)	1.21 (1.69 - 0.96)
Hypocotyle diameter (mm)	Hypoxia	3.69 (4.85 - 1.30)
	Cont.	2.45 (3.35 - 1.06)
	Ratio (hypoxia / cont.)	1.51 (2.01 - 1.13)

Conclusions

It is clearly that the secondary aerenchyma formed in soybean plays a role in supplying oxygen from aerial parts to roots and nodules, and there are interspecific and intraspecific differences in the secondary aerenchyma formation. Although the research for secondary aerenchyma in soybean plants is on the way, it may be able to breed soybean varieties with flooding tolerance.

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Agronomical Performances of Soybean Cultivated under Saturated Soil Culture on Tidal Swamps

M. Ghulamahdi[a], M. Melati[a], S. A. Aziz[a], A. Junaedi[a] , Sahuri[a], Y. Puspitasari[a], D. Sagala[b]

[a]*Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
[b]*Department of Agronomy, Faculty of Agriculture, Prof Azairin University, Bengkulu, Indonesia*
** Corresponding author: Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Kampus IPB, Dramaga, Bogor, Indonesia 16680*
Tel/fax : +62-251-8629353
mghulamahdi@yahoo.com

Abstract

Saturated soil culture (SSC) is a cultivation technology mantaining water depth constanly to make soil layer in the saturated condition. This paper resumes of two experiments to evaluate the effect of water depth and bed width, and leaching time and varieties of soybean cultivated under SSC on tidal swamp. The research was conducted at Banyu Urip, Banyuasin, South Sumatra from April to August 2010. In the first experiment, water depth in the furrow irrigation as main-plot consisted of 10 and 20 cm under soil surface (USS) and bed widths as sub-plot consisted of 2, 4, 6 and 8 m. The results showed that the highest grain yield was obtained on 20 cm USS water depth and bed width 2 m (4.15 ton/ha), it was significantly different from those at bed width 4 m (2.59 ton/ha), 6 m (1.84 ton/ha) and 8 m (1.74 ton/ha). The grain yield on water depth 10 cm USS and bed width 2 m obtained was 3.43 ton/ha, it was significantly different from those at bed width 4 m (2.46 ton/ha), 6 m (1.75 ton/ha) and 8 m (1.68 ton/ha). In the second experiment, leaching time as main-plot consisted of without leaching, every 2, 4 and 6 weeks, and soybean variety as sub-plot consisted of Tanggamus, Slamet, Willis and Anjasmoro. The results showed that interaction between leaching time and varieties did not significally affect grain yield, varieties responded leaching time differently. The highest grain yield was obtained by Anjasmoro variety with leaching time every 2 weeks (4.06 ton/ha) but it was not significally different from those with leaching time every 4 weeks (3.99 ton/ha) and every 6 weeks (3.93 ton/ha). From these experiments, water depth 20 cm USS and bed width 2 m may be recommended for SSC soybean cultivation on tidal swamp, and presumably, leacing time every six weeks will gain more effective economically and technically.

Keywords: leaching time, saturated soil, tidal swamp, water depth, bed width

Introduction

One alternative system to develop soybean cultivation in Indonesia is to optimize the use of marginal land, and tidal swamp is one of the potential ecosystem for future soybean production. Indonesia has about 20 million ha tidal swamps, 9 millions ha out of them is appropriate for agriculture, 2 milions is suitable for soybean (Noor and Sabur, 2007). The major constrain of producing soybean in tidal swamp due to its high pyrite content. When pyrite is oxidized, soil pH decreases. Djayusman *et al.* (2001) reported that high pyrite content suppressed the productivity of soybean on tidal swamps to only about 800 kg/ha. SSC is a technology in cultivation giving water permanently, maintains and keeps its depth constantly (about 5 cm USS). This makes soil layer in saturated condition. In SSC, watering is started from the beginning of growth to maturity stage. By keeping the water-table constantly, soybean will be avoided from negative effect of inundation on soybean growth, because soybean will acclimatize and improve its growth (Troedson *et al*., 1985).

Soil water management can be applied to reduce pyrite content where the soil is in reductive condition and able to support soybean growth. SSC technology is one of soil water managements studied in highland and succeed to increase soybean production (Indradewa *et al.*,

2004; Ghulamahdi *et al.*, 2006). This offers the chance to reduce the pyrite, hence increase soybean production on tidal swamps. Adisarwanto (2001) suggested for soybean cultivation using bed width less than 2 m. The addition of bed width will reduce the use of labor in the preparing land to make the trench, but to consider the ability of water seeped from the trench into the middle of beds. Indradewa *et al*. (2002) reported that the inundation in the trench with 3-4 m wide bed was the ideal plot. There was no difference in the influence of bed width on the growth and yield of soybean. Response of soybean to saturated condition varied between varieties and the later-maturing soybean was better than the earlier one (CSIRO, 1983; Ghulamahdi *et al*., 1991; Ghulamahdi, 2008; Ghulamahdi and Nirmala, 2008). Many varieties of soybean have been studied in their response on acid soil. Alihamsyah and Ar-Riza (2006) found that Tanggamus, Wilis and Slamet were varieties that could adapt well on inland. Leaching of land can decrease negative effect of poisonous material (Fe, Al, Mn) to the soybean growth. The frequency of water drainage from the trench will effect to the content of poisonous material. The objectives of the research were : 1) to study the effect of water depth and bed width , and 2) to investigate the effect of leaching time and varieties to the growth and production of soybean under SSC on tidal swamps.

Materials and Methods

This research was conducted on tidal swamps land in Banyuurip Village of Tanjung Lago Sub District, Banyuasin District, South Sumatera, Indonesia from April to August 2010. In the first experiment, water depth in the furrow irrigation as main-plot consisted of 10 and 20 cm under soil surface (USS) and bed widths as sub-plot consisted of 2, 4, 6 and 8 m. This experiment used Tanggamus variety. In the second experiment, leaching time as main-plot consisted without leaching, every 2, 4 and 6 weeks, and soybean variety as sub-plot consisted of Tanggamus, Slamet, Willis and Anjasmoro. Each main plot was surrounded by furrow irrigation. Water was given at planting time and kept until the maturity stage and made plots in wet condition. Two weeks before planting, plots were applied with 2 ton dolomite/ha, 400 kg SP18/ha, and 100 kg KCl/ha. Soybeans were sprayed with 10 g Urea/l water at 2, 4, 6 weeks after planting to support acclimatization.

At planting date, seeds were inoculated with *Rhizobium sp* and treated with insecticide with active agent Carbosulphan 25.53%. Seeds were planted in 2 x 5 m plot size, 20 x 25 cm planting distance, 2 seeds per hole. The observed variables were : nodule, root, stalk, and leaves dry weight at 6 weeks after planting (WAP); plant height, number of branch, fill pod, and empty pod per plant, seed productivity (ton/ha), and 100-seed dry weight at harvest time.

Results and Discussion

In the first experiment, there was the influence of water depth on the leaf, stalk, root and nodule dry weight at 6 WAP. The leaf, stalk, root and nodule dry weight were significantly higher in the water depth 20 cm USS than that ini 10 cm USS (Table 1). This was predicted because the root growing zone in the water depth 20 cm USS wider than 10 cm USS, so it provied adequate for maximum root growth. According to Suwarto *et al.* (1994) water depth significantly influenced on the leaf, stalk, root and nodule dry weight.

Water depth affected the plant height, number of branch and fill pod per plant, but there was no influence of water depth on the number of empty pod and 100 seed weight. Plant height, number of branch and fill pod and productivity of soybean were significantly higher in the water depth 20 cm USS than those in 10 cm USS (Table 2).

Table 1. The effect of water depth on leaf, stalk, root, and nodule dry weight at 6 WAP

Water Depth (cm)	Leave (g)	Stalk (g)	Root (g)	Nodule (g)
10	3.52b	3.64b	0.73b	0.33b
20	4.51a	4,84a	0.99a	0.48a

Note: numbers followed by the same letter on the same column are not significantly different with Duncan multiple range test at 5%.

Table 2. The effect of water depth on plant height, number of branch and fill pod, and productivity at harvest time

Water Depth (cm)	Plant Height (cm)	Branch/Plant	Fill Pod/Plant	Productivity (ton/ha)
10	70.66b	4.22b	67.83b	2.33b
20	73.86a	4.55a	71.83a	2.58a

Note: numbers followed by the same letter on the same column are not significantly different with Duncan multiple range test at 5%.

There was the influence of bed width on the leaf, stalk, root and nodule dry weight. The leaf, stalk, root and nodule dry weight were significantly higher in the bed width 2 m than that with the other bed widths (Table 3). This was predicted because the water seepage from the ditch into the middle of bed highest distributed on the bed width 2 m.

Table 3. The effect of bed width on leaf, stalk, root, and nodule dry weight at 6 WAP

Variables	Bed Width 2 m	Bed Width 4 m	Bed Width 6m	Bed Width 8m
Leave dry weight (g)	4.57a	4.29ab	4.03ab	3.17b
Stalk dry weight (g)	5.27a	4.48ab	4.09ab	3.11b
Root dry weight (g)	1.09a	0.83ab	0.78ab	0.72b
Nodule dry weight (g)	0.58a	0.40b	0.34cb	0.29c

Note: numbers followed by the same letter on the same line are not significantly different with Duncan multiple range test at 5%.

At harvest time, there was the influence of bed width on the number of branch, fill pod per plant, seed productivity, and 100 seed weight, but there was no influence on the plant height and empty pod. Number of branch, fill pod, seed productivity, and 100 seed weight were significantly higher in bed width 2 m than those in the other bed widths (Table 4). Indradewa *et al.* (2002) concluded that there was no difference in the effect of bed width on soybean growth and yield.

Table 4. The effect of bed width on number of branch and fill pod, productivity and 100 seed weight at harvest time

Variables	Bed Width 2 m	Bed Width 4 m	Bed Width 6 m	Bed Width 8 m
Branch/plant	4.71a	4.41ab	4.33ab	4.05b
Fill Pod/plant	80.17a	73.50b	65.17c	60.17d
Productivity (tones / ha)	3.79a	2.52b	1.79c	1.71d
100 seeds weight (g)	11.89a	11.30ab	10.98b	10.96b

Note: numbers followed by the same letter on the same line are not significantly different with Duncan multiple range test at 5%.

There was the influence of water depth and bed width interaction on the leaf, stalk, root and nodule dry weight. The leaf, stalk, root and nodule dry weight were significantly higher in the water depth 20 cm USS with bed width 2 m than those in the other treatments (Table 5).

At harvest time there was the influence of water depth and bed width interaction on the the plant height, number of branch, fill pod, seed productivity and 100 seed weight of soybean. There was no interaction effect of water depth and bed width interaction on the number of empty pods. Plant height, number of branch and fill pod, seed productivity and 100 seed weight were significantly higher in the water depth 20 cm USS with bed width 2 m than those in the other treatments (Table 6).

Table 5. The effect of interaction of water depth and bed width on leaf, stalk, root, and nodule dry weight at 6 WAP

Variables	Water Depth (cm) x Bed Width (m)							
	10x2	10x4	10x6	10x8	20x2	20x4	20x6	20x8
Leaf dry weight (g)	3,22bc	3,68bc	4,14bc	3,90b	5,92a	4,91ab	3,92bc	3,29bc
Stalk dry weight (g)	3,58bc	3,79bc	4,16bc	3,06c	6.96a	5,17ab	4,02bc	3,19bc
Root dry weight (g)	0,69b	0,71b	0,84b	0,65b	1,50a	0,94b	0,72b	0,82b
Nodule dry weight (g)	0,34bc	0,44b	0,30cd	0,22d	0,82a	0,37bc	0,37bc	0,36bc

Note: numbers followed by the same letter on the same line are not significantly different with Duncan multiple range test at 5%.

Table 6. The effect of interaction of water depth and bed width on plant height, number of branch and fill pod, productivity, 100 seed weight at harvest time

Variables	Water Depth (cm) x Bed Width (m)							
	10x2	10x4	10x6	10x8	20x2	20x4	20x6	20x8
Plant Height	73,55ab	72,83ab	68,06b	67,65b	77,70a	77,23a	71,14a	70,23b
Branch/plant	4.30ab	4.31ab	4.25ab	3.90b	4.68a	4.48ab	4.41ab	4.18ab
Productivity (tonnes/ha)	3.43b	2.46d	1.75f	1.68g	4.15a	2.59c	1.84e	1.74f

Note: numbers followed by the same letter on the same line are not significantly different with Duncan multiple range test at 5%.

In the second experiment, the leaching time did not affect the variables growth, yield component, and yield of soybean dry, but that only affected the nodule dry weight. The leaching time every 2 weeks gave the nodule dry weight per plant significantly different with that at every 4 and 6 weeks, but did not different with without leaching (Table 7).

Table 7. The Effect of leaching time on leaf, stalk, root, and nodule dry weight at 6 WAP

	Without	Every 2 Weeks	Every 4 weeks	Every 6 weeks
Nodule dry weight (g)	0.18ab	0.19a	0.11b	0.13b

Note: numbers followed by the same letter on the same line are not significantly different with Duncan multiple range test at 5%.

The variety affected the root, stalk dry weight, and seed productivity, but did not affect the other variables (Table 8). Stalk and root dry weight of Slamet were highest than those of the other varieties. The highest of seed productivity was obtained on Anjasmoro (Table 8).

Table 8. The Effect of variety on leaf, stalk, root, and nodule dry weight at 6 WAP

Variables	Tanggamus	Slamet	Wilis	Anjasmoro
Stalk dry weight (g)	4.86ab	5.60a	4.07b	3.85b
Root dry weight (g)	0.77ab	0.87a	0.69ab	0.63b
Productivity (ton/ha)	2.73b	2.39b	2.48b	3.83a

Note: numbers followed by the same letter on the same line are not significantly different with Duncan multiple range test at 5%.

The leaching time and variety interaction did not affect the seed productivity (Table 9). The highest grain yield was obtained by Anjasmoro variety with leaching time every two weeks (4.06 ton/ha), but it was not significally different from those with leaching time every four weeks (3.99 ton/ha) and every six weeks (3.93 ton/ha). This suggested that the leaching time every 6 weeks gained more effective economically and technically. This productivity of Tanggamus in 2010 was lower than 2009 (Ghulamahdi, 2009). It was predicted that the Tanggamus was more responsive to the higher solar radiation than lower solar radiation. The dry climate was in 2009, and wet climate was in 2010. Based on visualization observation in the field the size of leaf of Tanggamus in dry

climate was wider than that in wet climate. Irwan (2006) stated that at high temperatures and low humidity, solar radiation stimulated the emergence of flower buds into flowers.

Table 9. The effect of interaction on leaching time and variety on the seed productivity (ton/ha)

Leaching Time	Tanggamus	Slamet	Willis	Anjasmoro
Without	2.31	2.16	2.44	3.36
Every 2 weeks	3.08	2.41	2.46	4.06
Every 4 weeks	2.80	2.62	2.71	3.99
Every 6 weeks	2.73	2.40	2.29	3.93

Note: numbers followed without letter are not significantly different with Duncan multiple range test at 5%.

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Agronomic Performance of F7 Large Seed Soybean Breeding Lines in Medium Plains

Darman M. Arsyad[a,*] Asadi[b]

[a] *Central Institute for Agricultural Technology Assessment and Development, Bogor, Indonesia*
[b] *Research Institute for Agricultural Biotechnology and Agricultural Genetic Resources, Bogor, Indonesia*
** Corresponding author: Central Institute for Agricultural Technology Assessment and Development, Bogor, Indonesia*
Tel: +62-251-8351277; Fax: +62-251-8350928
dmarsyad@gmail.com

Abstract

Hybridization between different genotypes aims to obtain descents (breeding lines) which inherits with the good characters of both parents. One of the soybean breeding program is directed to obtain new varieties with higher yield potential, wide adaptation, desirable agronomic traits and large seed. A total of 60 F7 soybean breeding lines (origin crosses of Tanggamus x Local Tegal , Sibayak x Local Tegal, and Sibayak x Argomulyo), selected in 2007, and four large seed standard varieties (Argomulyo, Burangrang, Anjasmoro, and Panderman) were evaluated in rice fields, Sukasono Village, Sukawening Subdistrict, Garut District, West Jawa, Indonesia (at 700 m above sea level) on the early dry season (February-May) 2008. A randomized block design with three replicates was used. Experimental plot size was 0.9 x 4.0 m, spacing of 45 x 10 cm, one plant per hill. Intensive techniques cultivation involves fertilizing with 75 kg urea, 200 kg SP36, and 150 kg KCl per ha, controlling of weeds, pests and diseases were carried out. Irrigation was applied when there was no rain. The results showed that there was variability of seed yield and agronomic traits among the breeding lines tested. Among the 60 F7 breeding lines, eight lines were significantly better yield than Anjasmoro (best standar variety), namely U-719-1-1, U-509-1-1, U-714-1-1, V-159-1-3, V-426-1-2, V-421-1-1, V-563-1-1, and V-570-1-2 with a yield capacity ranged from 2.5 to 2.7 t/ha, meanwhile Anjasmoro yielded at 2.3 t/ha. Breeding lines of U-719-1-1 and U-714-1-1 had a moderate seed size (12.0 to 12.5 g/100 seeds); V-159-1-3 and V-570-1-2 had a slightly large seed (13.6 g/100 seeds), and U-509-1-1, V-426-1-2, V-421-1-1 and V-563-1-1 had a large seed (14.6 to 15.6 g/100 seeds). Breeding line with the U and V code were derived from Tanggamus x Lokal Tegal and Sibayak x Local Tegal crosses, respectively. Plant height was suggested as a criterion of selection for high yield.

Keywords: soybean, high yield, large seed

Introduction

The availability of improved varieties of high yielding, early maturity or moderate, and good seed quality are needed for increasing the domestic soybean production. Today the preferences of users (farmers and craftsmen tempeh and tofu) were more likely to require large varieties of soybean seed. Craftsmen tempeh and tofu has long accustomed to using large seed soybean imports, so that preferences are now a lot towards the large seed. To meet user demand, then breeding programs to produce varieties of soybean large seed in major need of attention. A number of varieties of soybean large seed has been available as Argomulyo, Burangrang, Anjasmoro, and Grobogan (Hermanto *et al.,* 2009).

In an effort to produce new varieties of soybeans are superior to varieties already exist, a number of crossing (hybridization) between the genotypes having high yield potential, adaptation is quite broad, many pods and smaller seeds with the genotypes of the large seed, few pods, narrow adaptation and relatively low yield potential was created in 2004 (Arsyad *et al.* 2005). Arsyad and Asadi (2011) reported that amount of 4800 F4 lines originated from five single cross combination were planted in ricefield, Sukawening, Garut District, West Java on early dry season 2007. By using pedigree method of selection, 1311 F5 lines were selected and grown in similar site on late dry

season 2007. Amount of 540 F6 lines were selected and grown in similar site on early rainy season 2007/2008. Sixty-two F7 lines were selected and 42 lines among them had high yield and large seed size.

This study aims were to: (a) obtain F8 lines better than comparable variety of Anjasmoro, having the higher yield potential, good agronomic traits, and has a large seed size or slightly larger, and (b) investigate the behavior of the relationship between traits in soybean breeding lines.

Materials and Methods

A total of 60 F7 soybean breeding lines (origin crosses of Tanggamus x Local Tegal, Sibayak x Local Tegal, and Sibayak x Argomulyo) selected in 2007, and four large seed standard varieties (Argomulyo, Burangrang, Anjasmoro, and Panderman) were evaluated in rice fields, Sukasono Village, Sukawening Subdistrict, Garut District (700 m above sea level) on the early dry season (February-May) 2008. A randomized block design with three replicates was used. Experimental plot size was 0.9 x 4.0 m, spacing of 45 x 10 cm, one plant per hill. Intensive techniques cultivation involves fertilizing with 75 kg of urea, 200 kg of SP36, and 150 kg of KCl per ha, controlling of weeds, pests and diseases were carried out. Irrigation was applied, if there is no rain. Observations were made on days to maturity, plant height, number of branches, number of pods, seed yield, 100 seed weight. Analysis of variances was performed on the characters observed and followed by LSD (Gomez and Gomez 1984), and inter-character correlation and path analysis followed Singh dan Chaudhary (1979).

Result and Discussion

Analysis of variances showed that there were significant different among the breeding lines tested against agronomic performances such as yield, seed size (weight of 100 seeds), plant height and number of pods per plant (Table 1). The range of seed yield of the 60 lines tested ranged from 1.53 to 2.65 t/ha, whereas the yield of four check varieties ranged from 1.47 to 2.30 t/ha. Large seeds (based on 100 seed weight) of 60 lines tested ranged from 10.3 to 20.7 g, and four check varieties had 100 seeds weight from 14.7 to 19.4 g. The breeding lines selected were those with 100 seed weight over 14 g. Based on these criteria, 32 lines were selected. The best check variety (Anjasmoro) with the yield of 2.3 t/ha and 100 seed weight was 14.7 g, while three other check varieties gave lower yield (1.5 to 2.0 t/ha).

By using Anjasmoro as a comparison, the eight lines significantly better, namely U-719-1-1, U-509-1-1, U-714-1-1, V-159-1-3, V-426-1-2, V-421-1-1, V-563-1-1, and V-570-1-2. Two lines, namely U-719-1-1 and U-714-1-1 had a moderate seed size (12.0 to 12.5 g/100 seeds), two other lines of V-159-1-3 and V-570-1-2 had a slightly large seed size (13.6 g/100 seeds), and four other lines of the U-509-1-1, V-426-1-2, V 421-1-1 and V-563-1-1 had a large seed size (14.6 to 15.6 g/100 seeds). There were 27 other lines that have a large seed size (> 14 g/100 seeds), but the yield was lower or equal to Anjasmoro.

The results of the preliminary yield test of F7 lines showed that there were the best five lines derived from cross of Sibayak x Local Tegal and three lines derived from cross of Tanggamus x Tegal, while lines derived from cross of Sibayak x Argomulyo nothing was selected. All F7 lines tested in this study had a yellow seed color, and the seeds was classified as moderate, rather large and large. Genotypes (parents) who are used to form the lines tested in this study had a medium seed size (Sibayak), rather small (Tanggamus) and large (Local Tegal and Argomulyo).

Table 1. Seed yield, 100 seed weight, number of plant harvested, plant height, number of pods per plant, and days to maturity of F7 soybean breeding lines in Garut, early dry season 2008

No.	Breeding line	Seed yield (t/ha)	100 seed weight (g)	Number of plant harvested per 3.6 m^2	Plant height (cm)	Number of pods per plant	Days to maturity
1	U-788-1-1	1.91	12.5	42	66	43	84
2	U-121-1-1	1.76	12.9	45	59	53	84
3	U-719-1-1	2.46	12.0	50	67	55	85
4	U-562-2-2	1.53	12.4	39	52	56	84
5	U-534-3-1	2.10	15.1	38	66	49	85
6	U-511-1-1	1.93	12.7	47	74	51	85
7	U-79-2-2	1.84	13.6	42	62	46	88
8	U-79-2-3	2.02	14.2	47	55	46	89
9	U-509-1-1	2.49	15.6	48	65	58	89
10	U-508-3-1	2.00	15.0	48	69	45	90
11	U-601-1-1	1.70	13.6	47	68	41	90
12	U-622-1-1	2.24	13.3	48	73	49	87
13	U-622-1-2	2.21	13.7	46	56	46	89
14	U-464-1-1	2.34	12.5	54	64	59	88
15	U-76-2-1	2.07	12.3	52	58	47	91
16	U-221-1-1	2.22	12.3	45	58	42	92
17	U-142-1-1	2.25	13.2	53	66	49	85
18	U-505-1-1	2.27	14.2	44	67	52	90
19	U-805-1-1	2.37	14.2	46	69	50	90
20	U-714-1-1	2.50	12.5	55	63	56	86
21	U-553-1-1	2.20	12.6	47	63	46	86
22	U-512-1-1	2.11	14.5	43	54	44	92
23	U-675-1-1	2.25	13.3	51	63	46	90
24	U-542-1-1	2.12	14.9	43	58	38	83
25	U-568-1-1	1.69	13.5	40	52	40	89
26	U-788-1-1	2.09	10.8	39	61	63	93
27	V-4-1-2	2.32	14.9	48	66	48	91
28	V-92-1-2	2.16	16.0	40	70	44	92
29	V-129-1-1	1.80	14.3	38	75	40	92
30	V-129-1-2	2.07	16.9	47	77	30	91
31	V-129-1-3	2.05	14.6	46	70	39	87
32	V-158-2-1	2.09	13.7	49	68	45	86
33	V-159-1-3	2.52	13.6	51	78	49	86
34	V-160-1-1	2.04	13.6	39	68	58	86
35	V-180-1-2	2.30	15.1	56	81	46	91
36	V-180-1-3	1.94	15.0	48	69	39	91
37	V-273-2-3	1.75	15.2	39	76	45	89
38	V-284-2-2	2.33	14.5	52	81	37	89
39	V-284-2-3	2.36	14.4	48	77	53	89
40	V-296-1-1	1.94	15.2	45	67	45	93
41	V-296-1-3	1.94	15.2	38	74	53	93
42	V-215-1-1	2.01	13.5	47	73	40	89
43	V-342-1-1	2.25	13.8	48	94	51	89
44	V-426-1-2	2.50	14.6	51	82	52	89
45	V-421-1-1	2.65	15.2	46	86	47	88
46	V-421-1-2	2.39	14.5	53	71	44	93
47	V-424-1-3	1.87	15.1	43	65	47	91
48	V-468-1-2	2.04	14.3	47	66	48	91
49	V-503-1-1	1.59	14.6	38	55	45	93
50	V-563-1-1	2.63	15.3	46	72	40	89
51	V-570-1-2	2.61	13.6	49	71	47	92
52	V-579-1-1	2.13	13.0	44	71	51	91
53	V-933-2-1	1.80	14.6	41	77	44	88
54	V-933-2-2	1.99	20.7	39	69	36	86
55	V-1118-1-1	1.88	16.2	42	66	44	82
56	V-1118-1-3	1.95	16.8	47	72	39	94
57	W-104-2-1	1.79	12.6	44	62	56	92
58	W-38-1-1	2.06	14.6	53	83	46	93

59	W-38-1-2	2.04	12.7	51	74	42	95
60	W-106-1-3	2.37	10.3	58	79	58	89
61	Argomulyo	1.47	15.9	51	52	22	80
62	Burangrang	2.00	15.7	49	74	40	81
63	Anjasmoro	2.30	14.7	44	76	35	85
64	Panderman	1.60	19.4	38	59	38	95
	Breeding lines	**	**	**	**	**	-
	LSD .05	0.12	1.3	10	12	14	-
	CV (%)	13.1	6.8	13.9	10.9	19.2	-

Breeding lines of U code was from Tanggamus x Tegal cross; Breeding lines of V code was from Sibayak x Tegal cross; Breeding lines of W code was from Sibayak x Argomulyo cross

In the plant breeding needs to investigate the relationship (correlation) between characters. If the selection is done on a character, we need to observe how they affect other characters (Burton, 1983). Analysis of correlation between traits in this study showed that seed yield had the strongest positive correlation with the number of plants in both F7 populations (lines) (Table 2). The greater the number of plants the higher plants and the higher the seed yield. The pattern of relationships among characters in Tanggamus x Tegal population seemed to differ from the Sibayak x Tegal population.

The study also found that seed yield did not correlate with weight (large) seed, but Susanto *et al.* (2001) reported that seed yield was positively correlated with large seeds. This suggested that there were opportunities to obtain lines of high yielding and large seeds. The results also found that the bigger the seed the less number of pods. This indicates that it would be difficult to obtain large seed lines by the high number of pods. To get maximum yield for the lines of large seed, let the optimum environmental conditions, so that seed filling goes well.

Dynamic relationship between the characters can be decomposed into two components, namely direct and indirect effects (Singh and Chaudhary 1979). The pattern of direct effect between the two populations seem to differ (Tables 3 and 4). In the cross of Tanggamus x Tegal, a strong direct effect was shown by the number of pods and number of plants, while in the cross of Sibayak x Tegal indicated by plant height and number of pods. Direct effect of pod number and plant number in the cross of Tanggamus x Tegal poorly supported by the results of the other components (Table 3). Direct effect of plant height in crosses Sibayak x Tegal also poorly supported by the results of the other components, but direct effect of pod number somewhat weakened by 100 seed weight (Table 4).

Characters that can be used as selection criteria for yield is the yield component that has a positive correlation with the yield and have a large direct effect on yield, and yield components which have a large positive direct effect (although less correlated with the outcome) (Board *et al.* 1997). Based on this, it appears that plant height and pod number are considered as selection criteria in soybean. In the field work selection, the use of pod number and plant height were also more practical compared with other characters.

Table 2. Correlation between characters of F7 soybean breeding lines in Garut, early dry season 2008

Character	Seed yield	100 seed weight	Number of plants	Plant height	Number of pods	Days to maturity
Seed yield		0,090	0,574**	0,348	0,327	0,146
100 seed weight	-0,197		-0,125	0,066	-0,365	0,012
Number of plants	0,662**	-0,272		0,325	0,174	0,016
Plant height	0,484**	-0,119	0,387**		0,190	-0,148
Number of pods	0,192	-0,526**	-0,050	0,084		-0,021
Days to maturity	-0,095	-0,074	0,040	-0,133	-0,062	

Value above and below diagonal represented for Tanggamus x Tegal (N=26) and Sibayak x Tegal (N=30), respectively
** significant at P. 0.01

Table 3. Direct effect (in diagonal) and indirect effect of yield component on yield of 26 F7 soybean breeding lines of Tanggamus x Local Tegal in Garut, early dry season 2008

Character	Number of plants	Plant height	Number of pods	100 seed weight	Days to maturity	Total effect
Number of plants	**0.544**	-0.133	0.159	0.008	-0,003	0,575
Plant height	0.177	**-0,408**	0,173	-0.004	-0,026	-0,088
Number of pods	0.095	-0.078	**0,911**	0,022	-0,004	0,946
100 seed weight	-0.068	-0.027	-0.333	**-0,060**	-0,002	-0,490
Days to maturity	0,042	-0,299	0,094	**-**0,004	**-0,036**	-0,203

Tabel 4. Direct effect (in diagonal) and indirect effect of yield component on yield of 30 F7 soybean breeding lines of Sibayak x Local Tegal in Garut, early dry season 2008

Character	Number of plants	Plant height	Number of pods	100 seed weight	Days to maturity	Total effect
Number of plants	**-0,022**	0.724	0.012	-0.029	0,002	0,687
Plant height	-0,009	**1,870**	0,020	-0.013	-0,006	1,862
Number of pods	0.001	0.157	**0,238**	-0,057	-0,003	0,336
100 seed weight	0.060	-0.223	-0.125	**0,108**	-0,003	-0,183
Days to maturity	-0,001	-0,249	-0,015	**-**0,008	**0,045**	-0,228

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Organically Production of Soybean Supported by Fertilizers Residue under Saturated Soil Culture

Maya Melati [a,*], Kalimatul Jumro [a]

[a] *Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Indonesia*
** Corresponding author: Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University (IPB).*
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, Indonesia.
Tel.: +62 251 8629353; fax: +62 251 8629353.
maya_melati05@yahoo.com

Abstract

Organic farming system can use natural on-farm inputs that are normally available at the production site. Saturated Soil Culture (SSC) is a technology in cultivation that gives water permanently, maintains and keeps its depth constantly; this makes soil layer in saturated condition. SSC technology can be implemented in land with poor drainage or in cultivating soybean on rice field in the period between two rice plantings when the soils may still be in water saturated conditions. The experiment was conducted to study the response of two soybean varieties to the residues of different types of fertilizer in an organic farming system. The experiment was carried out at IPB experimental station, Bogor, Indonesia, in October 2010-February 2011. Split plot design was used with types of fertilizer as the main plot (chicken manure, *Centrosema pubescens* Benth, and *Tithonia diversifolia* Hemsl.) and soybean varieties as the sub plot (Anjasmoro and Wilis). To study the effectiveness of fertilizers residue, the current experiment applied 50% fertilizer rates (10 t chicken manure only/ha, 5 t chicken manure mixed with 2.1 t *C. pubescens*/ha, and 5 t chicken manure with 2.1 t *T. diversifolia*/ha) of those applied in the previous soybean planting (May-August 2010). The results showed that chicken manure yielded the highest seed weight and filled pod number per plant. However, seed yields per hectare were not affected by fertilizer types. The soybean plant with the application of chicken manure, *C. pubescens*, and *T. diversifolia* produced 2.37, 2.42, and 2.43 t seed/ha, respectively. Wilis had higher number of filled pod per plant than Anjasmoro, but the yields per hectare of both varieties were not different. Production of Anjasmoro and Wilis was 2.43 and 2.38 t/ha, respectively.

Keywords: Centrosema pubescens *Benth, chicken manure,* Glycine max *(L.) Merr., green manure,* Tithonia diversifolia *Hemsl*

Introduction

National demand on soybean is about 2.2 million tons per year, but only 35-40% of this demand can be supplied from the domestic production, and the government has to import about 1.3 t soybean/year. Low productivity, decreasing agricultural land area, and limited access of farmers to technology and funding, are some of the factors restricting the national production of soybeans.

Organic farming is an alternative to currently used methods of farming; its use might increase soybean production. Currently the marketing of produce from organic farms is targeted only at consumers who are interested in it because they view it as being healthier. However, organic farming systems could be useful to farmers who have limited access to production inputs (e.g. inorganic fertilizers and pesticides) and funds. Because of the presumed lower productivity of organic systems as opposed to conventional farming the ability of organic systems to support food security is questioned; nevertheless organic farming may be able to provide local food security. Farmers can use on-farm inputs normally available to them on the site of production.

Organic farming system had been used to produce vegetable soybean with various types of fertilizer, for example chicken manure, green manure of *Calopogonium mucunoides*, *Centrosema*

pubescens, and *Crotalaria juncea*, rock phosphate, charcoal and ash or rice hull (Barus, 2005; Melati and Andriyani, 2005; Sinaga, 2005; Kurniasih, 2006). Some of those organic fertilizers had resulted significant differences in plant performances, but not in production. The experiment had also been conducted to produce dry seed of soybean to investigate whether organic farming system supported plant production not only at less mature seed but also at fully mature stage. Kurniansyah (2010) found that chicken manure only or its combination with green manure produced dry seed of 1.16-1.48 t/ha on upland, while Ramadhani (2011) found that soybean yield was 1.83-1.94 t/ha on soil with saturated soil culture technology.

Saturated Soil Culture (SSC) is a technology in cultivation that gives water permanently, maintains and keeps its depth constantly (about 5 cm under soil surface); this makes soil layer in saturated condition. In saturated soil culture, watering is started from the beginning of plant growth to maturity stage (Hunter *et al.,* 1980). By keeping the water-table constantly, soybean will be avoided from negative effect of inundation on soybean growth, because soybean will acclimatize and improve its growth (Troedson *et al.*, 1983). Ghulamahdi (2007) found that under SSC, growth and production of soybean was improved related to the increase of ACC, ethylene, glucose content, and neck diameter of roots, and the increase of nodules' weight, nitrogenase activity, and nutrient uptakes. SSC technology can be implemented in land with poor drainage or in cultivating soybean on rice field in the period between two rice plantings where the soils may still be in water saturated conditions. Ghulamahdi *et al.* (2009) also showed that SSC can be implemented in cultivating soybean in tidal swamp area with the production of more than double of those in upland.

The availability of nutrient provided by organic fertilizers is not as quick as from inorganic fertilizers; this may result in available nutrient in the following cropping season. Melati *et a*l. (2008) showed that the residue of fertilizers supported the production of organic vegetable soybean on upland soil. The current experiment studied the possibilities of producing dry seed of soybean with the residue of fertilizers under SSC technology. Two types of soybean cultivar (they differ in seed sizes) were used to investigate their responses to the treatments.

Materials and Methods

A field study was conducted in October 2010-February 2011 at the Cikarawang Experiment Station of Bogor Agricultural University (IPB), in Bogor, Indonesia. The soil is a silty clay loam soil. The experiment was the second crop sequence. The experimental design was a randomized complete block with spilt plot arrangement and three replicates. Types of fertilizer included in the study were chicken manure only (10 t/ha), chicken manure combined with *Centrosema pubescens* (5 + 2.1 t/ha), and chicken manure combined with *Tithonia diversifolia* (5 + 2.1 t/ha) were considered as main plots, and assigned to an area of 4 x 4 m. Subplots were assigned within each main plot, each differing by soybean cultivar, i.e. Anjasmoro and Wilis. Subplots dimensions were five 4-m long rows spaced 0.4 m apart and 0.1 m apart within row.

Those fertilizer rates were half of rates in the first crop sequence to evaluate the effectiveness of fertilizers residue. In the first crop sequence, the rate of chicken manure was applied at the rate of 20 t/ha followed Sinaga (2005) and the rates of green manure biomass was determined based on a study of Kurniasih (2006). It was expected that a yield of about 10 t biomass/ha was obtained from 25 kg seed of *C. pubescens.* However, dry conditions during the growth period of *C. pubescens*. (December 2009-April 2010) resulted in the production of only 3.5 t *C. pubescens* biomass/ha. Although *Tithonia diversifolia* could be easily collected from a nearby area, it was also applied at the rate of 3.5 t/ha to make appropriate comparisons with the use of *C. pubescens.* Besides those fertilizers according to the treatments, all plots were added with 1 t rice hull charcoal and 1 t dolomite per hectare. Green manure (combined with chicken manure, rice hull charcoal and dolomite) was applied 4 weeks before soybean planting, while chicken manure (plus

rice hull charcoal and dolomite) was applied 2 weeks before soybean planting. All materials were applied in planting rows; they were then below the position of soybean seed.

SSC technique was conducted by providing water permanently since 4 weeks after planting (WAP), maintained and kept water depth constantly at about 5 cm under soil surface in 20-cm depth furrow. Ghulamahdi (2007) found that plants normally experience chlorosis in saturated soil, therefore, in the current experiment liquid manure (1 L liquid manure/10 L water) was added as foliar application to the plants at the 3rd, 5th, and 7th day after irrigation began.

Other cultural practices were seed treatment by using *Rhizobium* inoculants with the rate of 6.25 g/kg seed, and the planting of *Tagetes erecta* and *Cymbopogon nardus* near soybean plants to control plant pest and diseases. *Tagetes erecta* and *Cymbopogon nard*us had been used in producing organic vegetable soybean (Kusheryani and Aziz, 2006).

Analysis of variance was used to analyze the data and Duncan' Multiple Range Test (DMRT) was used to compare means value.

Results and Discussion

Plant characters as shown in vegetative phase and yield component in generative phase were not different among fertilizer types. Variables were only different in number of filled pod and seed yield per plant with the highest values were in plants with the application of chicken manure only. Since the number of harvested plant in plot with chicken manure only was lower than those with other fertilizers, seed yields per hectare were not different among treatments (Table 1).

Table 1. Plant characters and production with three types of fertilizer

Variables	F test	Fertilizers			Means
		Tithonia diversifolia	Chicken manure only	*Centrocema pubescens*	
Vegetative phase					
Plant height at 7 WAP (cm)	ns	88.05	85.85	84.99	86.30
Plant height at harvest/ 14 WAP (cm)	ns	94.09	91.28	90.46	91.94
Trifoliate leaf numbers/plant	ns	18.5	19.5	18.3	18.8
Pest intensity (%)	ns	28.9	30.8	29.4	29.7
Disease intensity (%)	ns	8.8	10.6	8.5	9.3
Dry weight of leaves (g/plant)	ns	5.92	6.04	7.21	6.39
Moisture content of leaves (%)	ns	64.9	64.5	67.2	65.5
Nitrogen content in leaves (%)	ns	3.3	3.2	3.2	3.2
Phosphorus content in leaves (%)	ns	0.5	0.5	0.5	0.5
Potassium content in leaves (%)	ns	2.9	2.8	2.9	2.9
N uptake of leaves (mg/plant)	ns	19.47	19.66	23.04	20.72
P uptake of leaves (mg/plant)	ns	3.17	3.01	3.70	3.29
K uptake of leaves (mg/plant)	ns	17.26	16.99	21.52	18.59
Generative phase					
Dry weight of shoot (g/plant)	ns	20.77	26.60	26.25	24.54
Numbers of filled pod/plant	*	93.0b	111.7a	99.3b	101.3
Numbers of harvested plant/4.56 m^2	ns	118.8	104.0	112.0	111.6
Seed weight (g/100 seeds)	ns	15.17	15.83	16.00	15.67
Seed yield (g/plant)	**	25.33b	32.65a	28.25ab	28.74
Seed yield (g/4.56 m^2)	ns	1109.67	1082.50	1101.17	1097.78
Seed yield (ton/ha)	ns	2.43	2.37	2.42	2.41

Note: ns = not significant; * and ** = significant at $p < 0.05$ and $p < 0.01$, respectively. Numbers followed by letters indicating different at $\alpha = 5\%$ with DMRT.

Seed yield per plant may be related to the soil nutrient content. After the 1st crop season, C-organic, N-total, available P and K were higher in plots with the application of chicken manure only compared to those with other fertilizer treatments. These conditions were also detected in the 2nd crop sequence before soybeans were planted (Table 2). The amount of nutrients in the soil with chicken manure application had supported the seed yield of single plant.

Table 2. Soil characteristics

Treatments		pH H_2O	Walkly & Black	Kjeldhal	Bray I	N NH_4Oac pH 7.0				
Fertilizers	Varieties		C-org	N-Total	P	K	Mg	Ca	Na	KTK
			..(%)..	..(%)..	(ppm)	..(me/100g)..				
After 1st crop sequence										
Chicken m.	Anjasmoro	6.37	2.20	0.20	25.43	0.89	2.83	6.37	0.74	16.56
Chicken m.	Wilis	6.63	2.04	0.20	17.23	0.85	2.82	7.98	0.54	15.87
Tithonia sp.	Anjasmoro	6.53	1.84	0.18	9.20	0.86	2.60	7.42	0.59	15.80
Tithonia sp.	Wilis	6.67	1.89	0.19	15.57	0.60	2.41	6.66	0.51	14.77
Centrosema	Anjasmoro	6.40	1.76	0.17	6.15	0.69	2.74	6.98	0.45	16.68
Centrosema	Wilis	6.40	1.68	0.17	5.55	0.52	2.24	6.39	0.41	15.86
After application of fertilizers in the 2nd crop sequence										
Chicken m.	Anjasmoro	6.30	2.63	0.22	33.50	n.a.	3.06	7.94	0.61	17.76
Chicken m.	Wilis	6.50	2.55	0.22	28.80		2.90	7.82	0.48	18.36
Tithonia sp.	Anjasmoro	6.40	1.60	0.15	8.50		3.57	8.72	0.47	21.45
Tithonia sp.	Wilis	6.60	1.68	0.15	12.70		3.26	8.32	0.64	18.37
Centrosema	Anjasmoro	6.60	2.00	0.19	25.00		3.78	8.46	0.80	18.16
Centrosema	Wilis	6.30	1.92	0.18	28.70		4.76	9.23	0.75	21.76
After 2nd crop sequence										
Chicken m.	Anjasmoro	7.40	2.79	0.26	31.20	1.42	3.40	8.38	1.16	19.13
Chicken m.	Wilis	7.50	2.39	0.22	20.60	1.19	3.00	8.19	1.09	17.95
Tithonia sp.	Anjasmoro	7.60	2.23	0.21	14.40	1.11	2.90	7.76	1.06	17.76
Tithonia sp.	Wilis	7.50	2.15	0.22	9.00	0.86	2.65	7.42	0.70	16.78
Centrosema	Anjasmoro	7.20	2.55	0.23	5.20	0.93	2.55	7.34	1.08	18.35
Centrosema	Wilis	7.40	2.55	0.25	16.10	1.18	2.95	8.66	1.07	18.73

Note: Soil samples were composite of 3 replicates from the same treatment, therefore they were not statistically analyzed. n.a. = valid data were not available

There are some differences in plant characteristics and yield components of the two varieties in this experiment. These were accordingly with the characteristics of each variety. Anjasmoro has less number but larger leaves than those of Wilis, therefore, the nutrient uptake was higher in Anjasmoro. Anjasmoro has less number of filled pod but it has larger seed size, this resulted in similar seed yield of both varieties (Table 3).

Table 3. Plant characteristics and production of two soybean varieties

Variables	F test	Varieties		Means
		Wilis	Anjasmoro	
Vegetative phase				
Plant height at 7 WAP (cm)	*	83.32b	89.29a	86.31
Plant height at harvest/ 14 WAP (cm)	ns	91.98	91.91	91.90
Trifoliate leaf numbers/plant	*	20.3a	17.2b	18.80
Pest intensity (%)	ns	28.9	30.6	29.70
Disease intensity (%)	ns	8.8	9.9	9.30
Dry weight of leaves (g/plant)	ns	5.39	7.39	6.39
Moisture content of leaves (%)	ns	65.5	65.6	65.50
Nitrogen content in leaves (%)	ns	3.2	3.3	3.20
Phosphorus content in leaves (%)	ns	0.5	0.5	0.50
Potassium content in leaves (%)	**	2.7b	3.1a	2.90
N uptake of leaves (mg/plant)	*	17.47b	23.97a	20.72
P uptake of leaves (mg/plant)	*	2.77b	3.82a	3.30
K uptake of leaves (mg/plant)	*	14.41b	22.76a	18.59
Dry weight of shoot (g/plant)	ns	24.77	24.31	24.54
Numbers of filled pod/plant	**	115.4a	87.2b	101.30
Numbers of harvested plant/4.56 m^2	**	122.8a	100.4b	111.60
Seed weight (g/100 seeds)	**	12.11b	19.22a	15.67
Seed yield (g/plant)	ns	28.00	29.49	28.75
Seed yield (g/4.56 m^2)	ns	1087.11	1108.44	1097.78
Seed yield (ton/ha)	ns	2.38	2.43	2.41

Note: ns = not significant; * and ** = significant at $p < 0.05$ and $p < 0.01$, respectively.

Conclusions

The experiment concluded that in the second crop sequence, with half rates of fertilizer, all types of fertilizer and both varieties could be used to produce similar seed yield per hectare of organic soybean. In the second crop sequence, there was indication that soil characteristics had been improved under organic farming system.

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Factors Causing the Soybean Yield Gaps between Japan and USA

Keisuke Katsura [a,*], Koki Homma [a], Yohei Kawasaki [a], Larry C. Purcell [b], Randall L. Nelson [c], Taiki Yoshihira [d], Tatsuhiko Shiraiwa [a]

[a] *Graduate School of Agriculture, Kyoto University, Japan*
[b] *Department of Crop, Soil and Environmental Sciences, University of Arkansas, AR, USA*
[c] *USDA-Agricultural Research Service, University of Illinois, IL, USA*
[d] *Faculty of Dairy Science, Rakuno Gakuen University, Japan*
** Corresponding author: Experimental Farm, Kyoto University, 12-1, Hatchonawate, Takatsuki, Osaka, 569-0096, Japan.*
Tel.: +81 72 685 0134; fax: +81 72 683 1532.
kkatsura@kais.kyoto-u.ac.jp

Abstract

The average soybean yield in Japan is stagnated around 1.7 t ha^{-1}, which is quite low level compared to that in USA (around 2.7 t ha^{-1}). The objective of this study was to reveal the factors causing this yield gap between Japan and USA. To examine the variety effect on the yield gap, we conducted yield test at Osaka (central Japan, 34°51'N), Hokkaido (northern Japan, 43°03'N), Feyetteville, AR (south-central USA, 36°03'N), and Champaign, IL (mid-western USA, 40°06'N), in 2009 using 4–6 Japanese and 5–10 USA varieties. Averaged yield of USA varieties was 8–59 % higher than that of Japanese varieties in each experimental site, which means the yield potential of USA varieties was higher than that of Japanese varieties. To examine the environmental effect on the yield gap, we estimated the potential yield (Y_p) at Hokkaido, Shiga (central Japan, 35°16'N), Arkansas, AR (south-central USA, 34°30'N), and Champaign, IL, by simple simulation model, where we assumed that Y_p was determined by solar radiation, air temperature and growth duration. Estimated Y_p in USA (7.34 t ha^{-1} in Arkansas and 7.12 t ha^{-1} in Champaign, averaged over recent 30–year) was higher than that in Japan (5.84 t ha^{-1} in Shiga and 4.69 t ha^{-1} in Hokkaido), and this difference was mainly brought from the difference in solar radiation intensity. Ratio of the actual yield to the potential yield (Y_a/Y_p) was highest in Champaign (50.7 %, averaged over recent 5–year), followed by Hokkaido (44.6 %), Arkansas (38.9 %), and the lowest in Shiga (24.6 %). In addition, there were increasing tendencies in Y_a/Y_p in USA (0.23–0.50 % year^{-1}), while no such a tendency in Japan. The increasing tendencies in Y_a/Y_p in USA would be brought from the improvement of crop management in addition to breeding new varieties.

*Keywords: soybean (*Glycine max *(L.) Merrill), Yield*

Introduction

Improvement on soybean (*Glycine max* (L.) Merrill) production in Japan is strongly needed, because soybean self-sufficiency ratio in Japan is quite low (around 5%; MAFF, 2011a). Soybean yield in Japan has been stagnated around 1.7 t ha^{-1} (MAFF, 2011b) in recent years, while that in USA, the largest producer of soybean in the world, has increased steadily in the last few decades and reached around 2.7 t ha^{-1} (USDA-NASS, 2009). It is not clear, however, how varieties, environment and crop management have affected on this growing gap soybean yield between Japan and USA. The objective of this study is to clarify the factors causing soybean yield gaps between Japan and USA.

In the present study, we grew Japanese and USA commercial soybean varieties in northern and central Japan and mid-western and south-central USA, where are the major producers of soybean in Japan and USA. The objectives of this study were (1) to examine the yield potential (Y_p) of Japanese and USA varieties and study the varietal effect on the growing yield gap, (2) to estimate the changes of Y_p in northern and central part of Japan and mid-western and south-central USA using a simple simulation model, with the assumtion that Y_p was determined by solar radiation,

air temperature and growth duration, (3) to compare the Y_p in Japan and USA, and (4) to study the environmental and crop management effects on the growing yield gap between Japan and USA bycomparing the changes of Y_a/Y_p.

Materials and Methods

Eight Japanese and 14 USA commercial varieties (Suzukari, Enrei, Suzuyutaka, Tachinagaha, Sachiyutaka, Tamahomare, Toyomusume and Yuzuru for Japanese varieties and Athow, Omaha, Manokin, LD003309, 5002T, UA–4805, Osage, 5601T, Ozark, Hutcheson, Jack, Williams82, X34 and X88 for USA varieties) were used in the present study. 4–6 Japanese and 5–10 USA varieties were grown in Osaka (central Japan, 34°51'N), Hokkaido (northern Japan, 43°03'N), Feyetteville, AR (south-central USA, 36°03'N), and Champaign, IL (mid-western USA, 40°06'N) during the summer season in 2009. Crop managements, such as sowing date, planting density, fertilizer application rate, and so on, were based on the common practices in each experimental site. Experiments were consisted of 3-4 replications. Soybean yield was determined at maturity by harvesting more than 1m^2 area from each replication.

Based on the meteorological statistics and crop progress reports, changes of Y_p were estimated by the following simple simulation model.

$$Y_p = HI \cdot \sum_{n=1}^{d} \left[RUE \cdot \left\{ 1 - \exp\left(-k \cdot LAI \right) \cdot Rad \right\} \right]$$

where, HI is harvest index, RUE is radiation use efficiency, k is light extinction coefficient, LAI is leaf area index, and Rad is solar radiation. LAI was estimated by the logistic function of cumulative effective temperature with the base temperature being 8 °C. RUE in Japan was setup at 1.1 g MJ^{-1} for pre–R1+25–day period, and 0.77 g MJ^{-1} for post–R1+25–day period, based on the observed data under ideal conditions in Japan. RUE in USA was setup 10% lower than that in Japan, in consideration that RUE decreased under intense radiation environment. HI and k were assumed to be 0.5 and 0.6, respectively.

Potential yield (Y_p) was estimated for Shiga (central Japan, 35°16'N), Hokkaido, Arkansas, AR (south-central USA, 34°30), and Champaign, IL. Data from 1980 to 2009 for weather, crop calendar and statistics of soybean yield in each site were collected. Actual yield (Y_a) and Y_a/Y_p were analyzed for the field with and without irrigation system separately in Arkansas, because irrigation system has been rapidly spreading in south-central USA,

Results and Discussion

The seed yields observed in the variety tests are listed in Table 1. There was large variation in yield, which ranged from 2.12 to 5.91 t ha^{-1} depending on the varieties and sites (Table 1). Averaged yield of USA varieties (4.06–5.20 t ha^{-1}) was 8–59 % higher than that of Japanese varieties (2.63–3.80 t ha^{-1}) in each site, which means the yield potential of USA varieties was higher than that of Japanese varieties.

As for the meteorological statistics in the four assessed locations, there was not so big difference in effective growth period (98–105 days, from emergence to leaf yellowing) among the sites, while radiation intensity in USA (20.9–21.4 MJ m^{-2} d^{-1}) was more than 30 % larger than that in Japan (15.5–16.1 MJ m^{-2} d^{-1}) (Table 2). As a result, estimated Y_p in USA (7–8 t ha^{-1}) changed higher than that in Japan (5–6 t ha^{-1}), which showed the soybean potential yield in USA was higher than that in Japan, mainly because of solar radiation intensity (Figure 1). The values of Y_p on recent 30-year average in Shiga, Hokkaido, Arkansas, and Champaign were 5.84, 4.69, 7.34, and 7.12 t ha^{-1}, respectively.

Actual yield in Japan (1.56 and 2.17 t ha^{-1} on recent 30-year average, in Shiga and Hokkaido, respectively) was lower than that in USA (2.56, 1.72 and 3.04 t ha^{-1} on recent 30-year average, in Arkansas with irrigation system, Arkansas without irrigation system, and Champaign, respectively) (Figure 1). There were increasing tendencies in actual yield in all the sites, but the increasing rates were larger in USA (37.3, 20.2 and 35.1 kg ha^{-1} $year^{-1}$, in Arkansas with irrigation system, Arkansas without irrigation system, and Champaign, respectively) than those in Japan (0.9 and 12.5 kg ha^{-1} $year^{-1}$, in Shiga and Hokkaido, respectively). In addition, the increasing rates in Y_p was very small (14.5 and 35.1 kg ha^{-1} $year^{-1}$, in USA, which means that the changes in Y_p could not explain the recent soybean yield increase in USA.

Table 1. Yield (t ha^{-1}) of Japanese and USA soybean varieties grown under Osaka, Feyetteville, Champaign, and Hokkaido in 2009

	Osaka 34°51'N	**Hokkaido** 43°03'N	**Feyetteville** 36°03'N	**Champaign** 40°06'N
Japanese varieties				
Suzukari	3.64	4.51		2.85
Enrei	3.93		3.23	2.86
Suzuyutaka	4.13	3.09		2.68
Tachinagaha	3.10		3.44	
Sachiyutaka	4.16		3.60	
Tamahomare	3.44		3.48	
Toyomusume		3.28		
Yuzuru		4.32		2.12
Average	**3.73**	**3.80**	**3.44**	**2.63**
USA varieties				
Athow	5.07	4.87		3.88
Omaha	5.91	4.35		4.25
Manokin	5.23		4.70	
LD003309	5.06	3.13		4.66
5002T	4.69		5.73	
UA4805	5.15		4.44	
Osage	5.19		4.72	
5601T	5.50		5.32	
Ozark	5.19		4.23	
Hutcheson	5.03		4.85	
Jack		4.04		4.11
Williams82		4.20		3.95
X34 (LJ)		3.61		
X88 (LJ)		4.24		
Average	**5.20**	**4.06**	**4.86**	**4.17**

Table 2. Season of soybean cropping and its climate summary

	Latitude	Mean peak date*		Effective growth period** (days)	Total rainfall (mm)	Daily temperature		Daily solar radiation (MJ m^{-2} d^{-1})
		Planting	Harvesting			Max (°C)	Min (°C)	
Shiga, Jpn	35°16'	23 Jun	4 Nov	103	581	28.4	21.4	15.5
Hokkaido, Jpn	43°03'	23 May	9 Oct	102	333	23.9	16.7	16.1
Arkansas, USA	34°30'	24 May	13 Oct	105	261	32.4	21.0	20.9
Champaign, USA	40°06'	22 May	4 Oct	98	341	28.7	17.1	21.4

*, based on the crop progress report of Shiga and Hokkaido (1980-1997), East Illinois (1980-2005), and East Central Arkansas (1996-2008).

**, Emergence to leaf yellowing. The climate data were calculated for the effective growth period based on the meteorological statistics from 1980 to 2009 for the districts on Japan and USA respectively.

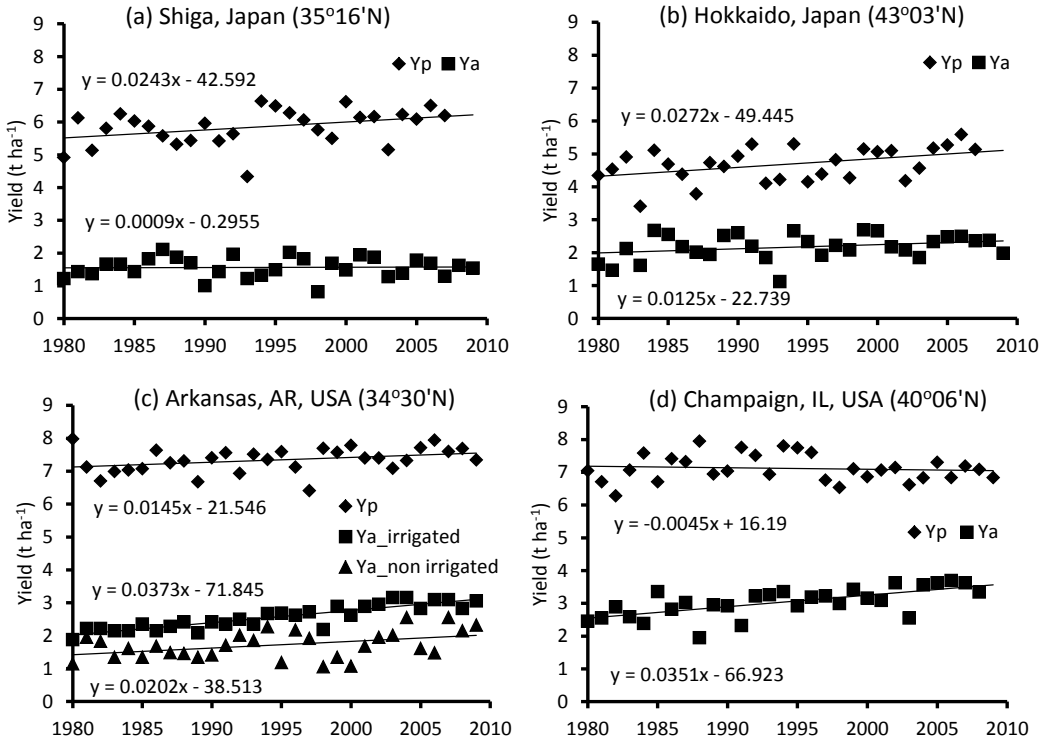

Figure 1. Changes of estimated potential yield (Y_p) and actual yield (Y_a) in (a) Shiga, (b) Hokkaido, (c) Arkansas, and (d) Champaign.

The Y_a/Y_p was the largest in Champaign (50.7 %, the recent 5 year average), followed by Hokkaido (44.6 %), Arkansas with irrigation system (38.9 %), Arkansas without irrigation system (26.5 %), and the lowest in Shiga (24.6 %) (Figure 2). As is the case in actual yield, there were increasing tendencies in Y_a/Y_p in USA (0.23–0.50 % year^{-1}), while no such tendency in Japan. We assumed that the increase in Y_a/Y_p in USA is brought from the improvement in crop management, such as increased adoption of irrigation system, introduction of early cultivation and raised bed planting, improvement in weed management, in addition to the developing and utilizing new high yielding varieties.

In the present study, we tried to reveal the factors causing the soybean yield gaps between Japan and USA focusing on environmental, varietal, and technological factors. Our results show that the greater amount of solar radiation is the major factor of higher yield in the USA and that the technological developments have caused the increase in yield gap between Japan and USA. There is still much room for improving soybean yield in Japan.

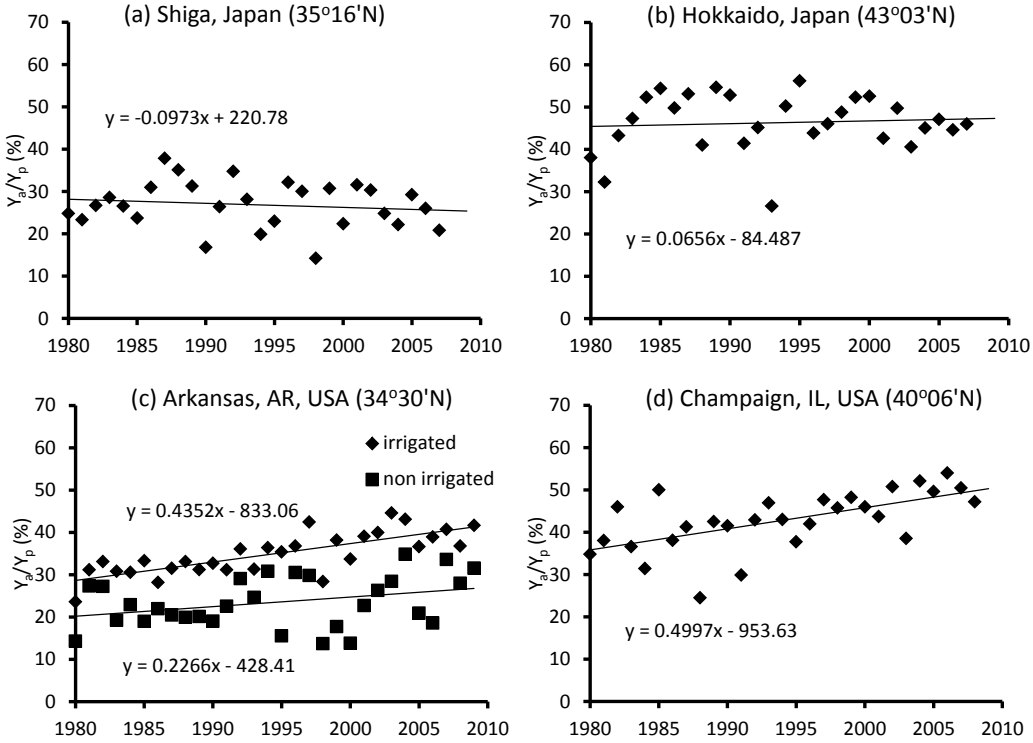

Figure 2. Changes of ratio of the actual yield to the estimated potential yield (Y_a/Y_p) in (a) Shiga, (b) Hokkaido, (c) Arkansas, and (d) Champaign.

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Photosynthetic Acclimation to Elevated CO_2 in Soybean

Mochamad Arief Soleh[a,b] *, Hai Yan[a], Tomohiro Konno[a], Makie Kokubun[a]
Ryoji Sameshima[c]

[a]*Graduate School of Agricultural Science, Tohoku University, Japan*
[b]*Faculty of Agriculture, Padjadjaran University, Indonesia*
[c]*National Agricultural Research Center for Tohoku Region, Japan*
moch_amir@yahoo.com

Abstract

Previous studies on plant responses to elevated atmospheric CO_2 concentration [CO_2] found the occurrence of elevated [CO_2]–induced photosynthetic acclimation. Soybean cultivar Enrei was grown in pots and was subjected to either ambient [CO_2] (ca. 380 µmol mol^{-1}) or elevated [CO_2] (ca. 580 µmol mol^{-1}) regime. The half of the plants were transferred to the other [CO_2] regime at flowering. Under elevated [CO_2], photosynthetic rate was markedly enhanced at the early growth stage, whereas it was decreased at the later growth stage on both transferred and untransferred plants, indicating that the acclimation occurred at the later growth stage of the plants regardless of the transfer. Electron transport rate (ETR) curve of the plants under elevated [CO_2] regime became plateau at about 300 µmol mol^{-1} intercellular [CO_2] at the later growth stage (35 days after transfer (DAT)), which was less than the plateaued value under ambient [CO_2] regime (320 µmol mol^{-1}), indicating that the acclimation of photosynthesis occurred due to decreased intercellular [CO_2] which might be restricted by the decrease in stomatal conductance, N or chlorophyll content in leaves. Moreover, the leaf N and chlorophyll content (SPAD reading) of the plants grown under elevated [CO_2] regime was lower than those of the plants grown under ambient [CO_2] regime at the later growth stage (80 days after sowing (DAS)/32 DAT). Thus the acclimation of photosynthesis of soybean appeared to be induced by decreases in stomatal conductance, N or chlorophyll content in leaves, although other factors such as starch accumulation in leaves at the later growth stage, which can restrict Rubisco activity in leaves, might be involved in the acclimation. Further research is needed to examine how other possible factors are involved in the acclimation of photosynthesis.

Keywords: elevated [CO_2]; photosynthesis.

Introduction

Increasing [CO_2] in the atmosphere will offset increased photorespiration by increasing photosynthesis, especially in plants with C_3 photosynthetic pathway. To examine environmental effects including the effect of increasing [CO_2] on soybean growth and development is very important, because plants develop and grow properly in proper conditions environmentally and genetically.

A number of previous studies showed that elevated [CO_2] affected photosynthetic behavior of crops such as soybean. Many previous studies showed positive responses of soybean to elevated [CO_2] (Rogers et al. 1997; Kimball et al., 2002; Pritchard and Amthor, 2005; Alagarswamy et al., 2006), but some other studies found down-regulation of photosynthesis under elevated [CO_2] (Sim et al. 1998; Sawada et al. 2001; Ainsworth et al. 2004; Kanemoto et al. 2009; Matsunami et al. 2009; Otera et al. 2011). The objective of the present study was to examine how the acclimation of photosynthesis occurs in soybean under elevated [CO_2].

Phenomenon of photosynthetic acclimation in plants can be ascribed to several factors. Several previous studies reported that acclimation of photosynthesis occurred for different reasons. Firstly, N limitation in leaves may cause photosynthetic acclimation (Kanemoto et al. 2009; Antal et al. 2010). Secondly, starch accumulation in leaves reduced Rubisco activity and induced photosynthetic acclimation (Sims et al. 1998; Sawada et al. 2001). Thirdly, balance in sink and

source affected photosynthetic acclimation (Rogers et al. 1998; Ainsworth et al. 2004). Fourthly, decrease of chlorophyll content or Rubisco activity in leaves may affect photosynthetic acclimation (Hotta et al. 1987; Sage et al. 1989). Finally, growth temperature induced photosynthetic acclimation in some C_3 species (Yamori et al. 2005).

Materials and Methods

A soybean cultivar Enrei (normally nodulating) was used in this study. Four seeds per pot (7 L) were sown on 6 June 2010 then thinned to one plant after emergence. Every pot was applied 6 g of compound fertilizer (N-P_2O_5-K_2O: 5-15-20) and 10 g of garden lime. The amount of each element per pot was N-0.3 g, P_2O_5-1.5 g, K_2O-1.2 g and SiO_2-2.8 g, Ca-0.4 g and Mg-0.4 g. The seeds were inoculated with *Bradyrhyzobium japonicum* strain. The soil used was low-humic Andosol type. The plants were grown in the temperature gradient chambers (Gradiotron) of National Agricultural Research Center for Tohoku Region, Morioka, Japan, during June to October in 2010. There were two levels of [CO_2] inside the chamber: ambient (380 μmol mol^{-1}) and elevated (580 μmol mol^{-1}). The half of plants were transferred into the different [CO_2] regime (from ambient to elevated (A-E) or from elevated to ambient (E-A)) at flowering (48 DAS (R1)). The [CO_2] and temperature around plant canopy were monitored every half hour by sensors which were installed above the plots.

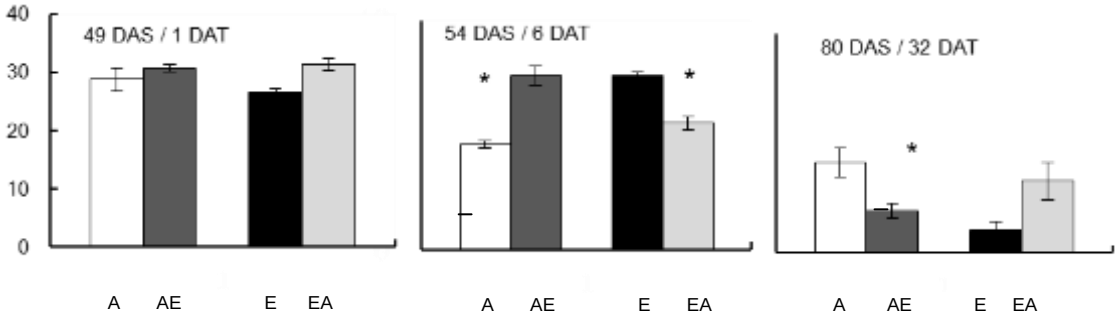
Measurement of photosynthesis was conducted at 2 DAT and 35 DAT. Chlorophyll fluorescence was determined by using portable photosynthesis system (LI-6400-40 with leaf chamber fluorometer, LI-cor Inc., NE, USA). The air flow to the chamber was controlled at 500 μmol s^{-1}. The measurements were conducted at saturated PPFD (1,500 μmol m^{-2} s^{-1}). The photosynthetic measurement was conducted at varying [CO_2] with a range of 100 – 1,250 μmol mol^{-1} (A-Ci curve). The values used for the calculation of ETR are as follows; maximal fluorescence (F*m*`), steady-state fluorescence (F*s*) and photosystem II efficiency (PhiPS2) with equation $\left[\frac{\mathrm{F\,m`}-\mathrm{F}s}{\mathrm{F\,m`}}\right]$. ETR was determined by the equation $\left[\frac{\mathrm{F\,m`}-\mathrm{F}s}{\mathrm{F\,m`}}\right].[f\,I\,\alpha_{leaf}]$,

where *f* is fraction of absorbed quanta that is used by PSII, and is typically assumed to be 0.5 for C_3 plants, *I* is incident PPFD (1,500 μmol m^{-2} s^{-1}), α_{leaf} is leaf absorptance which is assumed to be 0.85 (Genty et al. 1989). Measurements were conducted on the plants which were transferred (A-E, E-A) and untransferred (A-A, E-E). Two to three plants for each plot were used for the measurements.

Results and Discussion

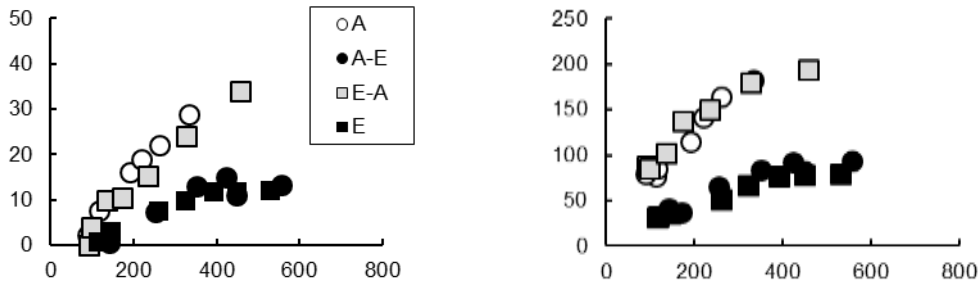
Under elevated [CO_2], photosynthetic rate was markedly enhanced at the early growth stage, whereas it was decreased at the later growth stage on both transferred and untransferred plants (Fig. 1), indicating that the acclimation occurred at the later growth stage of the plants regardless of the transfer. Electron transport rate (ETR) curve of the plants under elevated [CO_2] regime became plateau at about 300 μmol mol^{-1} intercellular [CO_2] at the later growth stage (35 days after transfer (DAT)), which was less than the plateaued value under ambient [CO_2] regime (320 μmol mol^{-1}) (Fig. 2), indicating that acclimation of photosynthesis occurred at about 300 μmol mol^{-1} intercellular [CO_2] probably due to decreased RuBP regeneration (Sharkey et al., 2007) which was affected by decrease in stomatal conductance (Fig. 3). Moreover, the leaf N and chlorophyll content (SPAD reading) of the plants grown under elevated [CO_2] regime was lower than those of

the plants grown under ambient [CO_2] regime at the later growth stage (80 days after sowing (DAS)/32 DAT) (data not shown).



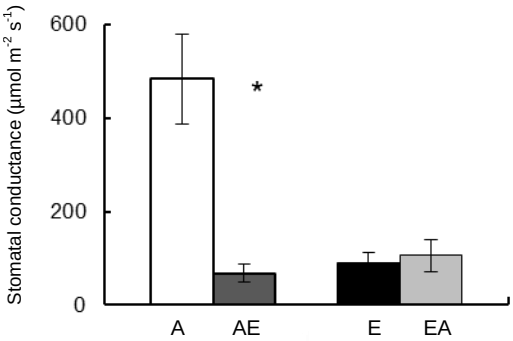
Measurements were conducted at saturated PPFD (1,500 µmol m^{-2} s^{-1}) under respective [CO_2] (A and E). Vertical bars indicate SE of three or five plants. *: significantly different at the 5% level between A and E [CO_2] regimes.

Figure 1. Photosynthetic rates of transferred (AE, EA) and untransferred (A, E) plants at the early growth stages (49, 54, and 80 DAS).



Fluorescence measurements were conducted at saturated PPFD (1,500 µmol m^{-2} s^{-1}).

Figure 2. Photosynthetic rate and electron transport rate at 2 DAT and at 35 DAT of transferred (A-E, E-A) and untransferred (A-A, E-E) plants.



Measurements were made in the morning at 80 DAS/32 DAT.

Figure 3. Effects of [CO_2] (A, E) on stomatal conductance of leaves.

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The Effect of Organic Materials and Decomposer on Soybean Growth and Production

Sandra Arifin Aziz[a*], Sri Ayu Dwi lestari[a]

[a] *Bogor Agricultural University,*
Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
** Corresponding author: Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor,*
Jl Meranti, Kampus Darmaga, Bogor, 16680, Indonesia
Tel.: +62 0251 8629353; fax: +62 0251 8629353
sandraaziz@yahoo.com

Abstract

Field experiment was set up in Cikarawang, Darmaga Bogor, Indonesia from December 2010 to April 2011. The objective of the study was to investigate the effect of chicken manure, rice straw, and green manure of *Tithonia diversifolia* with the application of decomposer, i.e. chicken manure liquid, chicken manure + *Tithonia diversifolia* liquids, and biofertilizer under organic farming system. A randomized block design was laid out with organic materials (chicken manure, rice straw and green manure of *Tithonia diversifolia*) as the first factor and decomposers (chicken manure liquid, chicken manure + *Tithonia diversifolia* liquid, and biofertilizer) as the second factor. The results showed that chicken manure gave the best effect on soybean growth and production. Soybean productivities from organic treatment chicken manure, green manure of *Tithonia diversifolia,* and rice straw were 1.00, 0.85, and 0.73 ton dry seed ha^{-1}, respectively. Biofertilizer gave better response on soybean growth and production components than chicken manure + *Tithonia diversifolia* liquid, chicken manure liquid and control, i.e. 089, 088, 0.82, and 0.98 ton dry seed ha^{-1}, respectively.

Keywords: organic soybean, decomposer, chicken manure, rice straw, Tithonia diversifolia *Hemsl*

Introduction

Increasing the inputs of nutrients has played a major role in increasing the supply of food to a continually growing world population. However, focusing attention on the most important nutrients, such as nitrogen (N), has in some cases led to nutrient imbalances, some excess applications especially of N, inefficient use and large losses to the environment with impacts on air and water quality, biodiversity and human health. In contrast, food exports from the developing to the developed world are depleting soils of nutrients in some countries. Better management of all essential nutrients is required that delivers sustainable agriculture and maintains the necessary increases in food production while minimizing waste, economic loss and environmental impacts. More extensive production systems typified by 'organic farming' may prove to be sustainable. However, for most of the developed world, and in the developing world where an ever-growing population demands more food, it will be essential to increase the efficiency of nutrient use in conventional systems (Gouldin *et al*., 2008).

The success of organic system in supporting national food security is still in doubt because the production of organic farming system is generally less than that of conventional farming. However, organic farming system may be able to support food security in local level because the continuity of plant production is possible. Farmers can use on-farm inputs that normally are available at production site and avoid the use of chemical fertilizer and pesticide which is relatively expensive and negative impact to the environment.

Animal and green manure can be used as organic fertilizer for production of organic vegetable soybean (Melati and Andriyani, 2005; Sinaga, 2005; Kurniasih, 2006). Chicken manure gave better result than sheep manure (Sinaga, 2005), while *Centrosema pubescens* was better

than *Calopogonium mucunoides* and *Crotalaria juncea* (Sinaga, 2005; Kurniasih, 2006). Chicken manure 10 ton ha^{-1} increased the vegetative growth and production in organic soybean (Melati and Andriyani, 2005).

In Indonesia, soybean is one of the crops used in the cropping pattern in the lowland. Arafah and Sirappa (2003) stated that rice straw should be incorporated to the soil after every harvest so it can be beneficial to the next crop in the cropping pattern. Indriani (2000) found that *Tithonia diversifolia* contains high nutrients, especially Nitrogen. This nitrogen can be useful in assisting decomposting bacteria in decomposition process.

One of the obstacles in organic agriculture is the decomposition process of the organic matter in the soil. Local microorganisms from various organic matters (fruits, plants, fish bones, dead animal and garbage) around us can be used as media for microorganism for decomposition process (Indriani, 2000). This effort can be used to optimize the growth and activity of the microorganism that decomposed the organic matter (Sutaryono dan Fauzi, 2007). Combination of biofertilizer and various organic matters influenced the physical and biological soil properties (Mezuan *et al.,* 2002). Bertham (2002) studied biofertilizer application that showed the increasing total pod number, filled pod number, and root nodule number in soybean. Further Hindratno (2006) found that decomposer application or chicken manure liquid as decomposer increased the plant height and fresh pod of vegetable soybean weight than without decomposer application.

This research was aimed to study the influence of organic matter, i.e. chicken manure, rice straw, and *Tithonia diversifolia* with decomposer, i.e. chicken manure liquid, chicken manure+*Tithonia diversifolia*, and biofertilizer (Bioextrim) to the organic soybean production.

Materials and Methods

The research was carried out at organic research station Cikarawang, Bogor, West Java, Indonesia, from November 2010 to April 2011. A randomized block design was laid out with organic materials (i.e. chicken manure, rice straw, and green manure of *Tithonia diversifolia*) as the first factor and decomposers (i.e. chicken manure liquid, chicken manure + *Tithonia diversifolia* liquid, and biofertilizer Bioextrim) as the second factor. Duncan's Multiple Range Test (DMRT) with α=5% and t-Dunnet test for comparison between treatments with control (rice straw without dekomposer) were used in this experiment.

Liming with 2 tonnes dolomit ha^{-1} and 2 ton ash ricehull ha^{-1} were given in row for soil conditioning 4 Weeks Before Planting (-4 WAP/Weeks After Planting). Organic matter as treatments were given in the form of 10 tonnes chicken manure ha^{-1}, 10 ton rice straw ha^{-1}, and 10 tonnes of *Tithonia diversifolia* ha^{-1} and decomposer in the form of chicken manure liquid, chicken manure + *Tithonia diversifolia* liquid, and biofertilizer. Coconut water and red sugar, i.e. 40 l and 4 kg, were used as the solution for each type of decomposer liquid and then fermented for 6 weeks (at -6 WAP) and given as soil drench.

Cymbopogon nardus and *Tagetes erecta* L. were used as the repellent plant (Kusheryani and Aziz, 2005). Extract of *Cymbopogon nardus*, *Cymbopogon nardus* + *Tithonia diversifolia*, and neem (*Azadirachta indica* A. Juss) were used as organic pest control and sprayed every week. Soybean of Wilis variety were planted 25 x 10 cm with the repellent plant in the middle of every plot.

Results and Discussion

The soil analysis before the experiment showed pH at 5.7 (slightly acid), C-organic at 1.20% (high), total N at 2.2 ppm (low), CEC low, base saturity of 29.26% (medium). Clay in texture (sand, loam and sand, i.e. 6.93, 23.26 and 69.81%, respectively). After the treatment the pH on control, *Tithonia diversifolia* and biofertilizer were 5.7 (slightly acid). On rice straw and chicken

manure liquid, and rice straw and biofertilizer the pH increased to 6.50, whereas the other six treatments were neutral to base (6.60-7.30). Low total nitrogen (0.12-0.22%), high C-organic was 1.20-2.39%, P availability was 2.80-10.20 ppm, low to high K at 0.29-0.94 me/100g, high base saturity was 64.04-96.15%.

Penta and tetra-foliate leaves found on 7 WAP, mostly on *Tithonia diversifolia* plots, with dark green colour, broad leaves and thicker canopy. On 5 WAP *Pseudomonas syringae* pv. glycinea were found on every plant. Saleh and Hardaningsih (2007) stated that this disease was mostly found in high altitude in Indonesia, with wet weather and high temperature.

Organic matter significantly affected seed growth percentage at 2 WAP, number of branches on 3, 5, 7, 9, 11, 13 WAP; number of tetra and penta-foliate on 7 WAP; shoot, root, nodule wet and dry weight on 7 WAP, disease prevalence on 8 WAP; insect infestation on 8 and 10 WAP; number of empty pod on 13 WAP; number of plant harvested; dry seed weight per 7.5 m^2; and soybean productivity (Table 1).

Table1. Organic matter on vegetative and generative variables

Variables	WAP	Organic Matter						Control
		Chicken Manure		Rice Straw		*Tithonia diversifolia*		
Growth percentage	2	336.33	b	369.22	ab	386.11	a	382.33
Plant height (cm)	13	12.79		12.88		13.53		12.59
Number of branches	11	51.06		52.22		49.76		49.85
Number of productive branches	13	3.17	a+	2.90	b+	2.83	b+	2.47
Leaf number	3	8.73	a+	6.69	bc	7.26	b	6.37
	11	10.54	a+	9.24	ab	8.43	b	8.63
Tetrafoliate	7	10.33	a+	9.12	ab	8.30	b	8.53
Pentafoliate	7	10.24	a+	9.06	ab	8.13	b	8.40
NAR (g/cm^2/weeks)	5-7	9.79	a	8.82	ab	7.94	b	8.27
	7-9	2.77		2.75		2.78	+	2.60
RGR (g/weeks)	5-7	6.59		5.92		6.07		6.00
	7-9	12.38		11.49		11.51		11.63
Nodule wet weight (g)	7	135.11	a+	77.33	c+	87.78	b+	68.00
Shoot dry weight (g)	7	37.11	a+	17.78	c+	24.56	b+	8.67
Leaf water content (%)	7	0.34		0.15		0.28		0.05
Insect infestation (%)	10	5.07	b	3.73	b	7.53	a+	4.70
Disease prevalence (%)	8	0.74	b+	0.51	c	1.03	a+	0.52
Number of filled pod		24.14		19.10		21.43		19.50
Number of empty pod		3.16	a+	1.74	ab	2.72	a	1.07
Seed dry weight (g)		46.69		33.28		40.36		36.58
Number of plants harvested		217.11	b	241.00	ab	249.89	a	255.33
100 seed dry weight (g)		9.59		9.15		8.98		8.65
Seed dry weight /7.5 m^2 (g)		752.44	a	550.67	b	636.78	ab	738.33
Productivity (ton/ha)		1.00	a	0.73	b	0.85	ab	0.98

Numbers followed by different letters in the same row significantly different at 5 and 1 % DMRT test; figures followed by (+) in the same row significantly different at 5% Dunnet test with the control.

Decomposer significantly affected number of branches on 3, 5 WAP; number of tetra and penta foliate on 7 WAP; Nett Assimilation Rate (NAR) on 7-9 WAP; shoot and root wet weight on 7 WAP; shoot dry weight on 7 WAP; disease prevalence on 8 WAP; and insect infestation on 8 WAP (Table 2). Combination of organic matter and decomposer significantly affected Nett Assimilation Rate on 7-9 WAP; shoot and root wet weight on 7 WAP; shoot and root dry weight on 7 WAP; and insect infestation on 10 WAP.

Table2. Decomposer on vegetative and generative variables

Variables	WAP	Decomposer						Control
		Chicken Manure Liquid		Chicken manure + *Tithonia diversifolia* liquid		Biofertilizer		
Growth percentage	2	357.67		372.33		361.67		382.33
Plant height (cm)	13	50.83		51.29		50.93		49.85
Number of branches	3	3.04	a+	3.01	a+	2.85	b+	2.47
	5	7.8	+	7.6	+	7.3		6.4
	11	9.3		9.5		8.6		8.4
Number of productive branches	13	8.9		9.2		8.5		8.3
Leaf number	3	2.8		2.8	+	2.7		2.6
	11	11.3		10.7		9.6		10.1
Tetrafoliate	7	102.1	a+	100.1	ab+	98	b+	68
Pentafoliate	7	27.7	a+	26.3	ab+	25.1	b+	8.7
NAR (g/cm^2/weeks)	5-7	$2.5x10^{-3}$		$1.9x10^{-3}$		$2.4x10^{-3}$		$4.5x10^{-4}$
	7-9	$2.8x10^{-3}$	a	$1x10^{-3}$	b	$7.6x10^{-4}$	b	$1x10^{-3}$
RGR (g/weeks)	5-7	0.26		0.23		0.29		0.05
	7-9	0.18		0.1		0.07		0.12
Shoot wet weight (g)	7	4.6	b	5.16	b	6.63	a+	4.7
Root wet weight (g)	7	0.65	bc	0.7	b	0.93	a	0.52
Shoot dry weight (g)	7	1.34	b	1.36	b	1.94	a	1.41
Nodule dry weight (g)	7	0.03		0.03		0.04	+	$9x10^{-3}$
Insect infestation (%)	8	20.89	a+	19.47	b+	21.98	a+	29.16
	10	6.89		7.69		7.67		5.48
Disease prevalence (%)	8	69.16	ab+	68.29	b+	69.65	a+	79.46
Seed dry weight (g)		41.49		40.3		38.54		36.58
Number of plants harvested		231.8		239.6		236.7		255.3
100 seed dry weight (g)		9.18		9.39		9.15		8.65
Seed dry weight /7.5 m^2 (g)		658		615.67		666.22		738.33
Productivity (ton/ha)		0.88		0.82		0.89		0.98

Numbers followed by different letters in the same row significantly different at 5 and 1 % DMRT test; figures followed by (+) in the same row significantly different at 5% Dunnet test with the control

The higher result on control plots seemed to be affected by the plot placement in the field that supplied more water in the beginning of the experiment than the treatment plots. Soil in the treatment plots were hard because of the changed condition from paddy field to the rain-fed

condition for the soybean planting. All the organic manure was recommended for soybean planting after paddy field. There was no need to apply decomposer after the paddy field for soybean planting with rice straw incorporation to the soil.

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Yield and Dry Matter Production of Japanese and US Soybean Cultivars under Drought Stress

Yohei Kawasaki[a*], Yu Tanaka[b], Keisuke Katsura[a], Tatsuhiko Shiraiwa[a]

[a] *Graduate School of Agriculture, Kyoto University, Japan*
[b] *Graduate School of Science, Kyoto University, Japan*
** Corresponding author: Graduate School of Agriculture, Kyoto University, Sakyo-ku, 6068502, Kyoto, Japan.*
Tel.: +81 75 753 6042; Fax: +81 75 753 6065.
kwsk77@kais.kyoto-u.ac.jp

Abstract

The difference in soybean yield between Japan and US is enlarging. The authors reported that the higher yields of US cultivars than Japanese cultivars were attributed to greater dry matter production and radiation use efficiency and that the US cultivars tended to have higher leaf stomatal conductance to water vapor (gs). These traits may cause different sensitivity to drought between cultivars. The objective of this study was to examine whether the yield response to drought differed between a Japanese cv. Tachinagaha (Tc) and a US cv. UA-4805 (UA) with low and high leaf gs, respectively. The two cultivars were grown on a drained paddy field in the Exp. Farm of Kyoto Univ. (Takatsuki, Japan) and the treatment with irrigation (Control) and without irrigation (Drought) was started at 20 days after emergence. The volumetric soil water content was on average 6% lower in the Drought than in the control plots. The leaf area development was inhibited only in UA under Drought. The gs was higher in UA than in Tc and it decreased in both cultivars by 34~38%. UA showed a greater mean seed yield and harvest index. Drought reduced mean seed yield and total dry weight of two cultivars. The yield reduction by drought in UA was associated with reduced radiation intercepted, while yield reduction in Tc was associated with reduced radiation use efficiency and harvest index. The significant G×E interaction was detected in harvest index and mean fraction of radiation intercepted. The two cultivars did not differ in yield response to drought, but they differed in the yield components affected by drought.

*Keywords: soybean (*Glycine max *(L.) Merrill), yield, genotype by environment interaction, drought*

Introduction

The difference in soybean yield between Japan and US is enlarging. The authors reported that the higher yields of US cultivars than Japanese cultivars were attributed to greater dry matter production and radiation use efficiency (Kawasaki *et al*. 2010) and that the US cultivars tended to have higher leaf stomatal conductance to water vapor (gs) (Tanaka *et al.* 2010). These traits may cause different sensitivity to drought between cultivars. The objective of this study was to examine whether the yield response to drought differed between a Japanese cv. Tachinagaha (Tc) and a US cv. UA-4805 (UA) with low and high leaf gs, respectively.

Materials and Methods

The two cultivars were grown on a drained paddy field in the Experimental Farm of Kyoto University (Takatsuki, Japan, Eutric Fluvisols) located at 34°51'N and 135°37E'. The date of sowing was 7th of July 2010 and the date of emergence was 12nd of July. Plant spacing was 0.7 by 0.15m. Fertilizers of N, P_2O_5, K_2O were incorporated into the soil before sowing at 3, 10 and 10 gm^{-2}, respectively.

The treatment with irrigation (Control) and without irrigation (Drought) was started at 20 days after emergence. In the Control, irrigation water was evenly applied using the plastic tube

(Sumisansui, Sumika Agrotech Co. Ltd.) extended on the ground in every other interrow. Irrigation was conducted when the soil matric potential declined lower than 50kPa. The volumetric soil water content was measured twice a week using EC-5 (Decagon Devices, Inc.).

The canopy coverage was measured by the digital image analysis using ImageJ (NIH, USA) (Purcell 2000, Shiraiwa *et al.* 2011). The total above-ground plant part was harvested at 35, 47, 61, and 74 days after emergence from a 1.26 m^2 land area and the dry matter weight was determined after drying at 80°C for 72h. The Leaf Area Index (LAI) was estimated by measuring leaf area of representative plants.

Results and Discussion

The volumetric soil water content was on average of 6% lower in the Drought than in the Control plots (Figure 1). The leaf area development was inhibited only in UA under Drought (Figures 2 and 3). The gs was higher in UA than in Tc and it decreased in both cultivars by 34~38% (Figures 4 and 5).

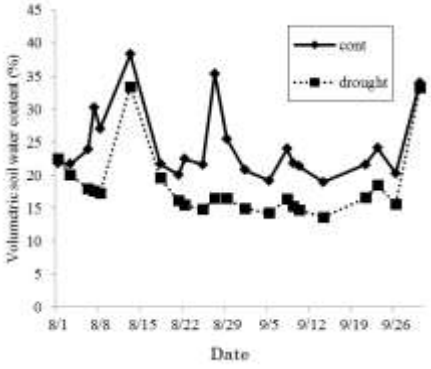

Figure 1. Change in volumetric soil water content measured by EC-5 (Decagon Devices, Inc.).

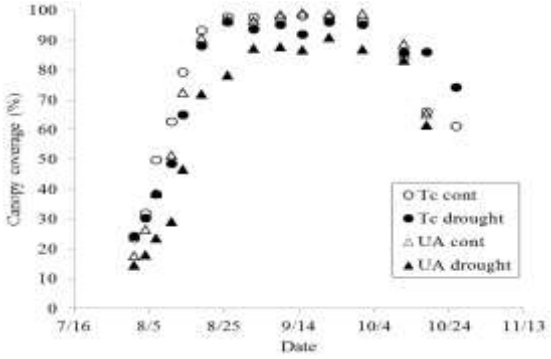

Figure 2. Change in canopy coverage measured by digital image analysis.

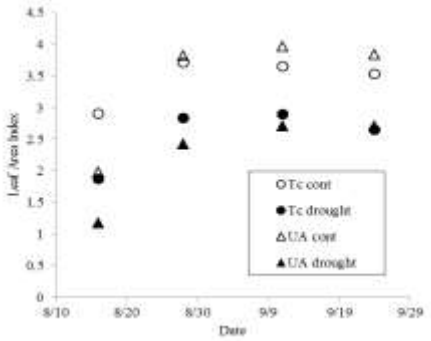

Figure 3. Change in Leaf Area Index.

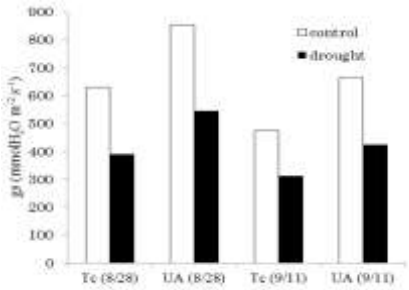

Figure 4. Stomatal conductance of Tachinagaha and UA-4805 under different soil conditions.

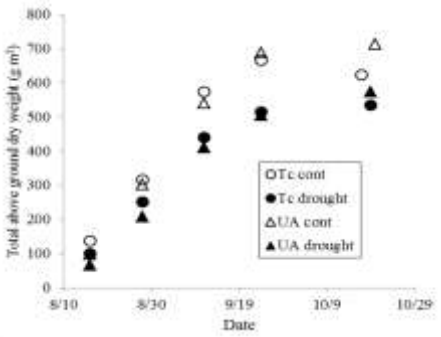

Figure 5. Change in total above ground dry weight.

UA showed a greater mean seed yield and harvest index in both of Control and Drought. Drought reduced mean seed yield and total dry weight of two cultivars. The yield reduction by drought in UA was associated with reduced radiation intercepted, while yield reduction in Tc was associated with reduced radiation use efficiency and harvest index. The significant G×E interaction was detected in harvest index and mean fraction of radiation intercepted (Table 1).

These results indicated that the two cultivars did not differ in yield response to drought, but they differed in the yield components affected by drought.

Table 1. Yield and yield components of drought experiment at Takatsuki in 2010

	Seed yield (t ha^{-1})	Total Dry Weight (t ha^{-1})	HI	Radiation Use Efficiency (g MJ^{-2})	Mean F (%)	Total radiation intercepted (MJ)	Total incident radiation (MJ)
Tachinagaha control	3.77	6.22	0.521	0.504	72.9	1233	1690
Tachinagaha drought	2.17	5.34	0.341	0.455	69.2	1170	1691
UA-4805 control	4.84	7.12	0.585	0.581	71.7	1225	1708
UA-4805 drought	3.65	5.74	0.531	0.572	58.6	1000	1705
Analisis of varience							
Cultivar	29.75 **	19.65 *	27.49 **	46.75 **	13.87 *	8.84 *	
Treatment	35.49 **	58.79 **	32.50 **	4.16 *	28.33 **	23.13 **	
cultivar x treatment	0.77 NS	2.98 NS	8.14 *	198 NS	8.72 *	7.33 NS	

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Selection of F4, F5 and F6 Soybean Breeding Lines for High Yield and Large Seed Size

Darman M. Arsyad [a]*, Asadi [b]

[a] *Central Institute for Agricultural Technology Assessment and Development, Bogor, Indonesia*
[b] *Research Institute for Agricultural Biotechnology and Agricultural Genetic Resources, Bogor, Indonesia*
** Corresponding author: Central Institute for Agricultural Technology Assessment and Development, Bogor, Indonesia*
Tel.: +62-251-8351277; Fax: +62-251-8350928
dmarsyad@gmail.com

Abstract

The first step in a cultivar development is to form a population with genetic variability for the characters of interest. This is done by hybridization of genetically different parents. Hybridization was made to combine the characters of high yield potential, wide adaptability, desirable agronomic characters, and large seed size by using cv. Tanggamus, Sibayak, Argomulyo, Anjasmoro, Panderman and Local Tegal. Amount of 4800 F4 lines originated from five single cross combination were planted in rice field, Sukawening, Garut District, West Java on early dry season 2007. Pedigree method of selection was used. Each line was grown in one row of three meters length and 40 cm between rows. There was a high variability on agronomic characters (plant height, pod number, seed yield and seed size). Amount 1311 F5 lines were selected and grown in the same location on late dry season 2007. Amount of 540 F6 lines were selected and grown in the same location on early rainy season 2007/2008. Sixty-two F7 lines were selected, and 42 lines among them had high yield and large seed size.

Keywords: high yield, large seed size, soybean breeding lines

Introduction

Breeding for high yield potential is generally the major goal in any breeding program including soybean. Breeding for high yield potential is inextricably linked with breeding for other characteristics. Today the preferences of users (farmers and craftsmen tempeh and tofu) were more likely to require large varieties of soybean seed. Craftsmen tempeh and tofu have long accustomed to using large seed soybean imports, so that preferences are now a lot towards the large seed.

To meet user demand, then breeding programs to produce varieties of soybean large seed in major need of attention. A number of varieties of large seed soybean have been available such as Argomulyo, Burangrang, Anjasmoro, and Grobogan (Hermanto et al., 2009).

As an effort to produce new varieties of soybeans that are superior to the existing varieties, a number of crossing (hybridization) between soybean genotypes of high yield potential, quite broad adaptability, many pods and smaller seed size with the genotypes of relatively low yield potential, narrow adaptability, few pods, and large seed size, and were carried out in 2004 (Arsyad et al. 2005).

This study aimed to: (a) obtain expected breeding lines better than the existing varieties, which possess higher yield potential, desirable agronomic traits and has a large or slightly larger seed size, and (b) study the behavior of the relationship among traits in soybean.

Materials and Methods

Plant Materials

Selection of F4, F5 and F6 soybean breeding lines was conducted in the rice field of Sukasono Village, Sukawening Subdistrict, Garut District, at the growing season I (April-June 2007), season II (July-September 2007) and the season III (September-November 2007), respectively. Selection materials that were used in the firs selection In the season I the 4800 F4 soybean breeding lines were grown and selected. Those breeding lines were derived from a combination of five single crosses, which were Tanggamus x Tegal, Tanggamus x Anjasmoro, Sibayak x Tegal, Sibayak x Argomulyo, and Sibayak x Panderman, made in 2005 (Arsyad et al. 2005). In season II,

the 1311 F5 breeding lines wich were selected from the F4 lines in season I, were grown and then as many as 540 F6 lines were selected. Those 540 F6 lines were grown in season III.

Methods

Pedigree method based selection was used in each generation (Fehr, 1987). In each season, each breeding lines was grown as a single row with 1.5 meters length. Spacing between rows (lines) were 40 cm and 10 cm within rows, and one seed/hole. Intensive techniques cultivation involves fertilizing with 75 kg urea, 200 kg SP36, and 150 kg KCl per ha, controlling of weeds, pests and diseases were carried out. Irrigation was applied, if there is no rain. The selection criteria was an ideal plant type,i.e. sturdy plants (not fall), plant height > 70 cm with 4-5 branches, medium leaf size, determinate type, more than 70 pods/plant and large pods. Selection procedure was done as follows: First, selected the good rows of plants, and secondly, selected the 3-5 plants in selected row that meet the selection criteria. Each selected individual plant was a new breeding line to the next generation. Data analysis was done by creating the frequency distribution of selected breeding lines, calculate the mean and standard deviation for the observed characters of each population, as well as t test for mean population comparation (Gomez & Gomez 1984, Sugiyono 2009).

Results and Discussion

Selection of F4 Lines

Before time to harvest, around 1600 lines was selected and then as many as 1311 lines was selected based on yield and seed quality. Only breeding lines derived from three crossing combinations were selected. Those were the crosses between Sibayak x Tegal (Code V), Tanggamus x Tegal (Code U) and Sibayak x Argomulyo (Code W). The other two crosses (Tanggamus x Anjasmoro and Sibayak x Panderman) derived inferior progenies. .

Frequency distribution of selected breeding lines based on crop yield are presented in Figures 1. The range of lines seem still very wide, ranging from less than 10 g to more than 50 g/line, with the highest frequency was in the range of 21-25 g/line. Genotype of parents produuced average yield of less than 20 g/genotype. This indicated the presence of transgressive segregation on the breeding materials. Variability was also found in the seed size (100 seed weight), plant height, and number of pods (Figure 2-4). The selection would be effective when there was wide phenotypic variability among breeding lines. This was understandable because the selected lines were still at F4 generation and was not selected since F2. The frequency of heterozygous individuals at a locus was 50% in F2 (Fehr 1983), and because the selection has not been done since the F2, therefore the heterozygote frequency in the F4 was still high (50%).

Breeding lines derived from Sibayak x Tegal cross have an average higher yields, larger seed, higher plant, but the number of pods is less than the breeding lines derived from Tanggamus x Tegal and Sibayak x Argomulyo (Table 1). No yield different between breeding lines derived from Tanggamus x Tegal and Sibayak x Argomulyo. Breeding lines derived from Sibayak x Tegal has larger seed size compared to the breeding lines derived from Tanggamus x Tegal and Sibayak x Argomulyo. Breeding lines derived from Sibayak x Argomulyo had a relatively smaller seed size compared to the other two cross combinations, but it had more pods per plant. It appears a tendency that the breeding lines of a large seed pods had fewer seeds, while breeding lines of moderate or relatively small seed pods had more seeds.

A total of 203 breeding lines have been identified with high yield capacity, which was more than 30 g/line (data not presented). A total of 99 breeding lines of which had large seed size (>13g/100 seeds), and the other 104 breeding lines had moderate or relatively smaller seed size. Selection will change the frequency of genotype and phenotype of a locus (Ferhr 1983). The basis of the expected effect of the selection is the change of gene frequencies (Falconer, 1967), but we do not know what the frequency of genes that changed, because the selection was only based on phenotypic selection. The selection would be effective to choose recessive alleles, as consequently in one generation all dominant alleles will be eliminated in the population. However, if the selection is intended to eliminate the recessive alleles, it would be more difficult because it is covered by dominant alleles in the heterozygous individuals (Fehr, 1983).

Although the selection in this study was only based on phenotypic values, it was expected to be quite effective to gain the best selected lines, which is expected to produce good progenies. The progress of the selection is determined by phenotypic variability, heritability values and selection intensity (Falconer, 1967). Agronomic characters such as plant height, number of branches and number of pods support high capacity of selected the breeding line.

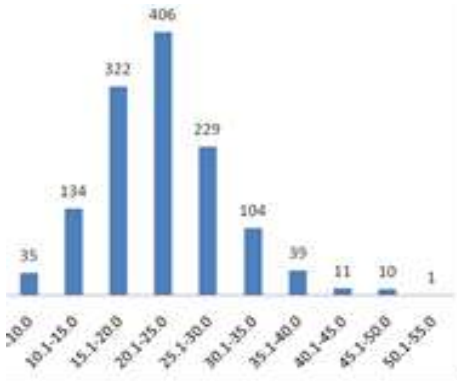

Figure 1. Frequency distribution of F4 soybean breeding lines for yield (g/plant), Garut, season I 2007.

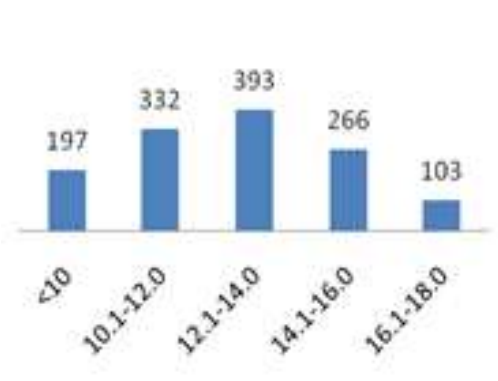

Figure 2. Frequency distribution of F4 soybean breeding lines for 100 seed weight (g), Garut, season I 2007.

Figure 3. Frequency distribution of F4 soybean breeding lines for plant height (cm), Garut, season I 2007.

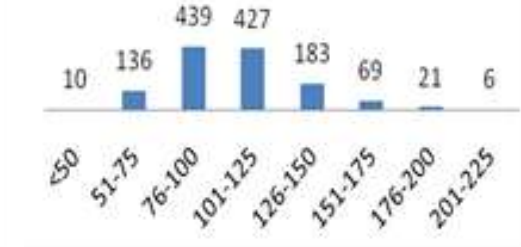

Figure 4. Frequency distribution of F4 soybean breeding lines for pod number per plant, Garut, season I 2007.

Table 1. Mean and standar deviation of seed yield, 100 seed weight, plant height, and number of pods per plant of F4 lines originated of three single cross in rice field at Sukasono Village, Sukawening Subdistrict, Garut District, early dry season of 2007

Population	Number of lines	Seed yield (g)	100 seed weight (g)	Plant height (cm)	Number of pods / plant
V (Sibayak x Tegal)	490	24a ± 7.1	14.1a ± 2.1	58a ± 9.2	97b ± 25.4
U (Tanggamus x Tegal)	400	22b ± 6.8	12.6b ± 1.9	51b ± 8.1	100b ± 24.4
W (Sibayak x Argomulyo)	401	22b ± 7.3	10.9b ± 2.2	56a ± 7.6	124a ± 29.5

Value in the same column followed by the same letter are not different at 0.01 probability level

Selection of F5 Lines

Before time to harvest around 700 lines was selected and, as many as 540 lines was selected based on yield and seed quality. Frequency distribution of breeding lines on several classes of seed yield is presented in Figure 5. The yield range of the breeding lines seemed quite wide, ranging from less than 10 g to more than 30 g/line, with the highest frequency present in the range of 11-15 g/line. The parent produced an average yield of less than 11g. A total of 191

breeding lines produced higher yield than that of the best parent (Tanggamus). This indicated the presence of the transgressive segregation, i.e. breeding line yields were higher than that of both parents. The occurence also the same as the seed size (100 seed weight), which was as many as 88 breeding lines contained a rather large to large seed size (Figures 6). Plant height and number of pods showed a fairly high variability (Figure 7 and 8).

Breeding lines derived from Sibayak x Argomulyo had the higher average yields, higher plant height, and more pods per plant than those of the breeding lines derived from Tanggamus x Tegal and Sibayak x Tegal (Table 2). In contrast, breeding lines derived from Sibayak x Tegal produced average seed size larger than that of the breeding lines derived from Tanggamus x Tegal and Sibayak x Argomulyo. It was a tendency that the breeding lines of larger seed correlated with fewer pods, shorter plants and lower grain yield. The selection would be effective when the high variability occurred among breeding lines. A total of 62 breeding lines had high yield capacity (Table 3).

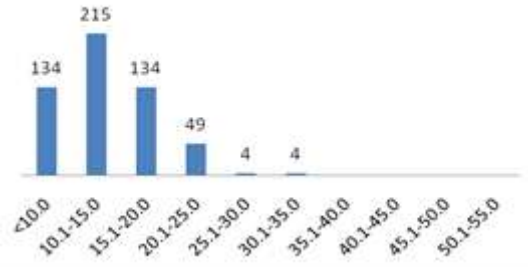

Figure 5. Frequency distribution of F5 soybean breeding lines for yield (g/plant), Garut, season II 2007.

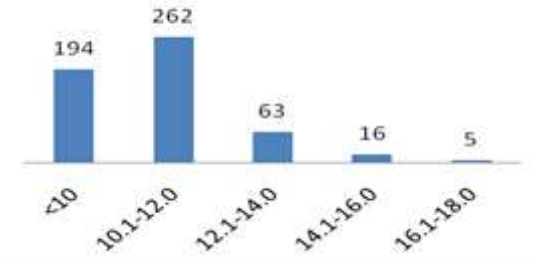

Figure 6. Frequency distribution of F5 soybean breeding lines for 100 seed weight (g), Garut, season II 2007.

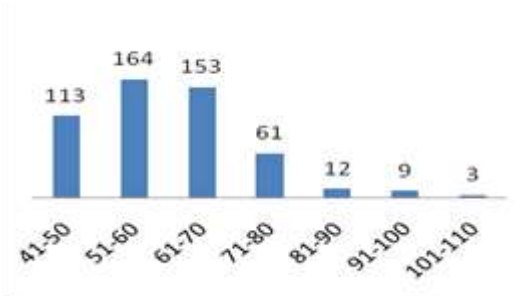

Figure 7. Frequency distribution of F5 soybean breeding lines for plant height (cm), Garut, season II 2007.

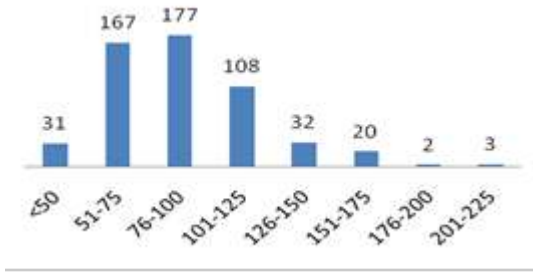

Figure 8. Frequency distribution of F5 soybean breeding lines for pod number per plant, Garut, season II 2007.

Selection of F6 Lines

Of the 540 F6 breeding lines that were selected in the experiment on season III 2007, 100 breeding lines were selected at the harvesting time, and the best 60 breeding lines were selected after processing. Of the 60 F7 breeding lines, as many as 42 breeding lines had the high yield capacity and relatively large seed (Table 4). Among the 42 breeding lines, 16 breeding lines were derived from the cross between Sibayak x Tegal, six breeding lines were derived from the cross between Tanggamus x Tegal, and 20 breeding lines were derived from teh cross between Sibayak x Argomulyo cross. Those breeding lines will be tested further in the preliminary yield testing on early dry season of 2008.

Table 2. Mean and standar deviation of seed yield, 100 seed weight, plant height, and number of pods per plant of F5 lines originated of three single cross in ricefield at Sukasono Village, Sukawening Subdistrict, Garut District, late dry season of 2007

Population	Number of lines	Seed yield (g)	100 seed weight (g)	Plant height (cm)	Number of pods / plant
V (Sibayak x Tegal)	200	14b ± 4.9	11.1a ± 1.5	64b ± 12.3	79b ± 25.5
U (Tanggamus x Tegal)	200	12b ± 4.1	10.8b ± 1.5	50c ± 7.8	79b ± 22.8
W (Sibayak x Argomulyo)	140	18a ± 4.6	9.9b ± 1.5	68a ± 8.7	119a ± 27.2

Value in the same column followed by the same letter are not different at 0.01 probability level

Table 3. Performance of the best 62 F5 breeding lines in ricefield at Sukasono Village, Sukawening Subdistrict, Garut District, season II 2007

Breeding line	Number of lines	Plant height (cm)	Number of branches per plant	Number of pods per plant	Seed yield (g)	100 seed weight (g)
V (Sibayak x Tegal)	20	62 (46-100)	6.8 (4-10)	107 (77-240)	23.3 (21.2-26.4)	11.7 (9.9-14.9)
U (Tanggamus x Tegal)	11	53 (40-61)	6.9 (4-10)	111 (75-145)	21.8 (20.6-23.4)	10.9 (8.4-13.3)
W (Tanggamus x Argomulyo)	31	69 (57-102)	6.5 (4-8)	148 (109-239)	23.8 (20.2-35.1)	13.0 (8.8-17.40
Tanggamus	-	50	5	68	10.7	9.2
Sibayak	-	56	5	86	10.0	9.8
Tegal	-	39	2	27	4.4	17.2
Argomulyo	-	40	5	52	8.9	13.3

Value in parenthese is a range

Table 4. Agronomic performance and seed yield of 42 F6 soybean breeding lines selected in ricefield at Sukasono Village, Sukawening Subdistrict, Garut District, season III 2007

No.	Breeding line	Plant height (cm)	Number of branches	Number of pods	Seed yield (g)	100 seed weight (g)
1	V-5-1-1-2	69	5	99	25.1	14.9
2	V-43-2-2-1	95	4	77	22.1	14.9
3	V-63-2-1-1	68	7	102	26.2	13.9
4	V-75-2-1-2	69	6	114	27.7	14.7
5	V-75-2-3-1	68	4	85	25.4	14.8
6	V-75-2-4-2	65	4	91	23.0	13.0
7	V-250-2-2-1	66	7	106	26.7	13.3
8	V-257-1-1-1	62	9	123	26.4	14.4
9	V-273-1-1-2	70	7	114	25.7	13.0
10	V-284-2-1-1	66	8	117	25.4	13.8
11	V-215-1-1-1	62	9	113	22.1	14.3
12	V-215-1-2-1	62	7	240	30.3	13.4
13	V-215-1-4-1	58	8	118	25.4	14.3
14	V-390-2-3-2	59	7	120	24.8	13.1
15	V-424-1-1-2	60	10	104	25.3	13.3
16	V-612-2-1-2	66	7	110	23.5	13.6
17	U-574-1-1-1	64	8	104	20.8	13.3
18	U-445-2-1-1	60	7	122	22.1	14.5
19	U-79-2-2-2	58	5	123	20.6	12.5
20	U-675-1-1-1	56	8	93	21.3	12.2
21	U-534-2-2-1	62	4	106	22.3	12.9
22	U-697-1-1-1	54	7	99	21.5	13.4
23	W-209-1-1-2	68	8	170	20.8	13.1
24	W-455-1-2-1	98	6	120	22.4	15.4
25	W-460-1-1-1	70	5	109	21.3	13.6
26	W-47-2-1-1	68	6	130	20.3	13.0
27	W-466-1-1-1	70	7	191	32.2	13.1
28	W-40-1-1-2	67	7	147	27.6	14.3
29	W-40-1-2-2	65	8	127	24.0	13.3

No.	Breeding line	Plant height (cm)	Number of branches	Number of pods	Seed yield (g)	100 seed weight (g)
30	W-165-1-1-1	75	7	168	25.7	13.7
31	W-284-1-3-1	72	8	239	34.3	13.4
32	W-537-1-1-1	67	4	103	21.2	13.6
33	W-267-2-1-2	74	5	100	20.0	14.5
34	W-530-1-2-1	67	6	145	25.3	13.3
35	W-308-2-1-2	71	7	180	35.1	13.3
36	W-258-1-1-1	86	5	127	24.0	13.9
37	W-271-1-1-1	70	8	158	21.1	12.6
38	W-519-1-2-1	79	8	175	25.1	13.1
39	W-169-1-2-1	68	6	160	30.8	12.7
40	W-304-1-2-1	64	7	138	22.3	12.5
41	W-304-1-3-1	66	7	176	21.2	13.3
42	W-197-1-1-2	59	6	142	23.3	13.8
43	Tanggamus	58	6	68	10.7	10.2
44	Sibayak	60	6	86	10.0	11.1
45	Tegal	45	3	36	14.2	17.0
46	Argomulyo	54	5	58	18.5	14.6
	V (16 lines)	67	6,8	114	25.3	13.9
		(58-95)	(4-10)	(77-240)	(22.1-30.3)	(13.0-14.9)
	U (6 lines)	59	6,5	108	21.4	13.1
		(54-64)	(4-8)	(93-123)	(20.6-22.3)	(12.2-14.5)
	W (20 lines)	71	6,5	150	24.9	13.5
		(59-98)	(4-8)	(100-239)	(20.0-35.1)	(12.5-15.4)
	Check (4 var.)	54	5	62	13.4	13.2
		(45-60)	(3-6)	(36-86)	(10.0-18.5)	(10.2-17.0)

V= Sibayak x Tegal cross
U= Tanggamus x Tegal cross
W=Sibayak x Argomulyo cross
Value in parenthese is a range

Acknowledgment

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Effect of Hydrogen Peroxide Spraying on Drought Stress in Soybean Plant

Yushi Ishibashi[a], Haruka yamaguchi[a], Takashi Yuasa[a], Mari Iwaya-Inoue[a], Susumu Arima[b,c], Shao-Hui Zheng[c]

[a] *Crop Science Lab., Faculty of Agricluture, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan*
[b] *Faculty of Agriculture, Saga University, Saga 840-8502, Japan*
[c] *Coastal Bioenvironment Center, Saga University, Karatsu, Saga 840-0021, Japan*
** Corresponding author: Yushi Ishibashi, Crop Science Lab., Faculty of Agriculture, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan. Tel.: +81 92 642 2824; fax: +81 92 642 2824.*
yushi@agr.kyushu-u.ac.jp

Abstract

We examined whether the spraying of soybean leaves with hydrogen peroxide (H_2O_2) would alleviate the symptoms of drought stress. Pre-treatment by spraying leaves with H_2O_2 delayed foliar wilting caused by drought stress compared to leaves sprayed with distilled water (DW). Additionally, the relative water content of drought-stressed leaves pre-treated with H_2O_2 was higher than that of leaves pre-treated with DW. Therefore, we analyzed the effect of H_2O_2 spraying on photosynthetic parameters and on the biosynthesis of oligosaccharides related to water retention in leaves during drought stress. Under conditions of drought stress, the net photosynthetic rate and stomatal conductance of leaves pre-treated with H_2O_2 were higher than those of leaves pre-treated with DW. In contrast to DW spraying, H_2O_2 spraying immediately caused an increase in the mRNA levels of *D-myo-inositol 3-phosphate synthase* 2 (*GmMIPS2*) and *galactinol synthase* (*GolS*), which encode key enzymes for the biosynthesis of oligosaccharides known to help plants tolerate drought stress. In addition, the levels of *myo*-inositol and galactinol were higher in H_2O_2-treated leaves than in DW-treated leaves. These results indicated that H_2O_2 spraying enabled the soybean plant to avoid drought stress through the maintenance of leaf water content, and that this water retention was caused by the promotion of oligosaccharide biosynthesis rather than by rapid stomatal closure.

Keywords: Drought stress, Galactinol, Hydrogen peroxide, Soybean

Introduction

Plants respond and adapt to water deficits at both the cellular and molecular levels by the accumulation of osmolytes and proteins that are specifically involved in stress tolerance. Drought stress is the primary cause of crop loss across the globe, reducing average yields in most major crop plants (Boyer 1982; Bray *et al*. 2000).

It is known that drought stress enhances the production of reactive oxygen species (ROS) in cellular compartments such as chloroplasts, peroxisomes, and mitochondria. If drought stress is prolonged, ROS production will overwhelm the scavenging action of the antioxidant system, resulting in extensive cellular damage and death (Cruz de Carvalho 2008). On the other hand, ROS are also known to function as signal molecules in plants (Foyer *et al*. 1997), controlling processes such as growth, development, responses to biotic and abiotic environmental stimuli, and programmed cell death (Bailey-Serres and Mittler 2006).

Abscisic acid (ABA), synthesized in response to drought stress, is known to induce stomatal closure and to reduce transpirational water loss (Schroeder *et al*. 2001). ABA activates the synthesis of ROS in guard cells by a membrane-bound NADPH oxidase, and ROS mediate stomatal closure by activating (through hyperpolarization) plasma membrane Ca^{2+} channels (Pei *et al*. 2000; Murata *et al.* 2001; Wang and Song 2008). In addition, it has been reported that hydrogen peroxide (H_2O_2), a type of ROS, is involved in the acclimation of *C. albidus* to summer drought (Jubany-Marí *et al.* 2009) and in that of maize (*Zea mays* L.) to salt stress (Azevedo Neto *et al.*

2005). H_2O_2 also increases the soluble sugar content of melon fruits (Ozaki *et al*. 2009). Other components may also be responsive to ROS as a part of a stress-activated signal transduction pathway. We therefore focused on ROS as signal molecules by examining whether exogenous H_2O_2 application (by spraying) could alleviate drought stress and by working to define the alleviation mechanism.

Materials and Methods

Plant materials

Soybean (*Glycine max* L. Merrill) cv. Fukuyutaka was used as the plant material. Three weeks after emergence, either 1 mM H_2O_2 or DW was sprayed only once onto the leaves of each plant (100 mL/pot), and then irrigation was stopped. Treatments with H_2O_2 or DW were always followed by drought stress. Measurements of all parameters were made after water had been withheld for 0, 2, 4, 6, and 8 days. For an additional control treatment, we included plants with no spray treatment and with irrigation maintained throughout the experiment. The following measurements consisted of four replicates.

Leaf relative water content

To evaluate leaf relative water content (RWC), 50 leaf discs (5 mm in diameter) from each plant were weighed to determine fresh weight (FW), then hydrated to full turgidity by being floated in DW for 24 h at 4 °C and weighed again to determine the turgid fresh weight (TW). Dry weight (DW) was determined by drying for 48 h at 90 °C. RWC was then calculated as [(FW-DW)/(TW-DW)]×100.

Photosynthetic measurements

Photosynthetic rate, stomatal conductance, and transpiration rate were measured in soybean leaves using an LCpro+ portable photosynthesis system (LCpro, ADC Bioscientific Ltd., UK) at room temperature (25 °C) in the morning (8:00-11:00 am). The quantum flux density at the leaf surface, flow rate, and leaf temperature in the chamber were maintained at 1500 μmol m^{-2} s^{-1}, 200 μmol s^{-1}, and 30 ºC, respectively. The rate of CO_2 assimilation in the chamber was measured at an ambient CO_2 concentration of 370 μL L^{-1}.

RT-PCR analysis

cDNA was synthesized from total RNA (1 μg) using Rever TraACE reverse transcriptase (Toyobo, Japan) according to the manufacturer's protocol. cDNA (1 μL) was amplified in a reaction containing 10 μL of Go Taq Green Master Mix (Promega, USA), 0.1 μL each of 50 μM forward and reverse primers, and 8.8 μL of water. The amplification was conducted using a Program Temp Control System Astec PC-320 (Astec, Japan) as follows: 1 min at 94 °C; 27 cycles of 15 s at 94 °C, 30 s at 58 to 62 °C, and 30 s at 72 °C; then 5 min at 72 °C. The specific primer sequences for GmGolS (Glyma10g28610.1, Phytozome) were 5′-GACAAGCTTAAGCAGCAGATGGGGCACGGA-3′ and 5′-ATCGGATCCTGCCAGCAGCAGTGCCCCCATAAG-3′; for GmP5CS (Genbank accession no. AY492005), the specific primer sequences were 5′-ATCAAGAGTTCCACTAAAATTCCTGTC-3' and 5'-TCATATGAGAAGGTCTCTGTGAGTGTAG-3'; for GmActin (Genbank accession no. V00450), the specific primer sequences were 5′-GCGTGATCTCACTGATGCCCTTAT-3′ and 5′-AGCCTTCGCAATCCACATCTGTTG-3′. The specific primer sequences for GmMIPSs were determined according to Chappell et al. (2006).

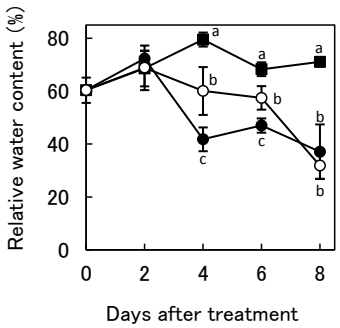
Myo-inositol and galactinol contents

Myo-inositol and galactinol levels were analyzed by HPLC using a 930-RI refractive index detector (JASCO, Japan) and a Shodex Asahipak NH2P-50 4E column (polymer-base, particle size; 5 µm, 250 mm × 4.6 mm i.d.) (Showa Denko K.K., Japan). Sugars were separated with acetonitrile-water (80:20, v/v) as an isocratic mobile phase at 0.8 mL/min using an 880PU pump (JASCO, Japan). The column was held at 40 °C.

Powdered freeze-dried leaves (50 mg) were extracted with 4 mL 80% (v/v) ethanol. The extracts were boiled for 20 min and centrifuged for 5 min at 25 000×*g* to produce pellets of insoluble material. The supernatant was removed and the pellet was extracted twice more using the same approach. The supernatants were then combined and dried. The residue was dissolved in 1 mL of DW and passed through a Sep-Pak C18 mini-column (Waters, USA). The extracts were filtered (0.45 µm) before HPLC injection.

Results and Discussion

The RWCs for the three treatments are shown in Figure 1. There were no significant differences in RWC between control, H_2O_2-treated, and DW-treated plants up to 2 DAT. At 4 DAT, the RWCs in control, H_2O_2-treated, and DW-treated plants were 80%, 60%, and 40%, respectively. The RWC at 6 DAT was also higher in H_2O_2-treated plants than in DW-treated plants. By 8 DAT, the RWCs for both the H_2O_2 and DW treatments were approximately 40%.



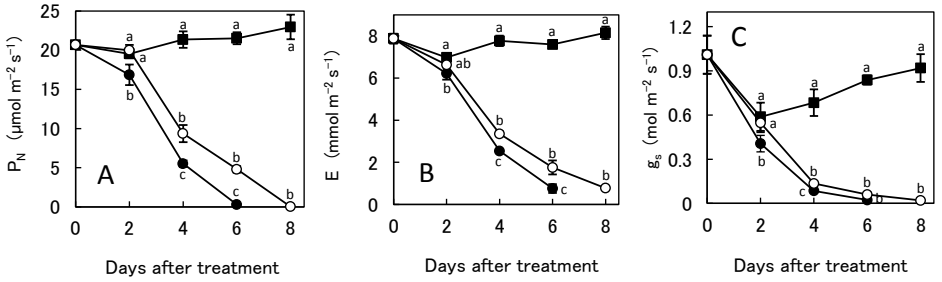
Closed squares indicate the control (irrigated, unsprayed) treatment. Open and closed circles indicate pre-treatment with H_2O_2 and DW respectively, followed by drought stress for the number of days indicated. For each day after treatment, means followed by different letters are significantly different at the 5% level as determined by Tukey's test (n = 4); error bars indicate standard deviations.

Figure 1. Relative water contents of soybean leaves during drought stress.

The net photosynthetic rate (P_N), transpiration rate (E), and stomatal conductance (g_s) during drought stress are shown in Figure 2. Here, although the P_N, E, and g_s significantly decreased during drought stress, these parameters were significantly higher in the H_2O_2-treated plants than in DW-treated plants. At 2 DAT, g_s levels in control, H_2O_2-treated, and DW-treated plants were 0.588, 0.508, and 0.323 mol m^{-2} s^{-1}, respectively (Fig. 2C). At 4 DAT, P_N levels in control, H_2O_2-treated, and DW-treated plants were 21.34, 9.36, and 5.51 µmol m^{-2} s^{-1}, respectively, and the levels of E were 7.76, 3.35, and 2.53 mmol m^{-2} s^{-1}, respectively (Fig. 2A, B).

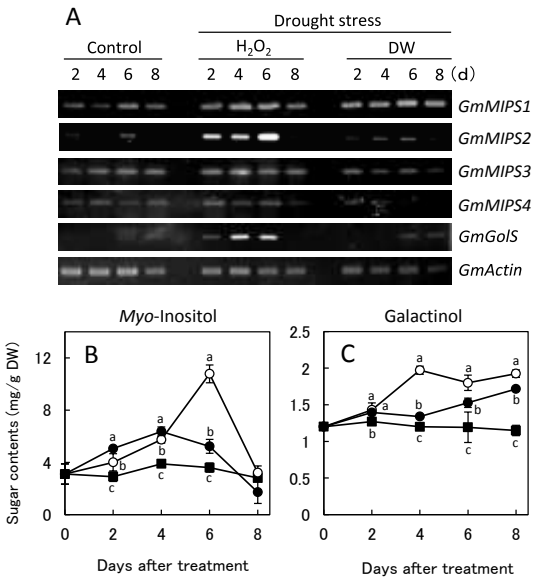
Myo-inositol plays critical and diverse biological roles in a myriad of cellular processes including signal transduction, stress responses, cell-wall biogenesis, growth regulation, and osmotolerance (Loewus and Murthy 2000). At 2, 4, and 6 DAT, *GmMIPS2* expression was markedly higher in leaves treated with H_2O_2 compared with control and DW-treated leaves (Fig. 3A). At 2 DAT, *GmMIPS2* expression was induced by H_2O_2 treatment but not by DW treatment. At 4 and 6 DAT, the expression of *GmMIPS2* mRNA in H_2O_2-treated leaves was remarkably higher than that in DW-treated leaves. In contrast, the expression of *GmMIPS1, -3,* and *-4* did not vary among the control, H_2O_2, and DW treatments. We also examined the expression of *galactinol synthase* (*GolS*),

which acts downstream of MIPS in the synthesis of raffinose-family oligosaccharides (RFOs). *GmGolS* expression was low in the control and DW-treated leaves but clearly higher in the H_2O_2–treated leaves, especially at 4 and 6 DAT (Fig. 3A).



(A) Photosynthetic rate (P_N); (B) transpiration rate (E); and (C) stomatal conductance (g_s). Closed squares indicate control (irrigated, unsprayed) treatment. Open and closed circles are H_2O_2 and DW pre-treatments, respectively, followed by drought stress for the number of days indicated. For each day after treatment, means followed by different letters are significantly different at the 5% level as determined by Tukey's test (n = 4); error bars indicate standard deviations.

Figure 2. Photosynthetic parameters during drought stress.



(A) Expression of *GmMIPS* genes and *GmGolS*. Control (irrigated, unsprayed); H_2O_2, H_2O_2 pre-treated; DW, DW pre-treated; d, days after treatment. *GmActin* was used as a control for loading. (B) *Myo*-inositol contents and (C) galactinol contents. Closed squares indicate the control (irrigated, unsprayed) treatment. Open and closed circles indicate pre-treatment with H_2O_2 and DW, respectively, followed by drought stress for the number of days indicated. Means followed by different letters are significantly different at the 5% level as determined by Tukey's test (n = 4); error bars

Figure 3. Gene expression of *GmMIPSs* and *GmGolS* and contents of *myo*-inositol and galactinol in leaves during drought stress.

We also found that the *myo*-inositol and galactinol contents in leaves pre-treated with H_2O_2 were increased compared with control and DW-treated leaves (Fig. 3B, C). *Myo*-inositol contents increased during drought stress, especially in H_2O_2-sprayed plants at 6 DAT, but decreased to starting levels by 8 DAT (Fig. 3B). The galactinol content in leaves pre-treated with H_2O_2 nearly doubled by 4 DAT, and the increased level was maintained until 8 DAT. On the other hand, the galactinol content in leaves pre-treated with DW increased until 8 DAT, but was significantly lower from 4 to 8 DAT than that seen in H_2O_2-treated plants (Fig. 3C).

Stress-inducible production of galactinol synthase (GolS) plays a key role in the accumulation of galactinol and raffinose, which function as osmoprotectants, under drought stress (Taji *et al*. 2002). GolS catalyzes the synthesis of galactinol from *myo*-inositol and UDP-galactose. We therefore examined the transcript levels of *D-myo-inositol 3-phosphate synthase1, -2, -3* and *-4* (*GmMIPSs*), which encode key enzymes in the synthesis of *myo*-inositol, and that of *GmGolS*, in H_2O_2-sprayed plants. We found that among the *GmMIPS* genes, only the transcript level of *GmMIPS2* increased after H_2O_2 spraying (Fig. 3A). Chappell *et al*. (2006) reported that *GmMIPS2* was poorly expressed in soybean leaves cultivated conventionally. The results reported here suggest that *GmMIPS2* is likely to be involved in drought stress signaling through ROS production caused by drought stress. Treatment with methylviologen, which enhances the production of O_2^- (another ROS), increased the transcript levels of *GolS* in *Arabidopsis thaliana* (Nishizawa *et al.* 2008). Overexpression of *AtGolS2* in transgenic *Arabidopsis thaliana* caused an increase in endogenous galactinol and raffinose, and showed improved drought tolerance (Taji *et al*. 2002). In soybeans, we have shown that H_2O_2 spraying increased the transcript level of *GmGolS* and the galactinol content in leaves (Fig. 3A, C). These results suggest that H_2O_2 spraying enabled the leaf to maintain a high level of RWC by regulating the osmolality in the leaf, consequently alleviating the effects of drought stress.

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Productivity of Several Lines of Soybean in Majalengka, West Java, Indonesia

Alia Astuti[a], Ence Darmo Jaya Supena[a,b], Suharsono[a,b,*]

[a]*Department of Biology, Bogor Agricultural University, Indonesia*
[b]*Research Center for Bioresources and Biotechnology, Bogor Agricultural University, Indonesia*
**Corresponding author: PPSHB IPB, Gd PAU, Jl. Kamper, Kampus IPB Darmaga Bogor 16680, Indonesia*
Tel: +62 251 8621257; fax: +62 251 8621724.
E-mail: sony-sh@ipb.ac.id; sony.suharsono@yahoo.com

Abstract

Soybean (*Glycine max* L. Merr) is a very important crop in Indonesia. The creation of new elite varieties is one approach to increase the national production of soybean. We have developed several potential lines of soybean to be released as a new varieties. Before releasing as new varieties, the productivity of these lines has to be evaluated in several locations. Therefore the objective of this research was to evaluate the productivity of six lines of soybean resulted from the cross between Slamet and Nokonsawon varieties, i.e. KH8, KH9, KH31, KH38, KH55, and KH71 in Majalengka, West Java, Indonesia. Four national elite varieties i.e. Anjasmoro, Slamet, Tanggamus and Wilis were used as standard. The experiment was conducted in two seasons by using randomized block design, with three blocks as replication. The result showed that based on the seed production per plant in two seasons, all six lines have higher productivity than Anjasmoro variety. The seeds of these six lines are bigger than that of standard varieties. Analysis of production stability in two seasons showed that KH71 is the most stable genotype in Majalengka. By comparing to Anjasmoro variety in two seasons, all six lines have a potential to be released as new varieties with high productivity and big seed.

Keywords: lines, productivity, seed size, soybean

Introduction

Soybean (*Glycine max* L. Merr) is a very important crop in Indonesia. Every year, Indonesia imports more than 1.2 million tons of grains of soybean for food. The creation of new elite varieties is one approach to increase the production of soybean in Indonesia. The creation of tolerant varieties to acid soil containing high concentration of aluminum is very important to extend the cultivation onto the marginal land with with this condition. Sunarto (1995) had created a variety of Slamet which is tolerant to acid soil. Eventhough variety of Slamet has a high productivity and is a tolerant to acid soil, it has a relatively small seeds and its hilum is black which are undesirable traits for tofu and tempeh industry. To improve these traits, we had crossed this variety with Nokhonsawon variety which has a big seeds, then followed by selection based on high productivity and seed size traits (Suharsono *et al*., 2006, 2007; Jambormias *et al*., 2011). From this cross, we obtained 18 potential lines to be released as a new variety.

Before releasing a line of soybean as new variety, the productivity of this line has to be evaluated in several locations. In this experiment, six of 18 potential lines of soybean were cultivated in the irrigated rice field in Majalengka, West Java, Indonesia. Majalengka is a center of soybean production in West Java. So, the objective of this research was to evaluate the productivity and seed size of six lines, i. e. KH 8, KH 9, KH 31, KH 38, KH 55, and KH 71, with four elite national varieties, i. e. Anjasmoro, Slamet, Tanggamus, and Wilis as standard in two seasons in Majalengka, West Java, Indonesia.

Materials and Methods

Six lines of soybean i.e. KH8, KH9, KH31, KH38, KH55, and KH71 were used in this experiment. Four national cultivars i.e. Slamet, Wilis, Anjasmoro and Tanggamus were used as standard. The experiment was carried out in Randomized Block Desain with three replications, so this experiment had 30 experimental units or plots. The size of plot is 4 m x 5 m, planting distance is 40 cm x 20 cm with two plants per hole, so the productivity per hectar is productivity per plant x 250,000. The plants were fertilized by 10 tons of manure, 100 kg urea, 150 kg SP3, and 100 kg KCl per ha in the beginning of cultivation. The evaluation of plant productivity was carried out by sampling. For sampling, 10 plants were ramdomly chosen per plot. Seed size was mesured by weighing 100 dry seeds which were ramdomly chosen.

The cultivation was carried out in two seasons, i. e. wet and dry seasons. The cultivation in wet season (season I) was caried out in December 2009 - March 2010, and in dry season was done in May - August 2010. The data of seed productivity per plant and seed size were analyzed by Duncan Multiple Range Test. The clustering of genotype based on seed productivity per plant and seed size was carried out by Important Performance Analysis (IPA). The analysis of stability of seed productivity in two season was carried out by Additive Main Effect Multiplicative Interaction (AMMI).

Results and Discussion

Seed productivity and seed size

The seed productivity of all genotype (KH lines and standard varieties) in the wet season was very high, between 22.9 and 35.6 g per plant, equivalent to 5.7-8.9 tons per ha. This productivity is higher than that in dry season that is between 15.35 and 20.45 g per plant equivalent to 3.8- 5.1 tons per ha (Table 1). Based on the description of Ministry of Agriculture (Deptan, 2011), the productivity of Anjasmoro, Slamet, Tanggamus, and Wilis is 2.25-2.3, 2.26, 1.22, and 1.6 tons per ha respectively. This productivity is lower than that of the same variety in Majalengka that is more than 5 tons per ha (Table1). This result indicates that the environment of cultivation in Majalengka was very favorable for soybean. The soil of this experiment has a pH 5.9 and contains 65,3 ppm of P which are very favorable for soybean growth.

Table 1. The productivity of several genotype in wet and dry seasons

Genotype	Productivity in wet season		Productivity in dry season		Average of Productivity	
	g/plant*	kg/ha	g/plant*	kg/ha	g/plant	kg/ha
KH8	24.74 ab	6,185	17.7 ab	4,425	21.22	5,305
KH9	30.58 bc	7,645	20.45 c	5,113	25.52	6,380
KH31	22.89 a	5,723	19.02 bc	4,755	20.96	5,240
KH38	29.36 b	7,340	19.43 bc	4,858	24.4	6,100
KH55	35.62 c	8,905	17.71 ab	4,428	26.67	6,668
KH71	30.27 bc	7,568	17.06 ab	4,265	23.67	5,918
Anjasmoro	23.43 a	5,858	17.30 ab	4,325	20.37	5,093
Wilis	29.64 b	7,410	16.19 a	4,048	22.92	5,730
Tanggamus	30.56 bc	7,640	15.35 a	3,838	22.96	5,740
Slamet	24.74 ab	6,185	15.42 a	3,855	20.08	5,020

*the number followed by the different letter in the same column is significantly different.

Comparing to Anjasmoro which is elite national variety, all lines have equal or higher productivity in wet and dry seasons. This result indicates that all lines have a potential as elite

national variety with a potential yield more than 5 tons per ha. In wet season, KH55 line has a highest productivity, and in dry season KH9 has a highest productivity.

If we compare between wet and dry seasons, all genotypes have a higher yield in wet season than in dry one. In this experiment, the precipitation during wet season in Majalengka was about 411,53 mm/month, and during dry season was about 202 mm/month. Based on Calvino & Sadras (1999), the precipitation more than 300 mm/month is better for production of soybean.

In average, all lines and Anjasmoro variety have a big seed, and bigger than the seeds of Wilis, Tanggamus and Slamet varieties (Table 2). The big size is very important in the production of tofu because the rendement is depend on the endosperm, and the endosperm of bigger seeds is bigger than small ones. So, based on the size of seeds, all lines have a potential to be released as a big seed varieties. As the yield, the size of seeds was also bigger in the wet season than that in dry ones. The size of seeds may be affected by the enironment of growth. KH31 and KH38 have bigger seeds compared to other genotypes.

Table 2. The size of seed of several genotype in two seasons

Genotype	Size of seed (g/100)		
	Wet season	Dry season	Average
KH8	21.76 e	16.80 bcd	19.28
KH9	22.88 e	15.50 b	19.19
KH31	21.71 e	19.50 e	20.61
KH38	22.82 e	18.46 cde	20.64
KH55	19.00 bc	16.03 b	17.52
KH71	20.74 de	18.70 de	19.72
Anjasmoro	18.50 c	16.60 bc	17.55
Wilis	14.42 ab	10.83 a	12.63
Tanggamus	12.60 a	10.76 a	11.68
Slamet	15.42 b	12.30 a	13.86

Clustering of genotype

In wet season, except KH31 line, other lines have higher seed productivity and seed size compared to Anjasmoro variety. In dry season, KH8, Kh31 and KH38 lines have higher productivity and seed size compared to Anjasmoro variety (Figure 1). This result indicated that KH8 and KH38 lines are consistently better than Anjasmoro variety.

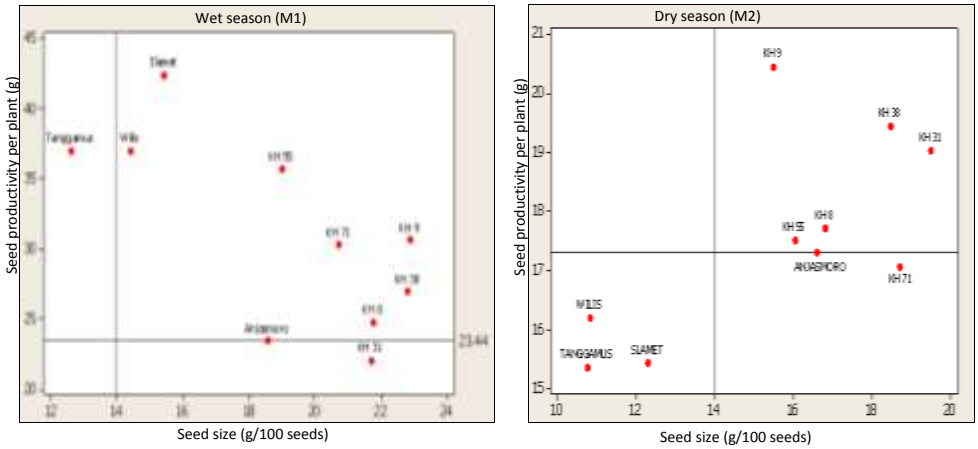

Figure 1. Clustering genotype of soybean based on seed productivity and seed size in wet and dry seasons.

Analysis of stability of seed productivity in two seasons showed that KH71 is the most stable genotype in Majalengka. KH9, KH38 and KH55 lines were more stable than Anjasmoro variety in wet and dry seasons than Anjasmoro Variety (Figure 2). If we consider to the seed productivity, the size of seeds and the stability of seed productivity, KH38 and KH71 are a potential lines to be developed to become elite national varieties.

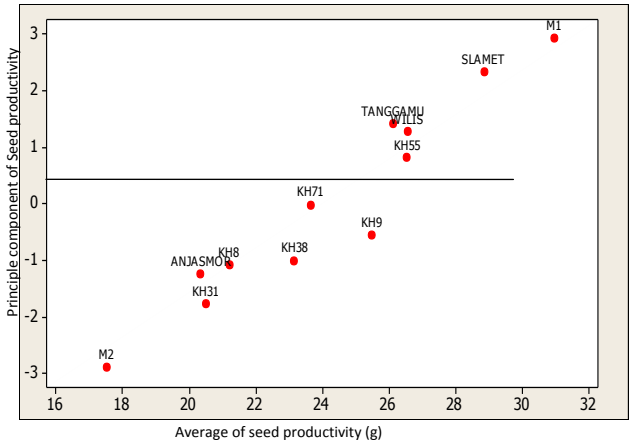

Figure 2. Stability of seed productivity in wet and dry season of soybean in Majalengka. M1= wet season, M2= dry season.

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Paclobutrazol Application Effectiveness on Growth of Two Peanut (*Arachis hypogaea* L.) Varieties

Iskandar Lubis[a,*], Aries Kusumawati[b], Munif Ghulamahdi[c], Heni Purnamawati[a], Yudiwanti WE Kusumo[d], Ahmad Ghozi Mansyuri[e], Sri Astuti Rais[f]

[a] *Laboratory of Crop Production, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
[b] *Depatment of Agronomy, Faculty of Agriculture, Andalas University*
[c] *Laboratory of Ecophysiology, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
[d] *Laboratory of Plant Breeding, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University*
[e] *Legume and Tuber Crop Research Center, Indonesian Ministry of Agriculture*
[f] *Gen Resource and Biotechnology Research and Development Center, Indonesian Ministry of Agriculture*
**Corresponding author: Lab of Crop Production, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University.*
Jalan Meranti 1, Kampus IPB Dramaga, Bogor 16680. Indonesia
Tel/Fax: +62-251-8629353.
iskandarlbs@yahoo.com

Abstract

Peanuts productivities in Indonesia during 1986–2003 ranged 0.7 to 1.2 ton/ha dry seeds, although some new varieties having potential yield from 2.0 to 2.5 ton/ha or more, farmers' productivity reached only 50–60% of the yield. This research was conducted to investigate the effectiveness of paclobutrazol on the growth and production of peanuts. Experiment was conducted at Bogor Agricultural University farms at Cikabayan. Planting was conducted in April to July 2009, using 2 peanut Varieties, namely Sima and Kelinci. Design of experiment was Split-split plot. Paclobutrazol was applied at 6 and 8 Weeks after Planting, WAP as main plot, Varieties (Sima and Kelinci) was used as sub plot and concentrations of Paclobutrazol (0, 100 and 200 ppm) was used as sub-sub plot with three replicates. Size of experimental unit was (3 x 5) m^2, with planting density of 125 000 plants/ha. Urea, SP36 and KCl were applied at planting date at 33.75 kg N, 36 kg P_2O_5 and 45 kg K_2O per ha, respectively. Observation covered growth and production, such as dry matter production, leaf area index, pod number, pod weight, non-structural carbohydrate and total nitrogen in the stem. The results showed that paclobutrazol concentration at 200 ppm increased pod weight and productivity of peanuts, however, did not affect the total soluble carbohydrate and nitrogen content in the stem at 10 WAP. Sima had vigorous growth than that of Kelinci variety showing bigger leaf area and higher chlorophyll.

Keywords : Peanut, paclobutrazol, growth

Introduction

Peanuts productivities in Indonesia during 1986–2003 ranged from 0.7 to 1.2 ton/ha of dry seeds (Kasno, 2004), although some new varieties having potential yield from 2.0 to 2.5 ton/ha or more have been released, farmers' productivity reached only 50–60% of the potential yield. Productivity is related with empty and or imperfect pod filling. Bell and Right (1998) found that peanut population in Indonesia is relatively high, however, many of pod produced empty or partly filled. Lukitas (2005) and Khasanah and Purnamawati (2007) reported that only 26-68% (in average 46%) of gynophores became filled pod. Physiological modification of plant growth by controlling the vegetative growth is one of method overcomes the problem. Growth regulator is used in order to maintain vegetative and reproductive growth balance. It can be suppressed the competition vegetative and generative growth which may decrease assimilate distributed to the sink (Cruz-Aguado *et al*., 1999; Arzani and Roosta, 2004).

Peanut is a semi determinate crop has vegetative growth continuously even if reach the flowering stage. In order to maximize assimilate for filling the pod, it is necessary to investigate source size modification and sink-source balance in peanut. Therefore, retardant as a growth regulator can be used for regulating plant growth balance. Banon *et al*. (2002) reported that one of growth retardant widely used is paclobutrazol. Moreover, Seeno and Isoda (2003) reported that foliar application of paclobutrazol at 100 and 200 ppm in the stage of initial pod formation and initial and medium pod filling increased seed production of peanuts.

This research aims were to investigate the effect of time application of paclobutrazol on the growth of peanuts; to optimize the paclobutrazol concentration to growth and production of peanuts; and to investigate the growth pattern and production of peanuts.

Materials and Methods

Experiment was conducted at Bogor Agricultural University farm at Cikabayan. Planting was conducted for four months from April to July, 2009. Analysis was done at laboratory of RGCI (Research Group of Crop Improvement), Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University. Materials for experiment were peanut seed of Kelinci and Sima varieties, Urea, SP36 and KCl as fertilizers, KCl was applied at planting date at 33.75 kg; N at 36 kg; P_2O_5 of 45 kg K_2O per ha, respectively. Paclobutrazol was used as retardant.

Design of experiment was Split-split plot design where Time of Paclobutrazol applications (at 6 and 8 Weeks after Planting, WAP) was used as main plot, Varieties (Sima dan Kelinci) was used as sub plot and Dosage of Paclobutrazols (0; 100 and 200 ppm) was as sub-sub plot with three replicates, Size of experimental unit was (3 x 5) m^2, with planting density of 125 000 plants/ha. Data were analyzed by F test at 5% level, and advanced test used Duncan Multiple Range Test (DMRT) in 5% level.

Observation included physiological character, growth and production. Growth component covered total dry matter, stem and leaf weight and leaf area index (LAI). Production component covered total number of pod, pod weight and productivity, while physiological character covered chlorophyll, total non-structural carbohydrate (TNC) and stem nitrogen content.

Results and Discussion

The results showed that the single treatment affected the growth of peanut. There was no interaction among time of application, concentration of paclobutrazol and variety. Time of application had significant effect on 8 WAP of peanut. Effect of variety was significant for plant height, total dry matter, culm and leaf dry weight, number of gynophores and pod, 100 seed weight and harvesting index, respectively. Paclobutrazol application had significant effect on plant height, stem dry weight, leaf number, pod number per plant, pod weight per plant, seed weight per plant, seed number per plant and plant productivity, respectively.

Leaf Area Index (LAI)

Statistical analysis showed that at 8, 10 and 12 WAP, LAI was significantly affected by variety. At 12 WAP, Sima had LAI of 4.48 which was significantly higher than that of Kelinci (3.55) (Figure 1). Slow initial growth in peanuts was performed by slow LAI development. Then LAI reached value of 5.5 and 7.0, respectively. Although LAI was higher, assimilate supply still restricted seed yield, this could be to its used for maintaining higher LAI and compensating dead leaf. Then growth of leaf may compete with the development of the sink organ (Gardner *et al.*, 1991).

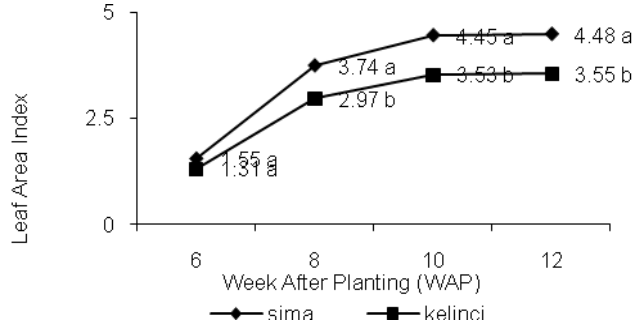

Figure 1. Leaf Area Index of Peanut varieties. Number followed by similar letter is not significant in 5% level.

Pod Number per Plant

Statistical analysis showed that time application of paclobutrazol and varieties had no significant effect on pod number per plant. There was only the concentration of paclobutrazol having significant effect on the pod number per plant. The highest number of pod represented by 200 ppm paclobutrazol reaching 14 pods per plant, while 0 and 100 ppm produced similar number (12) of pods per plant, respectively, as shown in Figure 2.

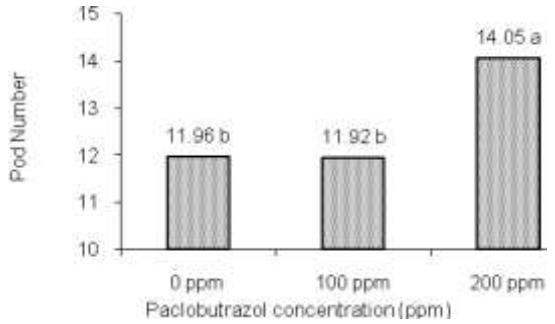

Figure 2. Total number of pod per plant after paclobutrazol treatment.

Productivity

Peanut productivity in this experiment represented by pod dry weight per ha. Statistical analysis showed that paclobutrazol concentration significantly affected peanut productivity. The highest productivity performed by 200 ppm paclobutrazol concentration which was 1.18 ton per ha, while concentration 0 and 100 ppm had no significant differences in productivity (Figure 3).

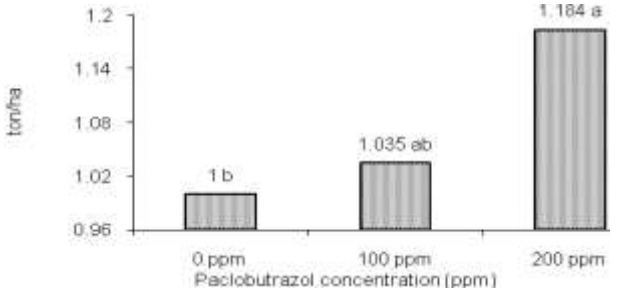

Figure. 3 Productivity after paclobutrazol treatment.

Chlorophyll content

Time of application and paclobutrazol concentrations did not have significant effect on the chlorophyll content. While variety significantly affected the chlorophyll content, and Sima has higher chlorophyll content than Kelinci (Figure 4). Yudiwanti (2007) stated that peanut which has dark green color has higher chlorophyll content and has high yield potential and tolerant to bacterial leaf blight. It is also supported by Taiz and Zeiger (2002) stated that highest content of chlorophyll visually performed by greenness of leaf, and dark green leaf will more efficient to catch solar radiation for photosynthesis.

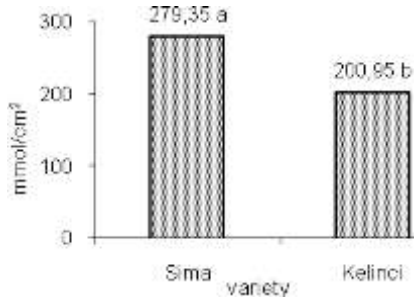

Figure 4. Chlorophyl content of peanut varietyof Sima and Kelinci.

Total Soluble Carbohydrate and Nitrogen Contents

There was no significant effect of paclobutrazol concentration on the content of total soluble carbohydrate (TNC) and Nitrogen content in the stem at 10 WAP, however, the content of TNC in 200ppm was absolutely higher than that of 0 or 100 ppm of paclobutrazol, and in the opposite nitrogen content in 200 ppm was absolutely lower than that at 0 and 100 ppm paclobutrazol, respectively (Figures 5a and 5b).

The effort for increasing potential yield of peanuts is important role in order to breakthrough barrier from physiological factors. The first step is to assess growth of yield organ, is it limited by availability of source or by sink capacity. Recently, genotype differences in allocation and mobilization pattern of assimilate in the stem of wheat has been sown (Cruz-Aguado et.al. 1999).

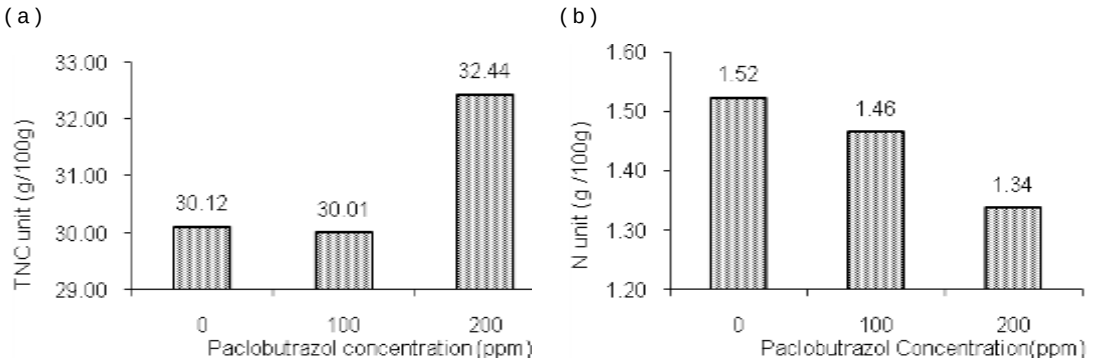

Figure 5. Soluble carbohydrate (a) and Nitrogen (b).content in the stem at 10 WAP as affected by paclobutrazol concentration.

Conclusion

Paclobutrazol concentration at 200 ppm increased pod weight and productivity of peanuts, however, it had no significant effect on total soluble carbohydrate and nitrogen content in the stem at 10 WAP. Sima had vigorous growth than that of Kelinci variety showed by bigger leaf area and higher chlorophyll content in the leaf.

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Effect of Source or Sink Restriction on Flowering, Podding, and Yield Performance in Field-Grown Adzuki Bean

Shinya Kasajima[a,*], Hirotake Itoh[a], Yasuo Nakamaru[a], Hozumi Yoshida[a], Hitoshi Sato[b]

[a] *Faculty of Bioindustry, Tokyo University of Agriculture, Hokkaido, Japan*
[b] *Hokkaido Research Organization Tokachi Agricultural Experiment Station, Hokkaido, Japan*
** Corresponding author: Faculty of Bioindustry, Tokyo University of Agriculture, Yasaka 196, Abashiri, Hokkaido 099-2493, Japan.*
Tel.: +81 152 48 3823
s3kasaji@bioindustry.nodai.ac.jp

Abstract

The effect of source or sink restriction on flowering, podding, and yield performance in field-grown adzuki bean cv. 'Erimo-shozu' was investigated. Defoliation and defloration treatments were performed as source and sink restriction during the flowering period. Defoliation treatment reduced the flower and pod numbers and shortened the period of flowering and podding. Defloration increased the flower and pod numbers. Defoliation treatment was shown to reduce the seed yield and yield components. The 100-grain weight in defoliation treatment was significantly the lowest among all treatments. These results suggest that the seed yield of adzuki bean depends on dry matter production during the flowering period. Furthermore, a compensatory effect in sink restriction indicated by an increase in flower number was also noted.

Keywords: adzuki bean, defloration, defoliation, flowering, podding, yield component

Introduction

Adzuki bean (*Vigna angularis* (Willd.) Ohwi et Ohashi) is the second most important leguminous crop in Japan after soybean (*Glycine max* (L.) Merr.). It is widely used for the production of a sweet bean paste called Ann, which is a major component in traditional Japanese sweets (Murata, 1999). It has been commercially produced in Hokkaido, Northern Japan, where crop breeding program is mainly carried out in that area (Shimada, 2006). In recent years, improvement of yield stability is an important issue to be considered since the production efficiency of adzuki bean grown in Hokkaido varies drastically each year because of higher rates of damage due to cold weather (Shimada, 2006). However, up-to-date information with regard to the yield-determining process of adzuki bean has been limited because of its minor role worldwide.

In adzuki bean, as in many other leguminous crops, knowing the habit of flowering and podding is considered to be important for analyzing the yield-determining process. Compared to the soybean, the adzuki bean has a long flowering period and a low podding efficiency (Takahashi, 1958). In general, source-sink relationships play an important role in the yield-determining process. For example, defoliation or defloration treatments of soybean plants accelerated the development of flower buds and podding (Saitoh *et al*., 2001). However, there is limited information about the effect of source or sink restriction on flowering, podding, and yield performance of adzuki bean. In order to evaluate the relative importance of source or sink on yield-determining process, we examined the changes in flower and pod numbers and yield components in field-grown adzuki bean plants as affected by defoliation or defloration treatments.

Materials and Methods

The field experiment was conducted in the Tokyo University of Agriculture farm at Abashiri, Hokkaido, Japan (43°53′44″N, 144°21′45″E). The soil type was andosol, and the preceding crop was barley (*Hordeum vulgare* L.). Adzuki bean cv. 'Erimo-shozu', which is a most famous leading variety of adzuki bean in Hokkaido, was used in this study. Four seeds per hill were seeded on June 2, 2010, at 16.6-cm spacing in approximately 3.5-m-long rows with 72-cm row spacing. The seedlings were thinned to two seedlings per hill three weeks after seeding. Compound fertilizer ($N:P_2O_5:K_2O$ = 5:25:14%) at 80 g m^{-2} was applied as the basal application. Although the summer air temperature in 2010 was higher than that in the average year, this experiment was performed under normal climatic conditions.

Source or sink restriction treatment was initiated on July 26, after flowering began. Source restriction treatment consisted of removing two lateral leaflets of every trifoliate leaf when flowering began. Sink restriction treatment consisted of removing every flower on each branch. After initiation, defoliation and defloration were continued until flowering ceased. Six experimental plots including a control were set up according to a randomized block design with two replications.

The change in flower and pod numbers was investigated for 5 plant samples in each plot. The flowering date was recorded on a small label (9 mm in width and 22 mm in length) for each plant, and then the label was wrapped around the pedicel of the flower. The labels were attached on all flowers that had formed pods by the time of maturity. The podding rate was calculated from the flower and pod numbers during the entire flowering period based on the label data. At maturity, 20 standard plants from 10 hills in each plot were sampled except for the plant with attached labels. After air-drying, the seed yield and yield components—namely, pod number, seed number per pod, and 100-seed weight—were examined.

Results and Discussion

The average temperatures during the vegetative and reproductive stages were 17.5°C and 19.6°C, respectively. The growth and yield of adzuki bean are known to be susceptible to cold weather damage (Shimada, 2006), e.g., the low temperature during vegetative growth induced abortion of main stem elongation, and low temperature before flowering induced podding injury in a previous study (Aoyama *et al*., 2009). In the present experiment, air temperature during every growth phase tended to be higher than that in the average year. The main stem length and top dry weight at maturity in control treatment condition were 83.4 cm and 640 g m^{-2}, respectively. It was confirmed that in the normal plant type, flowering and podding were not affected by low temperatures.

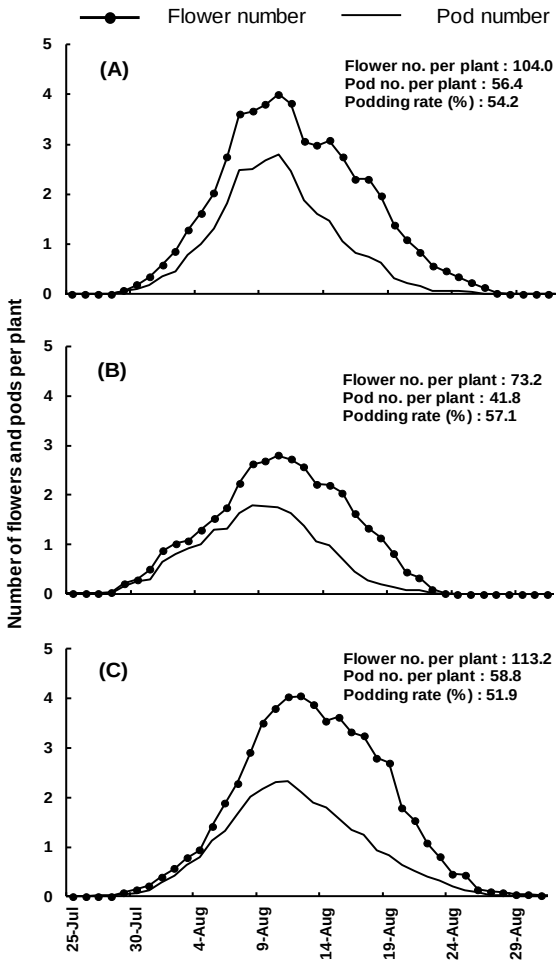
The change in flower and pod numbers per plant differed among treatments (Fig. 1). In control treatment, the cumulative flower and pod numbers per plant were 104.0 and 56.4, respectively (Fig. 1A). In source restriction treatment, however, the flower and pod numbers were reduced and the period of flowering and podding was shortened compared to control (Fig. 1B). Data indicated that dry matter production after flowering affects the flower and pod numbers. Furthermore, this study clarified that the reduction in dry matter production also affects the flowering and podding periods. Conversely, the flower and pod numbers in sink restriction treatment were higher than those in the control but the podding rate was lowest among all treatments (Fig. 1C). These results suggested that sink restriction treatment was compensated by a drastic increase in the flower number. In this study, sink restriction treatment consisted of removing all flowers from all branches. The effects of source and sink manipulations on the flower numbers of soybean varied with flower position between the main stem and branches (Saitoh *et al*., 2001). Thus, further studies are required to clarify the source-sink relationship between the main stem and branches of adzuki bean.

Table 1 showed the effect of source or sink restriction on the seed yield and yield components of adzuki bean. The seed yield tended to be lower in source restriction treatment than in control and sink restriction treatments, although no significant difference was observed. Similar tendencies were observed in yield components. The 100-seed weight in source restriction treatment was significantly lower than the weights in other treatments. The decline in 100-seed weight by defoliation suggested that the seed yield of adzuki bean might be limited by source size, which was consistent with previous reports on soybeans (Egli & Leggett, 1976; Saitoh *et al*., 2001).

Table 1. Effect of source or sink restriction on the seed yield and yield components of adzuki bean

Treatment	Seed Yield (g m^{-2})	Pod no. (no. m^{-2})	Seed no. per Pod	100-seed weight (g)
Control	358 a	487 a	5.52 a	12.9 a
Source restriction	305 a	438 a	5.42 a	12.1 b
Sink restriction	338 a	463 a	5.48 a	13.0 a

Values with different letters in a single column are significantly different at the 5% significance level by Tukey–Kramer's test.



Data shows 5-days moving average with 10 plants of 2 replicates. The flower number, pod number, and podding rate in each figure show the values of entire examination periods.

Figure 1. The change in flower and pod numbers per plant in control (A), source restriction (B), and sink restriction (C).

In conclusion, this study suggested that the seed yield of adzuki bean depends on dry matter production during the flowering period. A compensatory effect in sink restriction treatment indicated by an increase in flower number was also observed. Moreover, defoliation treatment shortened the flowering and podding periods. This result may be related to early maturity. In the future, we plan to conduct additional experiments using early maturing varieties.

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*In Japanese.
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Analysis of Genes Expressed during the Early Maturation of Sesame Seeds

Motonobu Yoshida, Akihiro Kishikawa, Takayuki Nakano, Eiji Tanesaka

Lab of Plant Breeding and Agrobiotechnology, Department of Agricultural Science
Kinki University
Nakamachi 3327-204, Nara 631-8505, Japan
Tel: 81-742 43 5245, Fax: 81-742 43 1155
yoshida_m@nara.kindai.ac.jp

Abstract

Sesame seeds of cultivated species, *Sesamum indicum,* contain abundant oil, in particular, large amounts of unsaturated fatty acid oleic and linoleic acids. Total amounts of oleic and linoleic acids in sesame seeds keep constant among varieties of *S. indicum*. However, the regulation of the mechanism is unknown. Therefore, this study attempted to clarify these points through transformation of *Arabidopsis thaliana* with novel genes in sesame seeds. Oil contents increase rapidly in sesame seeds within 4 weeks after flowering. A full-length cDNA library prepared from sesame seed of 1 to 3 weeks oldwas substracted with cDNAs from plantlets of 4 weeks after germination. The results showed that the cDNA library was expressed specifically during the early maturation.The 1,545 cDNA clones were sequenced. Of these, 30.3% of the clones were responsible for protein destination, 16.7% of the clones for the metabolism, and 6.9% of the clones for the cell growth and division. Among them, 13 genes for a transcription factor were identified, four were identified as a transcription factor involved in ethylene signaling. In addition, nine genes: osmotin-like protein, expansin-like protein, aquaporin-like protein, MADS-box protein 4, novel putative uncharacterized protein, ethylene response factor 2, ethylene responsive element binding protein 3, AP2 containing transcription factor and lipid transfer protein were analyzed by overexpression of *A. thaliana.* The *A. thaliana* overexpression strain for novel putative uncharacterized protein and aquaporin-like protein, respectively showed the increase of unsaturated fatty acids. The amount of oleic acid per seed grain in a strain for novel protein was 1.7 fold, and that of linoleic acid 1.5 fold, respectively.

Keywords: Sesamum indicum*, fatty acids, full-length cDNA, Arabidopsis transformation*

Introduction

Sesame seeds of cultivated species, *Sesamum indicum,* contain abundant oil, in particular, large amounts of unsaturated fatty acid oleic and linoleic acids. These unsaturated fatty acids lower the cholesterol level in the body (Satchithanandam *et al*., 1993). Because of their effects and antioxidant substances, attention has been paid to sesame seeds as health food (Budowski and Markley, 1951). Total amounts of oleic and linoleic acids in sesame seeds are constant among varieties of *S. indicum*. However, the regulation of the mechanism is unknown. It is necessary to clarify these points to breed novel varieties with high content of unsaturated fatty acids. Oil contents of sesame seeds increase rapidly within 4 weeks day after flowering (DAF). Therefore, this study attempted to analyze the function of novel genes expressed specifically during the early maturation of sesame seeds through transformation of *Arabidopsis thaliana* with novel genes of sesame seeds.

Materials and Methods

Plant materials and growth conditions

Sesamum indicum seeds were obtained from Toyama University. *Arabidopsis thaliana* plants were grown under continuous light at 22-23^{o}C and used for transformation.

Preparation of a full-lengh cDNA library and cDNA materials

Total RNAs were prepared from *S. indicum* seeds of 1 to 3 weeks old. A full-length cDNA library was prepared using pCMVFL3 vector as described previously (Uenishi *et al*., 2004). Total RNAs from young plants were extracted four weeks after germination. After purification of poly $(A)^+$RNA, cDNA materials were synthesized with cDNA synthesis kit M-MLV version (TaKaRa). A full-lengh cDNA library expressed specifically during the early maturation was prepared by subtraction of the following methods. A full-lengh cDNA library from *S. indicum* seeds of 1 to 3 weeks old was amplified with SP6 primer, T7 primer and the PCR programme which was 16 cycles of 98^{o}C for 10 sec, 56^{o}C for 5 sec and 72^{o}C for 3 min, followed by 72^{o}C for 3 min. They were used as a tester cDNA in subtraction. The subtraction was carried out with DsDD cDNA subtraction kit (Wako) using amplified cDNAs from a full-length cDNA library as a tester cDNA and cDNA materials from young plants of four weeks after germination as a driver cDNA. Subtracted cDNA clones were amplified with the PCR programme which was 35 cycles of 98^{o}C for 10 sec, 56^{o}C for 5 sec and 72^{o}C for 3 min, followed by 72^{o}C for 7 min. After removing cDNA clones of the lower molecular weight with Chroma spin^{TM}-200 (Clontech), they were selected in size with Chroma spin^{TM}-400, and pooled into four groups (fraction No. 4, fractions No. 5-7, fractions No. 8-14, fractions No. 15-30). Each pooled cDNA clone was amplified with *EX-taq* polymerase (TaKaRa), SP6 primer, T7 primer and the PCR programme which was 25 cycles of 98^{o}C for 20 sec, 50^{o}C for 30 sec and 72^{o}C for 3 min, followed by 72^{o}C for 5 min. They were ligated into pCR8/GW/TOPO vector (Invitrogen).

Sequence of cDNA clone

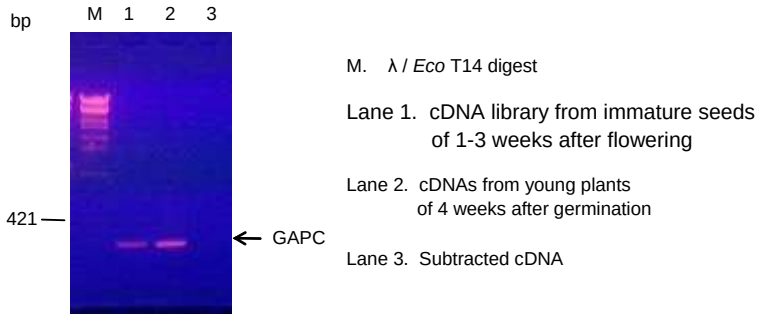
The cDNA clones from three groups (fraction No. 1-4, fraction No. 5-7, fraction No. 8-14) were sequenced with ABI Prism® 3100-Avant Genetic Analyzer (Applied Biosystems) using BigDye® terminator v3.1 cycle sequencing kit (Applied Biosystems). Homology analysis was carried out with BLASTX (DDBJ; http://www.ddbj.nig.ac.jp/welcome-j. html).

***Transformation of* Arabidopsis thaliana**

The cDNA clones ligated into pCR8/GW/TOPO vector were transferred into pBI-OX-GW (Inplanta Innovations Inc.) by using Gateway System (Invitrogen). Each cDNA clone transferred into pBI-OX-GW was used for the transformation of *Arabidopsis thaliana* which was done as described in a previous paper (Clough and Bent, 1998), with minor modification.

Results and Discussion

To obtain cDNA clones expressed specifically during the early maturation of *S. indicum* seeds, the subtraction was carried out using amplified cDNAs from a full-length cDNA library from *S. indicum* seeds of 1 to 3 weeks old as a tester cDNA and cDNA materials from young plants of four weeks after germination as a driver cDNA. The subtraction efficiency was confirmed with the PCR programme which was 22 cycles of 98^{o}C for 20 sec, 50^{o}C for 30 sec and 72^{o}C for 1 min, followed by 72^{o}C for 3 min. The primers were GAPC-F primer (5'-CCAACGCTAGCTGCACCAC-3') and GAPC-R primer (5'-AGGTCAACAACTGAGACATC-3') of the gene for cytosolic glyceraldehyde-3- phosphate dehydrogenase (gapC) which is one of house keeping genes (Figure 1). Signals were observed in both a full-length cDNA library from *S. indicum* seeds of 1 to 3 weeks old and cDNA materials from young plants of four weeks after germination, while no signals were observed in the subtracted cDNA library, suggesting that subtraction was efficiently performed.



GAPC : Cytosolic glyceraldehyde-3- phosphate dehydrogenase

Figure 1. Confirmation of subtraction efficiency.

The subtracted cDNA library was amplified and separated in size into four groups (fraction No. 4, fractions No. 5-7, fractions No. 8-14, fractions No. 15-30). Fraction No. 15-30 mainly contains cDNA clones of less than 400 bp. Therefore, 1,545 cDNA clones were sequenced from fractions No. 4-14. Properties of cDNA clones in fraction No. 4, fractions No. 5-7 and fractions No. 8-14 were summarized as the following; the average size 1,043.7 bp in fraction 4; 996.7 bp in fractions 5-7; 706.4 bp in fractions 8-14. The 1,545 cDNA clones were categorized and summarized with classification of each function (Table 1). The cDNA clones responsible for protein destination formed the largest percentage of 30.52%. Next, the cDNA clones responsible for metabolism formed the second largest percentage of 16.36%, the cDNA clones for cell growth, division were 7.01%, the cDNA clones for transcription and RNA processing were 4.48%. Unknown and novel cDNA clones were 30.39%. Comparison of our results with results by Suh *et al*. (2003) showed that subtraction was effectively performed from the fact that the occupation percentage of house keeping genes was low, and that of unknown novel genes was high at the present experiment. Of them, functional analysis of nine cDNA clones: osmotin-like protein, expansin-like protein, aquaporin-like protein, MADS-box protein 4, novel putative uncharacterized protein, ethylene response factor 2, ethylene responsive element binding protein 3, AP2 containing transcription factor and lipid transfer protein was carried out by overexpression of *A. thaliana.* Southern blot analysis in transformed *A. thaliana* using each DIG-labeled sesame gene as a probe showed that each sesame gene was indeed introduced into transformed *A. thaliana* (data not shown). The *A. thaliana* overexpression strain for novel putative uncharacterized protein and aquaporin-like protein showed the increase of unsaturated fatty acids in *A. thaliana* seeds. The amount of oleic acid per seed grain in the overexpression strain for novel putative uncharacterized protein was 1.7 fold, and that of linoleic acid 1.5 fold. In a strain for aquaporin-like protein, the amount of oleic acid was 1.4 fold, and that of linoleic acid 1.5 fold.

Tabel 1. Functional category of identified genes

Function	%
Protein destination	30.52
Metabolism	16.36
Cell growth, division	7.01
Transcription and RNA processing	4.48
Cell resue, defense, senescence and death	4.16
Transport	2.27
Signal transduction	2.14
Energy generation	1.10
Protein synthesis	1.04
Intracellular trafficking	0.32
Cellular organization/biogenesis	0.19
unknown	30.39

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Effects of Different Seeding Dates on Pattern of Internode Length in Sorghum Variety 'Kazetachi'

Akihiro Fujii[a*], Satoshi Nakamura[b], Mitsuo Saito[b] ,Yusuke Goto[a]

[a]*Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan*
[b] *School of Food, Agricultural and Environmental Sciences, Miyagi University, Sendai, Miyagi, Japan*
** Corresponding author: Graduate School of Agricultural Science, Tohoku University,*
1-1 Amamiya-machi, Tsutsumidori, Aoba-ku, Sendai, Miyagi, 981-8555, Japan
Tel.: +81-22-717-8639
b0am1131@s.tohoku.ac.jp

Abstract

Sorghum (*Sorghum bicolor* Moench) stems, which have many internodes, are the main organs harvested for feed and biofuel materials. Sorghum reportedly has three types, characterized by individual internode length based on the internode position from base to top: 1) ever-increasing type, with internode length increasing from the base to the top internode; 2) unimodal type, with internode length in approximately the middle internode position that is longer than in lower or upper positions, except at the neck internode of the panicle; 3) bimodal type, for which the internode lengths alternately increases and decreases twice before reaching the peduncle. Sorghum variety 'Kazetachi', an ultralate-maturity variety in Japan with strong lodging tolerance, showed patterns with many peaks. It is a multimodal pattern in internode length. Furthermore, this variety shows large variation in the number of peaks per plant and the internode position with a peak among plants. This report describes internode length pattern analysis of 'Kazetachi' and the assessment of seeding date effects on the pattern. Seeds were sown on 20 May, and 3 and 18 June in 2009 in the northern part of Japan. Internode lengths were measured at harvest in early November. Peaks in the internode length pattern of each plant were 4–7, including both distinct and vague peaks. Pattern analysis using the internode position with a distinct peak contributed to the distinction among patterns on each seeding date. Although internode positions showing a distinct peak differed among patterns of different seeding dates, the period during which the internode with distinct peaks elongated rapidly appeared to be almost identical among them, suggesting that the internode length in 'Kazetachi' is characterized more by environmental factors than by genetic factors.

Keywords: internode length, *multimodal pattern*, *pattern of internode length*, Sorghum bicolor *Moench*

Introduction

Sorghum (*Sorghum bicolor* Moench) stems, consisting of many internodes, are the main organ harvested for feed and making syrup. Sweet sorghum has attracted attention recently as a biomass energy crop because its stems accumulate large amounts of sugars, which are convertible into ethanol: bioethanol. However, little information about development of cultivation techniques to increase stem yield in sweet sorghum exists because most research efforts have emphasized increasing of grain yields for grain sorghum. Stem size of sweet sorghum is the important factor that determines the stem yield including sugar yield (Tsuchihashi and Goto 2004; Sato *et al*., 2008; Nakamura et al., 2009).Increasing sweet sorghum stem yields necessitates research into internode formation in stems. Three types of sorghum can be characterized by individual internode length based on the internode position from the base to top (Ayyangar *et al*., 1938). 1) For the ever-increasing type, the internode length increases from the base to the top internode. 2) The unimodal type has internode length in approximately the middle internode position that is longer than in lower or upper positions, except at the neck internode of the panicle. 3) In the bimodal type, the internode lengths increase gradually, then decrease and increase slightly, and decrease again, with one more increase before reaching the peduncle. However, 'Kazetachi', ultralate-maturity variety, with strong tolerance of lodging in Japan, shows new patterns with many peaks: a multimodal pattern of

internode length. This variety also shows large variation in the number of peaks per plant and the internode position, with the peak among plants (Figs. 1, 2) (Nakamura *et al*., 2011). This study assesses a method to analyze the internode length pattern in 'Kazetachi' and examines effects of the seeding date on the pattern.

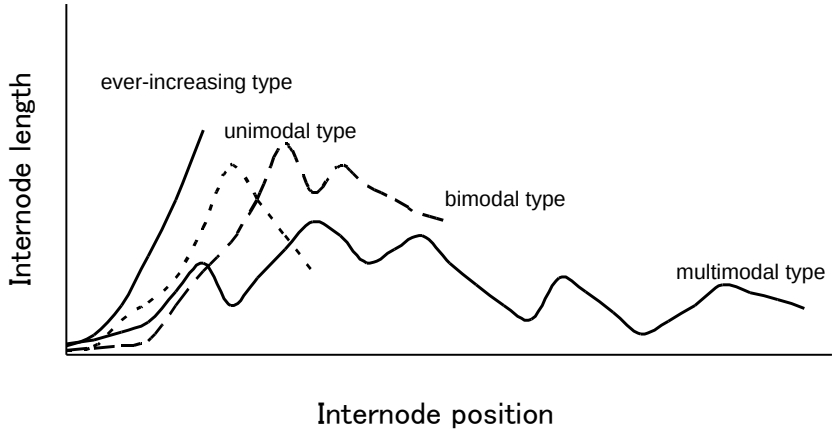

Figure 1. Schematic depiction of three internode length patterns—the ever-increasing, unimodal, and bimodal type—in sorghum, with the multimodal type shown for 'Kazetachi'.

Figure 2. Short internodes among upper internodes of a stem of sorghum variety' Kazetachi'.

Materials and Methods

This study was conducted at the experimental field of the School of Food, Agricultural and Environmental Sciences, Miyagi University, Japan. Seeds were sown on 20 May (S1), 3 June (S2), and 18 June (S3) in 2009 with three replications. Seedlings were thinned to one per hill at 0.85 m distance between the lines and to 15.0 cm between the plants. A basal fertilizer of 16–14–16 g NPK per m^{-1} was applied. The internode length was measured for each of the 18 plants at harvest in the beginning of November.

The (*n*)-th internode (IN n) was defined that between the (*n*+1)-th node and the (*n*)-th node where the (*n*)-th leaf is attached. The internode position of the peak in the internode length pattern was defined as the internode position that was longer than just upper and lower internodes.

Results and Discussion

The average of the total leaf number on the main stem was 30.7 ± 0.1 (S1), 29.8 ± 0.2 (S2), 29.1 ± 0.2 (S3). Figure 3 shows the internode length of each internode position through IN8–IN28 at each seeding date. The peaks per plant in the internode length patterns shown in S1, S2, and S3 were 3–5, from 3–6, and from 3–5, respectively. The average numbers of peaks were 3.6 ± 0.2 (S1), 4.4 ± 0.2 (S2), and 3.8 ± 0.2 (S3) (Fig. 4). Distinct and vague peaks existin the internode

length patterns of all plants, but the average patterns had very similar shapes. Three distinct peaks were observed in the average pattern, irrespective of the seeding date (Fig. 3). According to this result, these peaks were designated acropetally as peak I, peak II, and peak III. The respective internode positions with peak I in S1, S2, and S3 were IN12–IN14 (13.1 avg.), IN14–IN17 (14.7 avg.), and IN11–IN13 (12.0 avg.) (Fig. 5 and Table 1). The respective internode positions with peak II in S1, S2, and S3 were IN20–IN21 (20.4 avg.), IN18–IN21 (19.4 avg.) and IN16–IN20 (17.7 avg.). The respective internode positions with peak III in S1, S2, and S3 were IN25–IN27 (26.1 avg.), IN23–IN26 (24.8 avg.), and IN21–IN26 (23.4 avg.). These results show that the average internode positions of peak II and peak III shifted lower with a later seeding date, excepting peak I.

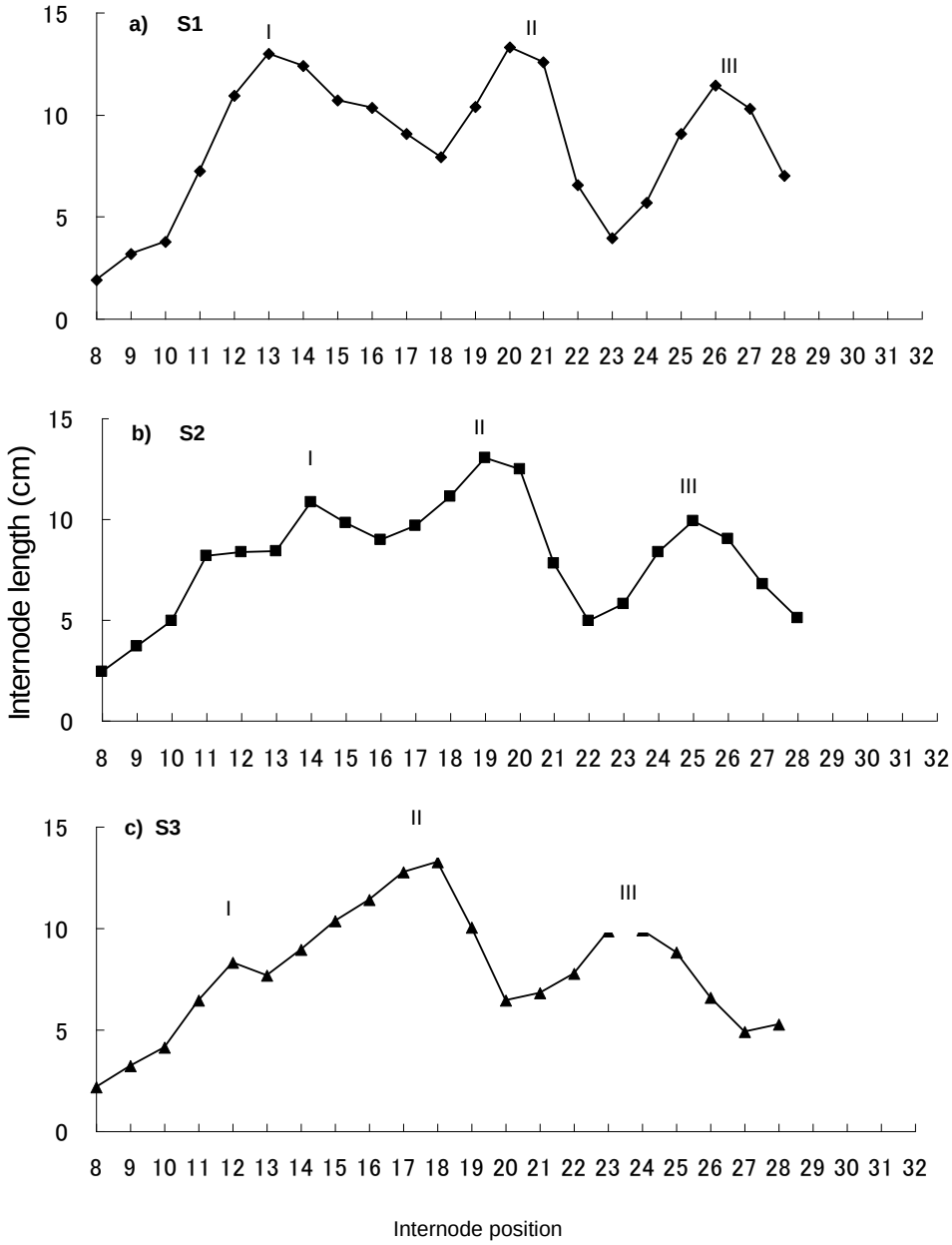

Figure 3. Internode lengths through IN8–IN28 in S1 (a), S2 (b) and S3 (c).

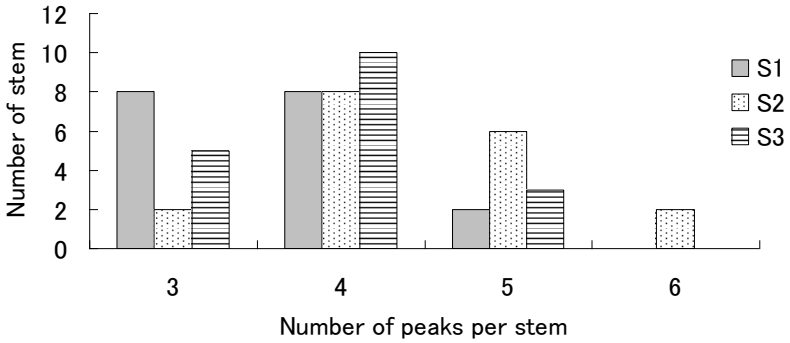

Figure 4. Number of internode length pattern peaks on each seeding date.

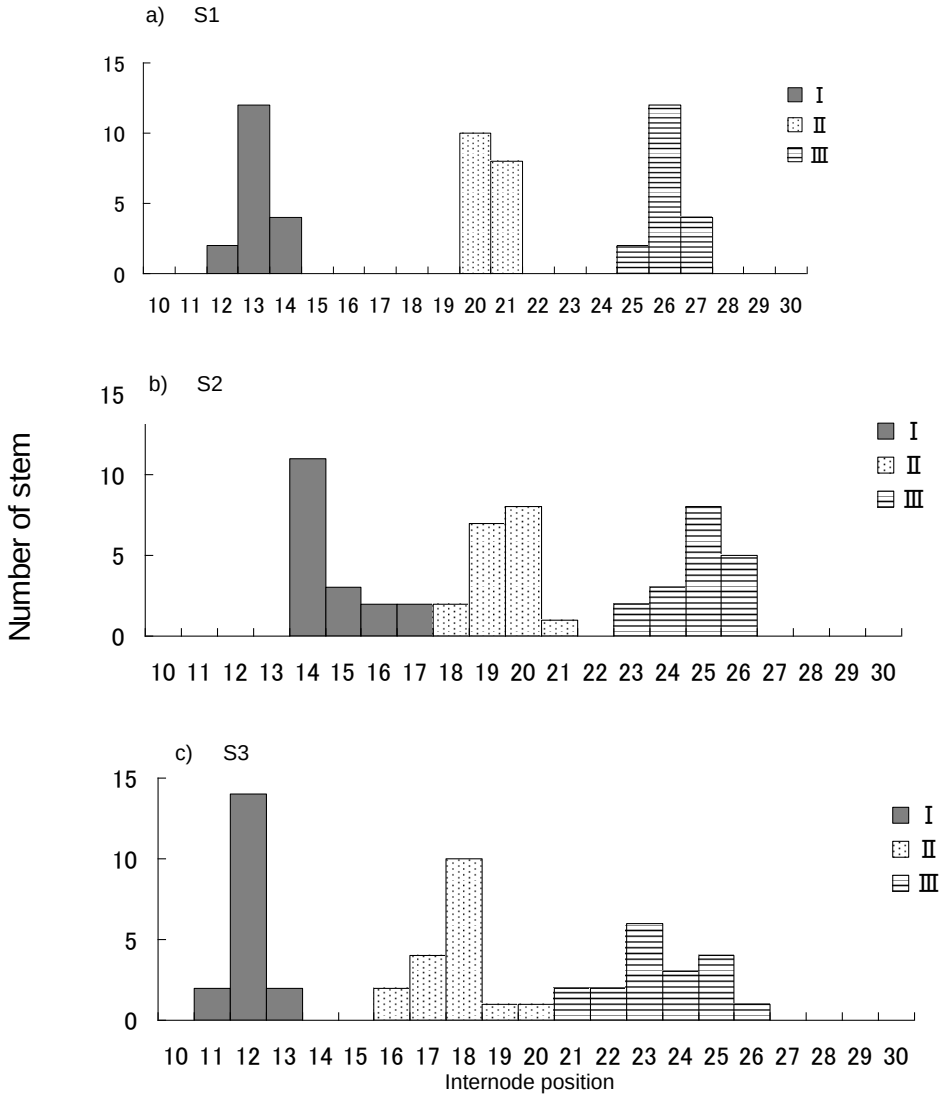

Figure 5. Internode positions with peak I – peak III in S1 (a), S2 (b), and S3 (c).

Table 1. Internode position and average internode position for peak I – peak III for S1, S2, and S3

plot	Range of internode position			Average of internode position		
	I	II	III	I	II	III
S1	12 – 14	20 – 21	25 - 27	13.1± 0.1	20.4± 0.3	26.1± 0.2
S2	14 – 17	18 – 21	23 – 26	14.7± 0.1	19.4± 0.2	24.8± 0.3
S3	11 - 13	16 - 20	21 – 26	12.0± 0.1	17.7± 0.2	23.4± 0.4

According to an investigation of the internodes as they increased rapidly in each plot on 5 September 2009, the positions of the internode in S1, S2 and S3 were estimated respectively as IN20, IN19, and IN18. These internode positions correspond to internodes with peak II in each plot, indicating that the internodes with peak II in S1, S2 and S3 were elongating at almost the same period. These results suggest that the internode length in sorghum variety 'Kazetachi' is characterized more strongly by environmental factors than by genetic factors.

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Pranajiwa as Raw Material for Bio-Oil

Endang Yuniastuti, Susanti Indriya Wati

Agricultural Faculty, Sebelas Maret University Surakarta, Indonesia
e.yuniastuti_ratu@yahoo.co.id

Abstract

The high increase in the number of human population every year causes the increase of human need for energy. However, it unfortunately still does not meet the production of energy sources.. This situation forces us to seek new sources of energies which are certainly eco-friendly, such as utilization of natural resources for biofuels. One of altenative solution is the use of Pranajiwa plants as a source for production of bio-oil. Pranajiwa or Pranajiwa (*Sterculia foetida* L.) is one species of plants in Indonesia originally from East Africa, Tropical Asia and Australia. This kind of tree crops with a kind of tall trees between 30-40 m, the tree is often found in forest. Pranajiwa seeds contain vegetable oils that can be used as an ingredient for industrial products such as cosmetics, soaps, shampoos, fabric softeners, paints, plastics and as an adaptive agent biodiesel. Pranajiwa seeds contain vegetable oils comprising a fatty acid that is sterculat acid. This plant has several advantages over other alternative crops as sources for oil production. Pranajiwa plants is not used for consumption that is dfferent from oil palm, sugarcane, coconuts, yams or cassava that has a primary function for consumption. Pranajiwa plants had fairly high oil yield that was greater than 40%. Event from a research conducted with the purification using ether, the yield of oil could reach more than 70%. Whereas using direct processing, the average oil yield rearched 45%. For biofuels, Pranajiwa seeds can then be extracted and used as raw material of bio-oil.

Keywords: Pranajiwa, bio-oil, alternative energy

Introduction

Increase in population leds to the increase of human need for energy. The energy currently comes from fossil and no longer it will run out. This situation encourages the Indonesian nation to immediately provide and build a populist-based alternative energy that is environmentally friendly by utilizing natural resources and existing plants as a source of energy that can be harnessed and developed. One form of energy is Biofuel (BBN).

Some plants such as jatropha, sweet potatoes, cassava, sugarcane, oil palm, coconut, sunflower, kapok, and pranajiwa or commonly known as a plant "Pranajiwa" can be developed as a biofuel or biofuel farming and to support energy programs or green energy. , The crop plant "Pranajiwa" *(Sterculia foetida* Linn.) is considered more appropriate to be used as Biofuel (BBN) or biofuels, especially as the bio-oil (lubricating oil) and bio-diesel (diesel substitute). This is supported by the fatty acid content which can be also used as an adaptive biodiesel.

However, the existence of plants "Pranajiwa" is now rarely found, even the presence status of plants "Pranajiwa" included a rare (Yuniastuti, 2008). This is partly because these plants have growing requirements at altitude less than 500 m above sea level, and exacerbated by logging and illegal use of timber for furniture materials or raw materials made of paper without rejuvenation. The remaining plants are only in sacred places because people are afraid to cut them down. Cultivation of crops "Pranajiwa" is very little or not at all and eventually the plant is just a legacy from ancestors that will be extinct. In this condition, it is necessary to give serious attention for "Pranajiwa" that has a potential as a producer of alternative energy (bio-oil). Cultivation "Pranajiwa" can be either generative or vegetative.

Pranajiwa *(Sterculia foetida)* is a kind of tree crops with high stature plants between 30-40 m, the tree is often found in the woods. Nomenclature is taken from the Roman myth, the name of god or *Sterquilinus Sterculius fertilizer.* Together with the species name, *foetida* (meaning, hard-smelling, foul) scientific name refers to the unpleasant odor released by these trees, especially flowers. In some areas in Indonesia, this plant is also used as a drug. Plant leaves can be used as an herbal remedy laxative, diuretic and mosquito deterrent; bark as a cure of some diseases; tree wood can be used as building construction; other than edible seeds can also be used as a cough medicine (Yuniastuti, 2010). Pranajiwa oil benefits directly related to the fatty acid content sterculat which is unsaturated fats in seeds (Anggraeni, 2005)

Pranajiwa as an alternative energy-producing plant has several advantages over other alternative energy crops, such as oil palm, sugarcane, coconuts, yams, and cassava. Pranajiwa is not a crop for consumptions; while oil palm, sugarcane, coconuts, yams or cassava has the primary function for consumption. Also compared to other alternative energy crops, Pranajiwa has high oil yield that is greater than 40%, even from research conducted by refining petroleum ether the yield can reach more than 70%. While using direct processing (compound) average oil yield reached 45%. As Fuel Plant (BBN), plant seeds and billowing retrieved and extracted can then be used as raw material for lubricating oils (bio-oil) (Yuniastuti, 2010).

Materials and Method

Locations of research

Research locations were in 10 districts in Central Java, namely:

1. Karanganyar District: Karangmojo, Karangwuni, Derman, Ngaliyan1, Bibis, Kodokan, Teak, Ngaliyan 2, Ngetal, Jetak
2. Sukoharjo District: Shower, Cangkol, Macanan, Temulus, Pondok 1, Pondok 2, Minggiran, Nderso, Palur, Triyagan.
3. Boyolali District: Tegalmuncar, Klabang, Kendal big, Tlatar, Jagoan, Tagung, Badan, Gatak Malangan, Pojok, Bulu
4. Purworejo District: Kroyo, Trukan, Doplang, Sindurjan, Tegal Kuning, Pogung Juru Tengah, Borowetan, Bedug, Paduroso, Bayan
5. Wonogiri District: Krisak, Bulak, Godean, Donorejo, Kaloran, Nambangan, Gerdu, Brajan, Gebang Wetan, Mblimbing
6. Klaten District: Kurung, Kalikebo, Jetis, Talang, Jerukan 1, Jerukan 2, Kebon, Sabrang, Pandanrejo, Planggu.
7. Sragen District: Plalar, Toyogo, Bero, Pengkol, Sambung Macan, Mojopahit, Pucang, Drojo, Tunjung Semi, Demakan.
8. Grobogan District: Gendingan, Tambirejo, Nambuhan, Bakalan, Candisari, Kepayu, Penawangan, Getasrejo, Kronggen, Rejosari.
9. Blora District: Brengus, Dlongos, Keboan, Gagakan, Pudak, Loworejo, Jambangan, Putat, Sukorame, Sambiroto
10. Semarang District: Ngener, Mbatan Kidul, Tawang, Reksosari, Muncar Jaten, Mrakas, Durenan, Sambiroto, Dadapayam

Analysis of Morphology, Cytology and Molecular conducted at the Central Laboratory of the Sebelas Maret University, Laboratory of Plant Breeding, Physiology and Biotechnology Faculty of Agriculture, Sebelas Maret University, Surakarta.

Plant materials

Plant materials used in this study were Pranajiwa or billowing plants *(Sterculia foetida Linn.)* existing in the region of Central Java which covers 10 districts of Sragen, Boyolali, Klaten, Wonogiri, Sukoharjo, Karanganyar, Semarang, Purworejo, Grobogan and Blora.

Activities of the experiment included:

1. Identification of Morphology
2. Cytology
3. Analysis of Chemical Components
4. Seeding and In Vitro Plant Propagation Pranajiwa *(Sterculia foetida* Linn)

Results and Discussion

In the habitus of the plants, grendruwo is a tree with height reaching 45 meters. Plant leaves of "Pranajiwa" grow at the end of branches, is compound leaves, and palmatus shaped leaves with 7-8 children (foliolum). unfoliolatus-shaped leaves, long strands of leaves (laminae) between 10-17 cm with a smooth leaf surface. Flower crop "Pranajiwa" grow at the end of branches and branching to form clumps, and yellow to purplish red. Flower is unisexual with diameters

ranged between 2 – 2.5 cm. "Pranajiwa" Fruits are large enough with a length of about 10 cm, oval-shaped, smooth and woody rind. Seeds of black fruit have a length of between 1.5 to 2.0 cm. "Pranajiwa" fruits are green when still young, then become red and brown when old and will fall to ground. "Pranajiwa" fruits are also unique and strange that are comprised of five lumps (locus), quite large, weighing 1-3 kg that further strengthens the community,s assumption that the fruit is a fruit of "Pranajiwa" (devil fruit). The seeds of "Pranajiwa" fruits will fall and not be utilized optimally because many people are afraid to use it because of the above reason.

Ecologically, the "Pranajiwa" plants serve as a micro-habitat of animals and certain animals. It is reported in Komodo Island the population of yellow-crested parrot *(Cacatua subphurea parvula)* are protected and use “Pranajiwa” trees as a nest. "Pranajiwa" plants have crowns and roots that are fairly large and it can function as a regulator of the hydrological cycle because the roots can hold soil water in a large enough capacity. Internally the "Pranajiwa" plants are difficult to breed through generative ways because its seeds have thick-though skin and dormancy (Sutopo, 2002). However, in plant nurseries soaking in warm water (temperature $\pm$ 45 °C) for 50-10 hours could help to break seed dormancy (Yuniastuti, 2008). "Pranajiwa" seeds soften the harsh experience, and can germinate 3 days after planting. In addition to the generative seed, multiplication can also made through *in vitro* approaches. This is done as a form of plant germplasm conservation of “Pranajiwa”. To overcome Pranajiwa from extinction, conservation efforts should be done through both in-situ and ex situ approaches.

Morphological identification of ”Pranajiwa” plants that were found in Central Java could be classified into two types, namely large fruit and fruit respectively. Grouping of seeds was based on the average weight of 10 seed parents between 1.5 to 2.0 grams (large seeds) and 1.0 to 1.5 grams (small seeds). From the observation, the flowers appeared on the last branch or subsidiary to the 4th to the 6th. Similarly, the leaves, which also appeared in young leaves of the branch to the 4th to the 6th. It was found that the percentage of fruit-set was quite low where in a flower stalk that contains 20-30 flowers is only 1-2 pieces standing. In some areas the observations that had been previously done for such research in Navan this plant was not found, it relates to altitude of the location that is less than in accordance with the terms of Pranajiwa crops to grow. Pranajiwa grow best at altitudes of less than 500 m above sea level. Meanwhile, in some locations of Semarang regency Pranajiwa also have not been found anymore because it was felled and no effort for the rejuvenation of this plant has been done.

Observations in some locations in Blora and Grobogan District Pranajiwa plants have been started to be used for reboitation by the District Government and Blora through the Grobogan Department of Public Works (MPW) by planting plants on the curb and the edge of the irrigation channels.

In May to July pranajiwa plants begin to form flowers and young fruits become old fruit, which is ready for harvesting. In general, pranajiwa plants flowering and fruiting are throughout the year. This situation allows pranajiwa plants to be intensively cultivated and used as biofuel feedstock (bio-oil).

Observation of pranajiwa chromosome number indicated that the number of chromosomes is 2n = pranajiwa 2X = 32. This number gives the assurance that the number of pra najiwachromosomes is 32 not 40. The number of chromosomes is reinforced by images of cells of different pranajiwa (Setyawan, 2009) The previous opinion that pranajiwa chromosome number was 40 may be due to that the number of chromosomes of the genus *Sterculia* is 40 (six of nine species of the genus *Sterculia* species that have been identified and has a number of chromosome 40 is *S. acerifolia* A. Cunn., *S.* A. St *Chicha* .- Hil., *S. rubiginosa* Vent., *S. striata* A. St .- Hil., *S. urens* Roxb., and *S. villosa* Roxb.). another reason is that the method used by previous researchers was less precise that might cause a low quality of the picture of chromosome (eg cell rupture that resulted in the chromosomes of neighboring cells join counted that made the chromosome number increased).

Karyotype was based on chromosome length, and shape of chromosomes. Based on the similarity of the size and shape of chromosomes could be seen that pranajiwa chromosomes are diploid. Based on the size and curve of the centromere is known that each pair of chromosomes pranajiwa is two. This is supported by the similarity of the size and shape of a sorted chromosomes that are homologues of each chromosome.

Standard chemical analysis consisting of analysis of terpenoid compounds, flavonoids, and steroids showed a very low content even not exist at all. Further analysis for tannins, saponins and alkaloids also showed a very low content. This could facilitate the purification pranajiwa seed oil as

a lubricating oil feedstock (bio-oil). Common proximate analysis consisted of water, fat content, protein, carbohydrate, ash, and others.

To determine the amount of oil content (yield results) seeds was carried by two methods that are compress (compound) and of purification with ether methods. On purification with ether, oil content (yield results) was obtained nearly 70%. While the direct compression method yield the results was obtained about 40%. High oil yield from pranajiwa seeds indicate a huge potential for further development as a lubricating oil (bio-oil).

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Selection and Evaluation Characteristics of Six Candidate Varities of Cucumber (*Cucumis sativus*) in the Dry Season Planting

Rahayu, S.T[a,*], U.Sumpena [a], Ali Asgar [a]

[a] *Indonesian Vegetables Research Institute (IVEGRI)*
** Corresponding author: Indonesian Vegetables Research Institute (IVEGRI)*
Jl.Tangkuban Perahu no 517 Lembang, Bandung
Tel.: 0222786245; fax: 0222786416.
ayyuks@gmail.com

Abstract

Cucumber is one of vegetables that are rich in vitamin C. The purpose of this study was to select and to evaluate physical and chemical characteristics of six varieties of cucumber that will be released as new varieties. Planting sited was chosen in plain medium during the dry season in Bandung. The design used was randomized block design with three replicates. Observation was done by determining physical characteristics including length, diameter, weight, texture, and colour of fruit. Chemical characteristics observed were moisture and content of vitamin C. The results showed that two varieties were not significantly different from the characteristics of the control, so both can be recommended to be released as new varieties.

Keywords: characteristics, cucumber, selection, varieties.

Introduction

Cucumber (*Cucumis sativus*) is one type of vegetable widely grown in Indonesia. Cucumber has a fairly broad market both modern and traditional markets. Cucumber production in Indonesia in 2009 reached 583,139 tons per hectare with an area of 56,099 hectares planted. Production mostly in West Java, Central Java and North Sumatra provinces (Anonymous, 2010). Production is still potential for further improved.

Cucumber planted in low, medium, or high plains with optimal rainfall ranges between 200-400 mm/month. Too high rainfall is not suitable for growing vegetables, as it will abort flowers (Sumpena, 2007). Cucumber grow well at the acidity of the soil ranged from 5.5 to 6.5, on alluvial, latosol, and andosol soil type. Cucumber planted in the lowlands can be harvested faster than cucumbers grown in the highlands. Cucumber harvest age ranged from 30 to 50 days after planting (Arief, 2009). Cucumber is one of horticultural commodity easy to handle because it is grown with simple maintenance, inexpensive, and short-lived cycle when it is compared to other vegetables such as tomatoes or peppers. Fluctuations in market prices are low compared to other vegetables, so it would be more profitable for farmers (Sumpena, 2007).

Cucumbers can be consumed directly in fresh as a salad and for pickles. The slicing cucumber are peeled, sliced and served with vinegar or dressing or as an ingredient of salads. In South-East Asia, the young shoots are eaten as raw or steamed (Siemonsma and Piluek, 1994). Cucumber is very important in South East Asia as it is in temperate regions. Breeding work should be aimed to produce improved cultivars resistence to diseases and pests attack in tropical lowland condition (Siemonsma and Piluek, 1994).

The nutrient content per 100 grams of cucumber is water at 96 g, protein at 0.6 g, fat at 0.1 g, carbohydrates at 2.2 g, Calcium at 12 mg, Iron at 0.3 mg, vitamin C at 12 mg. Cucumber fruit consists of 24 mg of P, 45 IU of Vitamin A, 0.03 mg of Vitamin B1, 0.02 mg of Vitamin B2, 0.3 mg of Niacin, and 15 mg at magnesium, respectively (Siemonsma and Piluek, 1994). In addition,

cucumber are also rich in fiber. The purpose of this study was to select and to evaluate physical and chemical characteristics of six varieties of cucumber that will be released as new varieties.

Materials and Methods

The research was conducted in plain medium during the dry season at Ciwastra, Bandung 400-700 m above sea level. Chemical analysis was performed at the laboratory of plant physiology at IVEGRI in Lembang, Bandung. Materials used in this experiment were three candidates of new varieties and three varieties of cucumber production of private firms as control.

Each treatment was planted in mulched beds at 9,6 m^2 using a plant distance of 40 x 60 cm. The fertilizer applied was NPK at 125 kg/ha and goat manure at 20 ton/Ha. The remaining dose given as a supplementary fertilizer twice during the plant growth. To control pests of plants (OPT) pesticides were used intensively twice a week, starting at two weeks after planting. Pesticides were adjusted to the type of pests that attacked.

Research was carried out by Randomized Design Group consisted of three replicates. Observation physical parameters included length, diameter, weight, colour, and texture of the fruit. Chemical parameters measured were moisture and vitamin C. Texture observation was done using a QTS 25 Texture Analysis, and colour observations used a chroma meter. Statistical tests were performed with the PKBTSTAT analysis followed by Tukey test at 5% level.

Results and Discussion

There are two types of cucumbers were observed in this study, i.e. green and white cucumbers. Green cucumber fruit is longer than the local cucumbers. Table 1 shows that the varieties of fruit length of variety H1 was not significantly different with the comparison varieties H5. Varieties H2, H3, and H4 were not significant different.

Tabel 1. Analysis variance for length, diameter, weight,colour, texture of cucumber fruit, moisture, and vitamin C of six virieties

Variety	Length (cm)	Diameter (cm)	Weight (g)	Colour (N/mm)	Texture (mm/10sec/50g)	Moisture (%)	Vitamin C (mg/100g)
H1	20.89^{a}	3.37^{b}	121.47^{a}	0.89^{b}	0.12^{ab}	95.30^{b}	19.16^{b}
H2	16.53^{b}	3.87^{a}	146.70^{a}	0.93^{b}	0.12^{ab}	95.68^{a}	17.87^{c}
H3	14.43^{bc}	4.22^{a}	150.44^{a}	1.05^{a}	0.14^{a}	95.14^{b}	19.06^{b}
H4	16.44^{b}	3.93^{a}	144.98^{a}	0.94^{b}	0.14^{a}	95.38^{ab}	17.28^{c}
H5	21.27^{a}	3.27^{b}	140.83^{a}	0.89^{b}	0.10^{b}	94.49^{c}	20.06^{a}
H6	12.66^{c}	3.83^{a}	145.63^{a}	1.05^{a}	0.12^{ab}	95.66^{a}	17.86^{c}
HSD 5%	2.19	0.40	71.08	0.10	0.03	0.31	0.79

Table 1 also shows that the diameter of varieties H1 was not significantly different with H5, meanwhile among varieties H2, H3, and H4 were not significantly different. Diameter would affect the weight of fruit. Type of fertilizer affect the production of cucumber (Sutater and Supriyadi, 1989). Cucumber weight of six varieties were not significantly different at level 5% at Turkey test. Cucumber weight affected the total production of cucumber. Factors planting and type of fertilizer used affected plant growth and produced cucumber (Hilman and Rosliani, 2004). Irigation and composition of the N on the fertilizer also affected the weight of cucumber (Zhan *et al*., 2011).

The colour of varieties H1, H2, H4, and H5 were not significantly different from each other, they were more dominant to the colour of green. Variety H3 was not significantly different compared with variety H6, having the dominant colour of white. Cucumber colour ranges from dark green, green, light green, whitish green to white depending on the type varieties (Sumpena, 2007).

Texture of varieties H1, H2, and H6 were not significantly different among them. Similar results were also found for varieties H3, H4, and H5. Higher texture value indicated softer texture, and conversely lower texture values indicated tougher texture. Texture value difference was also influenced by varieties and fertilization. K elements made harder texture, while the elements of N make the texture softer (Wiranatakusumah *et al,* 1992). Texture of consumer software was not preferred. Storage temperature affected the texture of the cucumber. Storage at low temperature will maintain the texture of the cucumber (Kohyam *et al*, 2009).

The highest moisture content was found in the variety H2, while the lowest was found at variety H5. Variety H1 was not significantly different compared with variety H3. Moisture will affect the shelf life of these products. The presence of water in food is a good medium for the growth of microorganisms. The high moisture is held within the product by osmotic force within cells, mostly as free water, althought a small proportion is chemically bound, therefore, this is more tightly held and more stable (Will, *et al*., 1989). Variety H5 had the highest of Vitamin C content , while variety H4 was the lowest. The vitamin C in vegetables is affected by the type of varieties and types of seasons. In the rainy season Vitamin C content is higher level compared with in the dry season (Hanson *et al*, 2011).

Conclusions

Caracteristic cucumber was effected by the kind of varieties. Varieties H1 and H3 had good caracteristics to be released in public, because they had low moisture and high in the Vitamin C content.

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Blue Light Induced the Stem Growth in Vegetable Water Spinach

Futoshi Sasaki[a,*], Mikio Sekinuma[a], Futoshi Kato[b] and Naoto Inoue[b]

[a]*Interdisciplinary Graduate School of Science and Technology, Shinshu University, Minamiminowa, Japan*
[b]*Faculty of Agriculture, Shinshu University, Minamiminowa ,Japan.*
Tel.: +81 265 77 1314.
10st504f@shinshu-u.ac.jp

Abstract

A local variety of water spinach in Taiwan was grown under various artificial light conditions to clarify the suitable growth condition in a plant factory. In this experiment, water spinach shoots were grown under various artificial light spectra controlling plant growth. Eight plots with conditions of 8 different light qualities were used to compare the growth of the water spinach plant. Different light qualities were established by combining 3 types of fluorescent light selected from 4 types of light emitted by white, blue, purplish-red, and far-red fluorescent lamps, respectively, and irradiation treatments were performed for 10 days in each plot. The results showed that the plant length and stem diameter increased in the plot in which 1 white and 2 blue fluorescent lamps were set. Increase in plant length and stem diameter were positively correlated with the energy of blue light (400-480 nm), but were negatively correlated with energy of green, red and far-red light (520-780 nm). The size of the stem cavity negatively correlated to the amount of energy of red light (600-660nm) and positively correlated to the amount of energy of blue light (480 nm). The results suggest that blue light promotes and red light inhibits stem growth of the water spinach.

Keywords: artificial light, light quality, plant length, stem diameter, water spinach

Introduction

Water spinach (*Ipomoea aquatica* Forsk.) is a popular vegetable in Japan because of its good texture and high nutrient value. This vegetative has a high content of protein (2.2 g/100 g), calcium (74 mg/100 g) and fiber (3.1 g/100 g) (JATCC, 2009). Moreover, many researchers have evaluated the chemical characteristics of this functional food (Miean and Mohamed, 2001; Prasad *et al*., 2005; Dasgupta and De, 2006). However, the duration of cultivation of this plant is limited to summer because the frost resistance of this plant is low. For obtaining a stable yield throughout the year, water spinach must be cultivated in an artificial environment that provides suitable conditions for its growth. The species can grow easily in hydroponic cultures, and it shows a high nutrient absorption. Therefore, the best growth conditions (light, temperature, nutrition) required for this species must be investigated in detail to maximize the production and sales of this vegetable. In this experiment, the light spectra that controls stem growth were investigated to establish a year-round culture under low energy input condition.

Materials and Methods

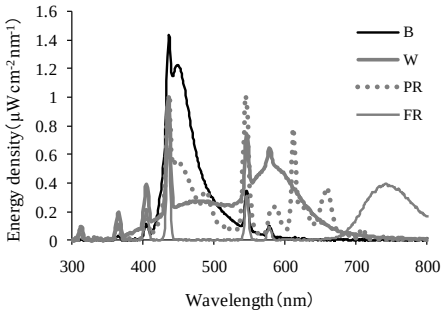
Plant materials

A local variety of water spinach from Taiwan was used in this indoor experiment. The seeds were scarified and soaked in water for 3 days, and the germinated seeds were sown on culture soil in 12 cm poly pot. After sowing, the seedlings were grown under the light of fluorescent lamps (FL40SBR-A, NEC Co. Ltd., Japan) for 7 days. After 7 days, we removed these fluorescent lamps and set other fluorescent lamps producing different light colors, therefore, these seedlings were grown under different light quality conditions by using artificial light.

Treatments and analytical method

Four different fluorescent lamps (white, blue, purplish red and far red) were used in different combinations to provide different lightings in 8 plots (L1-L8). Fig.1 shows the light spectrum of the 4 fluorescent lamps. The spectral distribution of the light in each plot was measured with a spectroradiometer (HSU-100S, Asahi Spectra Co. Ltd., Japan). Table 1 shows the energy distribution in each spectral band (UVA, B, G, R and FR). The air temperature was maintained at 27°C in the room, and all fluorescent lamps were continuously irradiated. The photosynthetic photon flux density (PPFD) was adjusted to 200 μmol m^{-2} s^{-1} at the top of the seedlings in each plot. Ten days after the treatments, the main stem length, stem diameter and cavity size were measured (Figure 2), and the average of all these parameters were calculated from the data of 15 stems.

The correlations between the morphological traits of the stems and the light quality in each plot were obtained. The analytical method of statistics referred to Kasajima *et al*. (2007). The ratio of energy (RE) was defined as the light energy in a specified wavelength width (50 nm) to the total light energy in the whole spectral range (250-1000 nm).



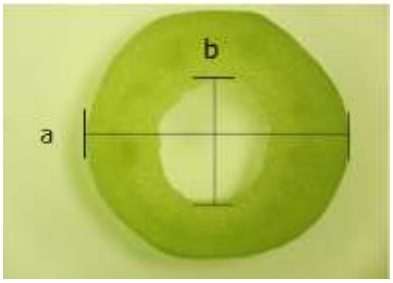
B = Blue (Caribbean Blue, 15W, Sudo Co. Ltd., Japan), W = White (Mellow White, 15W, Toshiba Co. Ltd., Japan), PR = Purplish red (Exotic Rose, 15W, Sudo Co. Ltd., Japan),
FR = Far-red (FL20SFR74, 20W, Toshiba Co. Ltd., Japan)

Figure 1. The emission spectra of four kinds of fluorescent lamps used in the experiment.

Table 1. The light quality in experimental plots (L1-L8)

Plot	Combination of lamps[1)]	RE in each spectral region[2)]				
		UVA	B	G	R	FR
L1	W + W + W	0.018	0.314	0.457	0.182	0.020
L2	W + PR + PR	0.007	0.410	0.351	0.220	0.010
L3	W + B + B	0.007	0.697	0.224	0.051	0.004
L4	W + PR + B	0.007	0.523	0.317	0.132	0.011
L5	W + W + FR	0.010	0.263	0.345	0.146	0.188
L6	W + PR + FR	0.005	0.302	0.307	0.155	0.210
L7	W + B + FR	0.010	0.390	0.265	0.090	0.204
L8	PR + B + FR	0.004	0.491	0.183	0.139	0.170

1) Refer to Figure 1.
2) Ratio of energy in UVA (320-400nm), B(400-500 nm), G(500-600 nm), R(600-700 nm) and FR (700-800nm) range to that in whole spectral range (250-1000 nm).



a, Stem diameter, b, Cavity size

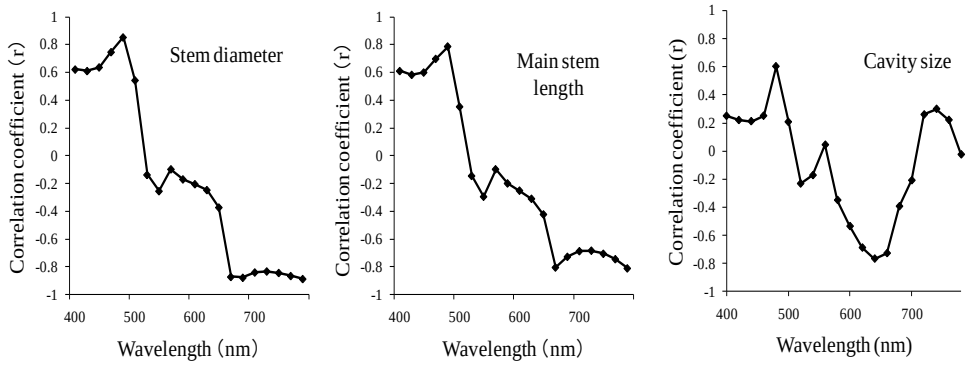
Figure 2. Stem-cross section in water spinach.

Results and Discussion

The plant length and stem diameter of water spinach increased in the plot in which 1 white and 2 blue fluorescent lamps were set (L3 in Table 3). Figure 3 shows the correlation coefficient between REs in various spectral ranges and the morphological traits of stems. Plant length and stem diameter were positively correlated with the energy of blue light (400-480 nm), however, they had negative correlation with the energies of green, red and far-red lights (520-780 nm). In particular, stem diameter was significantly correlated with the energies of light at wavelengths of 480 nm and 660 nm. The size of the stem cavity was negatively correlated to the amount of energy of red light (600-660nm) and positively correlated to the amount of energy of blue light (480 nm).

These results showed that the stem diameter, stem length and cavity size growth were promoted by blue light and inhibited by red light. Blue light had a positive effect on the stem growth of water spinach cultivated under artificial light conditions. However, some studies showed different results. Minanizawa and Kitta (1977) reported that greenish blue light of 450-550 nm suppressed the growth of mulberry (*Morus alba* L.). In morning glory (*Ipomoea nil* L), the elongation of plant length decreased under the blue light compared with that under green or red lights (Yamazaki *et al*., 2003). Kasajima *et al*., (2008) reported that red and green light play an important role in regulating hypocotyl elongation in common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* Gaertn.). In petunia (*Petunia* × *hybrid* Vilm.), blue light had no effect on the plant height (Fukuda *et al*., 2002). These reports suggested that the light response systems of some plants differed from those of water spinach.

On the other hand, stem elongation of eggplant (*Solanum melongena* L.) and sunflower (*Helianthus annuus* L.) were promoted under blue light (Hirai et al., 2006). Goto (2003) summarized the findings of previous studies in order to evaluate the significant effects of the quality of artificial light on plant growth and reported that blue light had various effects on plant growth. Therefore, these reports and our results suggest that the effects of light quality on stem growth differed among plant types and species. In future, these factors should be studied in detail for controlling the plant growth under artificial light.



Significant level: |r| > 0.83 (p < 0.01)

Figure 3. Correlation coefficient between RE in various spectral ranges and morphological traits of stem.

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Effects of Genotypes and Storage Time on Quality Parameters of Chinese Flowering Cabbage (Caisim) Planted in Subang

Rahayu,S.T.[a*] **R.Kirana[a]**

[a] *Indonesian Vegetables Research Institute (IVEGRI)*
Jl.Tangkuban Perahu no 517 Lembang Bandung 40391
** Corresponding author: Jl.Tangkuban Perahu no 517 Lembang, Bandung, Indonesia*
Tel.: 0222786245; fax: 0222786416.
swarnit@yahoo.com

Abstract

Chinese flowering cabbage (*Brassica rapa parachinensis)* is one type of leaf vegetables which are rich in fiber, minerals, and vitamins. Postharvest handling techniques and storage will affect the quality of chinese flowering cabbages. This experiment aimed to determine the effect of variety and storage time on quality parameters of chinese flowering cabbages. Chinese flowering cabbage was planted in Subang. The experiment was designed using Randomized design group (RAK) with three replications and two factors. The first factor was variety consisting of 10 varieties and the second factor was storage time were 5 and 10 days. Observations were made on 0, 5, and 10 days. Chinese flowering cabbage was placed in a cold storage at a temperature of 10^{o}C with humidity at 98%. Observing parameters included texture, colour, moisture, ascorbic acid, fiber, and chlorophyll content. The results showed that three varieties on storage for 5 days had good quality parameters.

Keywords: chinese flowering cabbages, quality, storage time, variety

Introduction

Chinese flowering cabbages that is one type of leaf vegetables grows well in Indonesia. Chinese flowering cabbage production and other types of mustard in 2009 were 562.838 tons with a planting area of 56,414 hectares. The plants are most widely grown in West Java, Central Java and North Sumatra provinces (Anonymous, 2010).

There were 22 varieties of chinese flowering cabbages or caisim that have been released by private sectors through introduction. Eventhough IVEGRI has not released any variety of chinese flowering cabbages, the seeds of chinese flowering cabbages line (LV-145) have been distributed to users. LV-145 already has a uniform appearance, but its quality has not been documented well.

Chinese flowering cabbages contain many vitamins and minerals that essential are for our body needs. The nutrient content per 100 gram edible fresh portion moisture 95 g, protein 1.2 g, fat 0.2 g, carbohydrates 1.2 g, vitamin A 5800 IU, vitamin B1 0.04 mg, Vitamin B2 0.07 mg, Niacin 0, 5 mg, ascorbic acid 53 mg, 102 mg Ca, Fe 2 mg, mg 27 mg, P 37 mg, K 180 mg, Na 100 mg. The brassica family includes broccoli, cabbage, kale, cauliflower and brussels sprouts, to prevent many common diseases such as cancer, heart disease, diabetes and hypertension (Siemonsma and Piluek, 1994).

The purpose of storage to maintain the price stability, inhibit the development of biological agents and preserve the quality of the produce in minimal moisture of loss and reduce the respiration (*Dris et al*.,2003).

The purpose of this experiment was to determine the quality of LV-145 and other chinese flowering cabbages line as a preliminary testing.

Materials and Methods

The preliminary testing of chinese flowering cabbage quality was conducted in IVEGRI Research Station in Subang, 100 m above sea level. The treatments were 10 genotypes (five IVEGRI line and five released varieties as control). The experiment was designed using Randomized Design Group (RAK) with three replications. Differentiation among treatments to determine (genotypes) using the F test at 5% level of test. If there was a significant the analysis was continued using LSD test. Plants were grown in mulched beds measuring $3m^2$ using a plant distance of 20 cm x 20 cm with a population of 60 plants for each replication. The first application of fertilizers given were goat manure (10t/ha) and half dose of NPK 16:16:16 (1.5 t/ha). The remaining dose of NPK was given twice as a supplementary fertilizer during the plant growth. In addition, lime, dolomite (1 ton/ha), was applied a week before planting. Pesticides were applied twice a week to control pests, starting two weeks after planting. Pesticides were adjusted to the type of pests that attacked.

Chinese flowering cabbages were kept in cold storage at a temperature of 10^0 C with RH 98%. Observations were conducted on 0, 5 and 10 days. Physical and chemical analyses were conducted in IVEGRI Laboratory. Physical analysis covered texture and colour, while chemical analysis included moisture, ascorbic acid, chlorophyll content, and fiber.

Results and Discussion

Table 1. Analysis of variance of texture, colour, moisture, ascorbic acid,chloropyll, and fiber for genotype

Genotype	Texture (mm/sc/gr)	Colour (N/mm)	Moisture (%)	Ascorbic acid (mg/100gr)	Chlorophyll (µg/mg)	Fiber (%)
1	2.07^{a}	1.18^{a}	92.37^{c}	93.94^{d}	262.42^{cd}	0.99^{ef}
2	2.08^{a}	1.21^{a}	92.36^{c}	94.12^{d}	216.63^{d}	1.10^{d}
3	2.02^{a}	1.26^{a}	92.97^{a}	81.74^{g}	232.05^{d}	1.18^{c}
4	2.05^{a}	1.20^{a}	93.13^{a}	87.04^{f}	245.84^{cd}	1.14^{cd}
5	2.03^{a}	1.23^{a}	92.60^{b}	92.72^{d}	340.11^{b}	1.38^{a}
6	2.02^{a}	1.13^{a}	92.27^{c}	90.25^{e}	376.50^{b}	1.00^{ef}
7	2.16^{a}	1.23^{a}	92.74^{b}	86.27^{f}	245.74^{cd}	0.96^{f}
8	2.00^{a}	1.22^{a}	92.66^{b}	96.19^{c}	282.82^{c}	1.04^{e}
9	2.04^{a}	1.20^{a}	92.18^{c}	106.62^{b}	347.62^{b}	1.32^{b}
10	2.09^{a}	1.21^{a}	91.39^{d}	112.24^{a}	538.55^{a}	1.36^{ab}
HSD 5%	0.30	0.16	0.21	1.84	50.17	0.05

Table 2. Analysis of variance of texture, colour, moisture, ascorbic acid, chlorophyll, and fiber for storage time

Storage Time	Texture (mm/sc/gr)	Colour (N/mm)	Moisture (%)	Ascorbic acid (mg/100gr)	Chlorophyll (µg/mg)	Fiber (%)
0 hari	1.81^{b}	0.89^{c}	92.10^{b}	77.43^{c}	53.99^{c}	0.98^{c}
5 hari	1.91^{b}	1.25^{b}	93.41^{a}	85.80^{b}	498.70^{a}	1.00^{b}
10 hari	2.45^{a}	1.49^{a}	91.89^{c}	119.11^{a}	373.79^{b}	1.46^{a}
HSD 5%	0.12	0.06	0.08	0.73	19.88	0.02

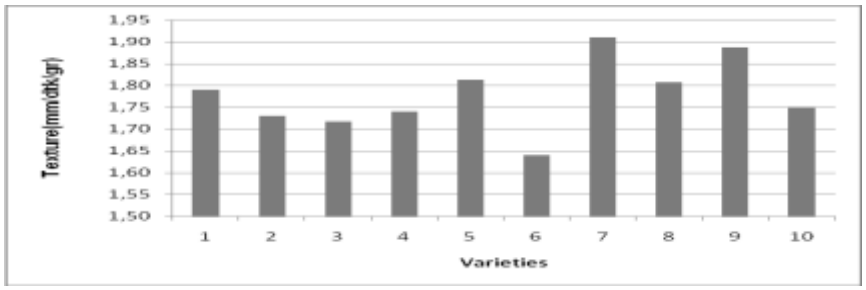

Figure 1. Effect of genotypes on chinese flowering cabbage texture.

Figure 1, the hardest texture was shown by genotype number 6, while the value of the soft texture was shown by genotype number 7. However, from the results of LSD test at 5% level shows that there is no significantly different for all varieties (Table 1). Duration storage affected the texture of chinese flowering cabbage. This was indicated by the value of texture where 5 days storage was significantly different from 10 days storage (Table 2). The longer storage time, the more evaporation from the material occurs that is due to the process of respiration.

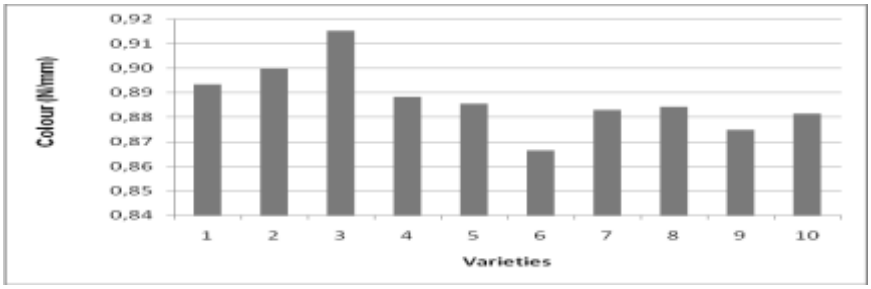

Figure 2. Effect of genotypes on chinese flowering cabbages colour.

Figure 2 shows that genotype number 6 had the lowest value of the colour (dark green), whereas genotype number 3 had the highest colour value (light green).However, the test of LSD at the level of 5% all types of varieties were not significantly different in colour values. The colour influenced by the content of chlorophyll is one of the parameters that affect the appearance of the product. Genotype number 3 had lower chlorophyll content than genotype number 6. Furthermore, colour values were significantly different for 0, 5 and 10 days-storage. During storage, chinese flowering cabbage colour changes because the process of respiration continues. Table 3 shows the genotypes no 3,4, and 5 were not significantly different from control genotypes number 9 and 10 for the colour values in 5 days-storage.

Table 3. Texture, colour, moisture, ascorbic acid, chlorophyll, and fiber for storage time * genotype

ST*Var	Texture (mm/sc/gr)	Colour (N/mm)	Moisture (%)	Ascorbic acid (mg/100gr)	Chlorophyll (µg/mg)	Fiber (%)
ST 0 D VAR1	1.79^{ghij}	0.89^{e}	92.68^{cd}	76.34^{kl}	45.91^{k}	0.92^{jklm}
ST 0 D VAR2	1.73^{hij}	0.90^{de}	92.06^{fghij}	78.44^{jk}	54.67^{k}	0.97^{jkl}
ST 0 D VAR3	1.72^{ij}	0.91^{cde}	92.55^{cde}	73.19^{lmno}	43.27^{k}	0.85^{mno}
ST 0 D VAR4	1.74^{hij}	0.89^{e}	92.81^{c}	72.36^{mno}	41.44^{k}	0.90^{jklm}
ST 0 D VAR5	1.81^{fghij}	0.89^{e}	91.78^{ijk}	88.36^{h}	50.89^{k}	1.01^{ij}
ST 0 D VAR6	1.91^{bcdefghij}	0.87^{e}	91.72^{ijk}	67.34^{p}	58.06^{k}	0.95^{jklm}
ST 0 D VAR7	1.91^{bcdefghij}	0.88^{e}	91.68^{ijk}	76.34^{kl}	55.91^{k}	0.96^{jklm}
ST 0 D VAR8	1.81^{fghij}	0.89^{e}	91.90^{ghijk}	75.24^{klmn}	57.46^{k}	0.98^{ijk}
ST 0 D VAR9	1.89^{cdefghij}	0.87^{e}	92.23^{efgh}	81.44^{ij}	59.18^{k}	0.88^{klm}
ST 0 D VAR10	1.75^{hij}	0.88^{e}	91.62^{kl}	85.22^{hi}	73.14^{k}	1.35^{de}
ST 5 D VAR1	1.88^{defghij}	1.24^{abcd}	91.82^{hijk}	97.25^{g}	551.44^{de}	1.08^{hi}
ST 5 D VAR2	2.17^{abcdefghij}	1.24^{abcd}	93.88^{b}	71.63^{no}	339.03^{ghi}	0.77^{no}
ST 5 D VAR3	1.84^{efghij}	1.29^{ab}	94.05^{ab}	72.68^{lmno}	393.23^{fg}	1.21^{fg}
ST 5 D VAR4	1.97^{abcdefghij}	1.27^{ab}	94.32^{a}	75.82^{klm}	375.21^{fgh}	0.94^{jklm}
ST 5 D VAR5	1.84^{efghij}	1.27^{ab}	94.02^{ab}	84.71^{hi}	577.60^{cd}	1.33^{de}
ST 5 D VAR6	1.64^{j}	1.12^{bcde}	93.88^{b}	71.11^{op}	410.63^{fg}	0.75^{o}
ST 5 D VAR7	2.04^{abcdefghij}	1.24^{abcd}	93.72^{b}	75.81^{klm}	409.41^{fg}	0.59^{p}
ST 5 D VAR8	1.81^{fghij}	1.25^{abc}	93.72^{b}	83.14^{i}	457.04^{ef}	0.87^{lmn}
ST 5 D VAR9	1.92^{bcdefghij}	1.26^{ab}	92.65^{cde}	100.91^{g}	629.84^{bcd}	1.19^{gh}
ST 5 D VAR10	2.02^{abcdefghij}	1.29^{ab}	92.08^{fghi}	124.96^{d}	843.56^{a}	1.30^{def}
ST 10 D VAR1	2.55^{a}	1.40^{ab}	92.63^{cde}	108.24^{f}	189.89^{j}	0.96^{jklm}
ST 10 D VAR2	2.35^{abcdefgh}	1.48^{a}	91.15^{m}	132.28^{b}	256.19^{ij}	1.56^{b}
ST 10 D VAR3	2.50^{abcd}	1.56^{a}	92.31^{defg}	99.35^{g}	259.65^{ij}	1.47^{bc}
ST 10 D VAR4	2.45^{abcde}	1.44^{ab}	92.27^{defg}	112.94^{e}	320.86^{ghi}	1.58^{b}
ST 10 D VAR5	2.43^{abcdef}	1.54^{a}	92.00^{fghijk}	105.10^{f}	391.84^{fg}	1.79^{a}
ST 10 D VAR6	2.49^{abcd}	1.42^{ab}	91.20^{lm}	132.29^{b}	660.82^{bc}	1.31^{def}
ST 10 D VAR7	2.52^{ab}	1.55^{a}	92.81^{c}	106.67^{f}	271.89^{hij}	1.33^{de}
ST 10 D VAR8	2.40^{abcdefg}	1.51^{a}	92.37^{def}	130.20^{bc}	333.96^{ghi}	1.26^{efg}
ST 10 D VAR9	2.32^{abcdefghi}	1.48^{a}	91.65^{jk}	137.52^{a}	353.84^{fghi}	1.88^{a}
ST 10 D VAR10	2.50^{abc}	1.47^{a}	90.47^{n}	126.54^{cd}	698.94^{b}	1.41^{cd}
HSD 5%	0.62	0.34	0.43	3.87	105.60	0.11

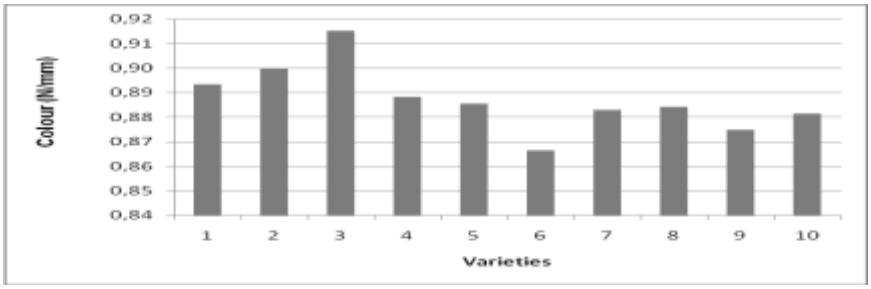

Figure 2. Effect of genotypes on chinese flowering cabbages colour.

Figure 2 shows that genotype number 6 had the lowest value of the colour (dark green), whereas genotype number 3 had the highest colour value (light green).However, the test of LSD at the level of 5% all types of varieties were not significantly different in colour values. The colour influenced by the content of chlorophyll is one of the parameters that affect the appearance of the product. Genotype number 3 had lower chlorophyll content than genotype number 6. Furthermore, colour values were significantly different for 0, 5 and 10 days-storage. During storage, chinese flowering cabbage colour changes because the process of respiration continues. Table 3 shows the genotypes no 3,4, and 5 were not significantly different from control genotypes number 9 and 10 for the colour values in 5 days-storage.

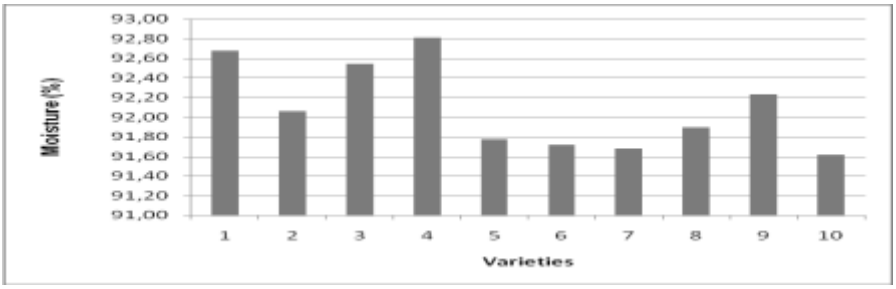

Figure 3. Effect of genotypes on chinese flowering cabbages moisture.

Chinese flowering cabbage moisture was significantly affected by genotypes. Figure 3 shows genotype number 4 was the highest water content, whereas the lowest levels of water contained was genotype number 10. Moisture was inversely related to chlorophyll and fiber content of which is a component of dissolved solids in chinese flowering cabbages. The moisture of genotype number 5 was not significantly different from control genotype number 7 and 8. Table 2 also shows that moisture values were significantly different between 5 and 10 days-storage.

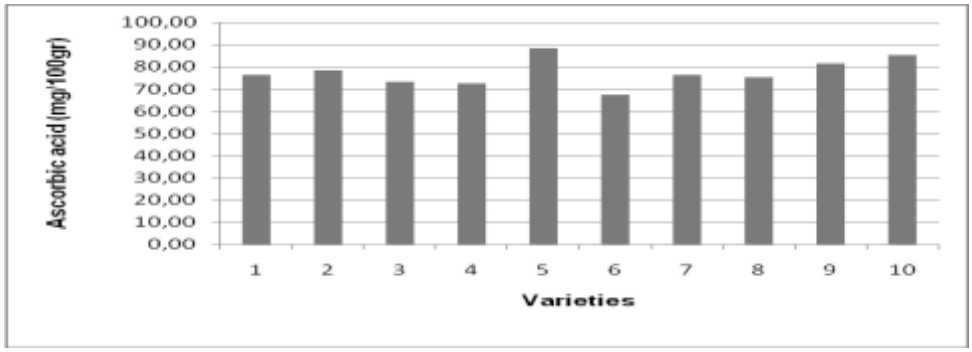

Figure 4. Effect of genotypes on chinese flowering cabbages ascorbic acid

Figure 4 shows that the ascorbic acid content in genotype number 5 was the highest value. Ascorbic acid is affected by the type of varieties and seasons (Hanson *et al*.,2011). Genotypes number 1,2 and 5 were not significantly different in ascorbic acid contents. Ascorbic acid is one of the components with high nutritional value in chinese flowering cabbages as other cabbage type vegetables such as broccoli (Jagdish Singh *et al*.,2007). During storage ascorbic acid becomes unstable (Hounsome *et al*., 2009) as also indicated in Table 2. However, genotypes number 3.5, 6, and 7 showed the values of ascorbic acid contents were relatively stable for 5 days-storage (Table 3).

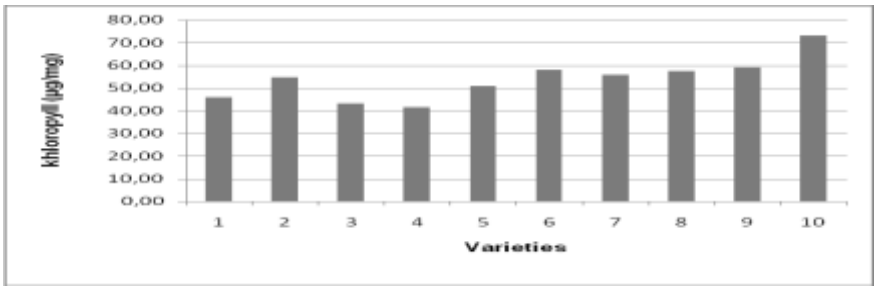

Figure 5. Effect of genotypes on chinese flowering cabbages chlorophyll content

Genotype number 10 had the highest chlorophyll content (Figure 5). The chlorophyll content affected the colour of the leaves that made it become greener. The chlorophyll content of genotype number 5 was not significantly different from control genotype number 6 and 9. While genotypes number 1 and 4 were not significantly different from control genotype number 7. The content of chlorophyll rapidly declines during storage. Storage in the dark room will decrease the speed of leaf colour change from green to yellow (Zhang *et a*l., 2011, In Press).

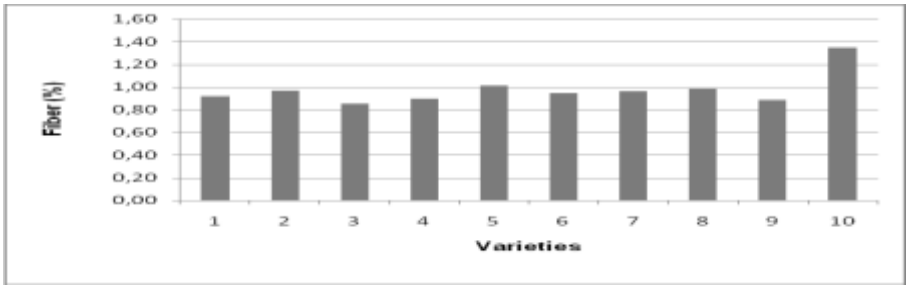

Figure 6. Effect of genotypes on chinese flowering cabbages fiber

Fiber content of genotype number 10 had the highest value. Genotype number 5 was not significantly different from genotype number 10, as well as the interaction 5 days-storage and genotype varieties were not significantly different for number 5 and 10.

Conclusion

1. Genotypes of chinese flowering cabbage gave effects on moisture, ascorbic acid, chloropyll, and fiber of chinese flowering cabbages
2. Storage time gave effects on texture, colour, moisture, ascorbic acid, chlorophyll, and fiber of chinese flowering cabbage.
3. Three genotypes still had good qualits up to 5 days on cold storage.

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Influence of Organic Fertilizer on Growth and Vitamin E Content of Traditional Vegetable, *Codonopsis lanceolata*

Futoshi Kato [a,*], Naoto Inoue [a], Seishi Yoshida [b]

[a] *Faculty of Agriculture, Shinshu University, Nagano, Japan*
[b] *Nagano Prefecture Vegetable and Ornamental Crops Experiment Station, Nagano, Japan*
** Corresponding author: Faculty of Agriculture, Shinshu University. 8304 Minamiminowa, Nagano 399-4598, Japan.*
Tel.: +81 265 77 1409 ; fax: +81 265 77 1409.
fkato@shinshu-u.ac.jp

Abstract

Codonopsis lanceolata (Sieb. et Zucc.) Trautv. is wild vegetable found in East Asian countries and considered valuable, especially among the Korean people. The effects of organic fertilizer application on the yield and quality of rhizome were investigated under Andosol condition. Commercial barnyard manure fermented with crushed bark, beef cattle dung, and leaf mold of Japanese oak were applied at the rates of 0, 5, 10, 15, and 20 $g \cdot m^{-2}$. Barnyard manure enhanced the fresh weight of rhizome, but decreased the vitamin E content. The C/N ratio in the soil surface up to the depth of 30 cm, which was controlled by the application ratio of barnyard manure and leaf mold, drastically influenced the rhizome yield. Total N, soluble P, exchangeable K, Ca, and Mg, however, did not significantly affect the rhizome yield. There was significantly negative correlation between the fresh weight of rhizome and the α-tocopherol (vitamin E) content at harvest time. The highest vitamin E content was observed with the leaf mold application rate of 10 $g \cdot m^{-2}$, and the contents achieved at 9–10 $mg \cdot DM^{-1}$. The nitrogen supply from the soil to plants influenced primarily the yield and quality as vegetable or drug. The vitamin E content under higher soil C/N ratio was one of the highest among commercial vegetables in the common Japanese food market.

Keywords: α-tocopherol, East Asia, fertilizer application, wild vegetable

Introduction

Food must always be safe and nutritious. Traditional vegetables grown on organic farms are becoming popular in markets in East Asian countries. The organic cultivation of vegetables has increased because organic farming benefits human health and is also effective for the conservation of agricultural environments.

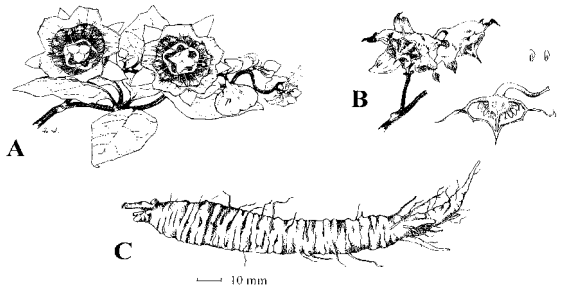
Codonopsis lanceolata (Sieb. et Zucc.) Trautv. is a perennial climbing herb that is a popular fancy vegetable in high demand among organic foods in Korea. On the other hand, it is recognized as a rare and quaint wild plant mainly inhabiting mountainous areas in Japan (Figure 1). *C. lanceolata* is chemotaxonomically similar to *Codonopsis pilosula* (Wang *et al*., 1995), which is a major source of the traditional Chinese drug “dangshen” (Iwai *et al.* 1992; Namba *et al.*, 1992a, 1992b). The natural population of *C. lanceolata* is distributed throughout shaded and humid sites in Korea, China, and Japan. Many farmers domesticate, produce, and sell *C. lanceolata* for its rhizome vegetable, which is used in a distilled spirit, traditional medicine, or as a foodstuff in Korea. The Korean common name for this plant is “deo-deog.” Korean and Chinese people have been carefully protecting and fostering *C. lanceolata* as a useful vegetable and drug since ancient times (Park and Lee 1991; Pemberton and Lee 1996; Sakamoto 1998).

C. lanceolata is also used as a minor vegetable and in a distilled spirit in Central Japan (Inoue 1998a; 1998b). Furthermore, the native Ainu people use it as a familiar vegetable, drug, and medicinal plant (Shiraoi Ainu Museum 1989; Annetai *et al*. 1996; Fukuoka and Sato 1995; Hayashi 1968). The common Japanese name for *C. lanceolata* is “tsuru-ninjin;” “tsuru” means vine and

“ninjin” means rhizome. The Ainu names are “chir-muk” and “tope-muk”. Local names in Central Japan include “tou-do,” “to-dog,” and “jii-sob”.

The cultivation area of *C. lanceolata* has increased to over 1000 ha in South Korea because the edible rhizome has a good aromatic flavor (Lee *et al*. 1996), good texture, and high antioxidant activity (Maeng and Park 1991). However, the chemical composition of the rhizome fluctuates according to the cultivation conditions; at present, the optimal soil conditions for producing high-quality rhizomes are unknown. For providing a high-quality vegetable for healthy functional foods, productivity testing is important to clarify the effects of organic fertilizer application rate on the yield and vitamin E content, which is responsible for the antioxidant activity of *C. lanceolata* rhizomes.

In this study, field experiments were carried out to investigate the effects of commercial barnyard manure fermented with crushed bark, beef cattle dung, or leaf mold of Japanese oak on the rhizome yield and α-tocopherol (vitamin E) content in *C. lanceolata* rhizomes at the end of 1 growing season.



A: inflorescence and vine; B: capsule and single seed; C: rhizome
The plant sample was collected atu Yong Weoul in Kangwon province of Korea

Figure 1. *Codonopsis lanceolata* (Sieb. et Zucc.) Trautv. (family Campanulaceae).

Materials and Methods

The experimental field was located at Shinshu University, Minamiminowa Village, Nagano Prefecture, Japan (alt. 740 m, 35°N and 138°E). The soil taxonomy and texture are Andosol and clay loam according to the FAO/UNESCO and international systems, respectively. Commercial barnyard manure that was fermented with crushed bark (80%), cattle dung (20%), and leaf mold of Japanese oak was prepared before seeding. Table 1 shows the chemical compositions of the soil and organic fertilizer. Two organic fertilizers were applied at rates of 0, 5, 10, 15, or 20 $g \cdot m^{-2}$ and plowed to a depth of 35 cm below the soil surface just before transplanting the seedlings. The experimental design was a random block design with 4 replicates. For all treatments, the plot size was 100 × 100 cm with 15 cm spacing between individuals. A nylon net was placed to allow the vines to climb.

Samples from the local population in Young Weoul, Kang Weon Province, South Korea were prepared, and seedlings with 4 leaves were transplanted into each plot in early May. Rhizomes were harvested in early November.

Soil samples were collected to a depth of 30 cm before and after fertilization and air-dried at room temperature. The total carbon and nitrogen contents of the soil were determined using a C-N corder (Yanaco, MT700). Ammonium-N and NO_3-N in the soil were extracted with 2 N KCl solution and measured by Bremner’s method. Soluble P in the soil was determined by Truog’s method. Exchangeable K, Ca, and Mg were extracted with 1 N acetic ammonium solution and measured by

atomic absorption spectrochemical analysis. Total α-tocopherol (as vitamin E) content in the rhizome was measured by high-performance liquid chromatography (HPLC).

Table 1. Chemical properties of soil fertilizer

Constituent	Soil	Organic fertilizer	
		Barnyard manure	Leaf mold
pH (H_2O)	6.3	7.1	6.8
EC (ms)	0.06	2.2	0.5
CEC (me)	22.5	36.7	44.6
Total N	0.5	2.2	0.9
Total C	9.3	23.8	23.8
C/N ratio	17.3	10.9	25.9
Inorganic N (NO_3)	2.4	11.6	0.2
Inorganic N (NH_4)	0.7	5.8	1.8
Soluble P	17.7	787.8	58.9
Exchangeable K	41.6	56.6	25.1
Exchangeable Ca	398.4	302.3	239.1
Exchangeable Mg	50	29.2	18.7

Table 2. Correlation between chemical component in soil and rhizome yield

Constituent	Correlation coefficient	significant
Total N	0.58	P < 0.100
Total C	0.22	
Soulble P	0.47	
Exchangeable K	0.23	
Exchangeable Ca	0.05	
Exchangeable Mg	0.04	
C/N ratio	-0.90	P < 0.001

Results

During the growth period, the precipitation on the site was 600 mm, mean air temperature ranged from 10–24°C, and mean solar radiation was approximately 15000 $J \cdot cm^{-2} \cdot day^{-1}$.

The application ratio of barnyard manure and leaf mold strongly influenced the rhizome yield at harvest time. The barnyard manure increased the rhizome yield, whereas fermented leaf mold decreased the yield. The correlation between the soil chemical conditions after fertilization and rhizome weight at harvest time was analyzed using all of the data (Table 2). There was a significant negative correlation between C/N ratio and rhizome weight (Figure 2). On the other hand, total N, total C, soluble P, and exchangeable K, Ca, and Mg in the soil after fertilization did not significantly affect the rhizome yield. The C/N ratio in the soil from the surface to a depth of 30 cm, which was controlled by the application ratio of barnyard manure or leaf mold, strongly influenced the rhizome yield.

Barnyard manure increased the fresh weight of rhizomes but decreased the vitamin E content (Figure 3). There was a significant negative correlation between the fresh weight and vitamin E content of rhizomes ($p < 0.001$). The highest vitamin E content occurred with the application of 10 $g \cdot m^{-2}$ leaf mold (9–10 $mg \cdot DM^{-1}$). The nitrogen supply from the soil to plants primarily influenced the yield and quality of the rhizome as a vegetable or drug. The vitamin E contents produced under higher soil C/N ratios are one of the highest among commercial vegetables available in the Japanese food market.

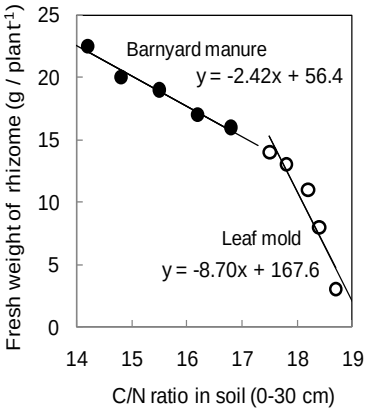


Figure 2. Relationship between C/N rations soil and rhizome yield.

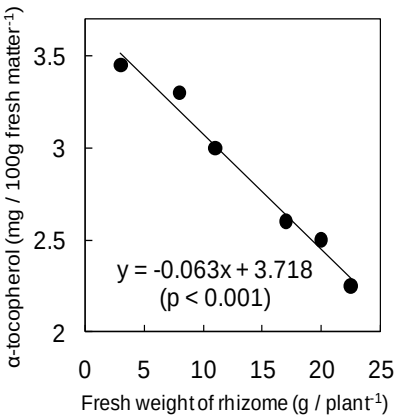


Figure 3. Relationship between rhizome yield and α-tocopherol.

Discussion

The natural habitat of *C. lanceolata* is cool shaded forest floor in mountainous areas in Korea and Japan. In this experiment, moderate amounts of rhizomes were harvested in only 1 growing season and despite a lack of shade, suggesting that the climate of the experimental field is favorable for both vegetative growth and rhizome production. Lee *et al*. (1996) reported that shading decreases the growth but improves the aromatic constituents. Our results and the previous report suggest that shading and the fermented organic fertilizers favor production of high-quality vegetables.

Our survey in South Korea (Sakamoto *et al.*, 1998) revealed that organic farmers in Kangwon Province have developed an organic fertilizer consisting of saw dust, rice bran, and rice chaff; it is mixed and fermented over 1 year and applied at a rate of 6 $g \cdot m^{-2}$ before seeding. The key technique of the organic manure production is considered to maintain the soil C/N ratio around 17% in the case of Andosol soils according to our results.

Wang *et al.* (1995, 1996) report that *C. lanceolata* is chemotaxonomically similar to *C. pilosula* and contains many polysaccharides that exhibit immunomodulatory effects. Maeng and Lee (1991) also report that the ethanol extract from *C. lanceolata* rhizome possesses effective antioxidant activity that is stronger than that of extracts from *Panax ginseng* C. A. Meyer. In addition, they point out that *C. pilosula* rhizome exhibits protective action on experimentally induced gastric ulcers in rats (Wang *et al.*, 1997). These reports and our present results suggest that the rhizome of *C. lanceolata* is a healthy food. Despite the trade-off between rhizome yield and quality, organic farming with an organic fertilizer that supplies nitrogen slowly is considered valuable in commercial food production.

C. lanceolata is also a traditional wild vegetable eaten by the Ainu people in Northern Japan. The screening research of the chemical composition in wild plants used by the Ainu revealed that the species has the highest vitamin E content among 67 tested species (Annetai *et al.*, 1996). It is also reported that most edible wild plants have vitamin E contents less than 0.5 $mg \cdot 100\ g^{-1}$ fresh weight; on the other hand, vitamin E content of the rhizome of *C. lanceolata* is 2.38 $mg \cdot 100\ g^{-1}$ fresh weight. Our results show that greater amounts of vitamin E are present in smaller rhizomes produced under higher C/N ratios due to the fermented leaf mold. Since the vitamin E contents of *C. lanceolata* are one of the highest among commercial vegetables in the Japanese food market, the vegetable is expected to be gradually accepted as a worthwhile healthy food.

Many wild species of Campanulaceae are believed to be useful ingredients in drugs that maintain the human health while being functional foods among Asian peoples. For example, many species of *Platycodon*, *Campanula*, and *Adenophora* are popular as traditional crude drugs and foods. Many Korean people and researchers of Korean herbal medicines believe that *C. lanceolata* is a functional food that has anti-cancer activity. Hata *et al.* (1998) point out that many edible wild plants in Akita prefecture, Japan induce cell differentiation, including the activity of the human leukemia cell line (HL60), which is a useful model system for drug screening. However, Inoue (2003) reports that the ethanol-soluble fraction from the rhizome of *C. lanceolata* does not exhibit the nitroblue tetrazolium reduction activity for the human promyelocytic leukemia cell line HL60 or neutrophil activity. In addition, the rhizomes do not inhibit melanine synthesis in mouse melanoma cell line B16. More investigation is required from other perspectives regarding the anti-cancer activity of *C. lanceolata* rhizomes to establish its status as a healthy food.

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We thank emeritus Professor Dr. Sadao Sakamoto of Kyoto University and Professor Dr. Choel-Ho Park, Kang Weon National University in Korea.

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Review on the Effect of Postharvest Treatment on Potato Quality

Ali Asgar

Indonesian Vegetables Research Institute
Jl. Tangkuban Perahu No. 517 Lembang 40791
Tel.(022)2786245, PO BOX 1586 Bandung, Indonesia
asgar1957@yahoo.com

Abstract

Research on tuber quality, storage, and processing was carried-out. Research emphasized on the evaluation of clones for processing quality, on-farm evaluation of promising storage technologies, and application of techniques to control postharvest losses due to pest and diseases, transportation, storage and marketing to aid in the management control at pest and disease and in the process upgrade of participating of laboratories, stores and farmers practices. Research on improving of processing method is needed to increase the quality of product including flavor. Research on packaging is mainly concentrated on the development of suitable packaging system for the product.

Keywords: post-harvest treatment, potato, quality, income.

Introduction

Among major vegetables, potato is potential to increase small farmer income, since potato is economically competitive vegetable. Potatoes are highly demanded by food industries such as french fries restaurants and potato chips industries. As estimated by World Bank, vegetable and fruit consumption in Indonesia increased approximately 3.9% during the period of 1995-2010 (Pasandaran & Hadi 1994). Demand projection to overall vegetable increases by 4.1% every year (8.2 to 12.3 million tons every year, Van Lishout, 1992).

The major challenge for Indonesian agriculture is how to produce high quality of potato at affordable prices that fit into the demands as rapidly growing population. It was clear that AARD plays an important role on vegetables quality. Postharvest technology increases product quality and reduces losses. Loss assessment of poor postharvest technology is estimated about 20 – 40% (Winarno & Aman, 1981).

Basic research program has to emphasize on strengthening the vegetable quality by improving efficiency of farming techniques, utilizing of the valuable domestic resources wisely to endure food safety, efficiency on management, food security and agribusiness development. Determination of some pre and postharvest treatment were evaluated for potato quality. Pre-harvest treatment commonly concerned with variety and harvest time, whereas postharvest concerned with fresh handling and processing.

In Indonesia, there are a few research on postharvest aspect of potato have been carried out. Postharvest experiments so far concentrated on the storage and handling of seed and potato tubers. In this paper we summarized the postharvest treatment affected the quality of potato.

Material and Methods

Several reports provided by IVEGRI were available fragmentally. Reserach started from potato varieties up to postharvest have been done, however, they need more improvement. Sequence of postharvest information were hardly found, however, some crop varieties, some postharvest experiments on various crops and some agronomic aspect methods were described

briefly. Field testing design commonly used were randomized block design, whereas in the laboratory most research were carried-out by complete randomized design with sufficient replication related to number of treatments. Postharvest treatment methodology related to the some vegetable varieties testing were mainly based on physical characterization, chemical analysis method and organoleptic test.

For potato varieties testing, 2-5 g sample was placed in 250 ml flask added with 50 ml of alcohol 80% and stirred for 1 h, then they were filtered and cleaned. The filtrate of 250 ml contained carbohydrate. Starch in filtrate as a residue were put on filter paper, and washed 5 times in 10 ml of ether, then let these samples to evaporate and they were rewashed by 150 ml of alcohol 10% to obtain free dissolved carbohydrate. Residue was removed and placed in an erlenmeyer flask through washing by 200 ml of aquadest then added with 20 ml of HCl 25%, covered with cooler lid, and boiled for 30 min. After that they were cooled down by NaOH 45% and diluted to 500 ml. Filtered and determined the sugar content as glucose from the filtrate. Determination of starch content was calculated by glucose weight times factor 0.9. Crop hardness was measured by penetrometer, and organoleptic test was carried-out by hedonic method.

Results and Discussion

Storage of seed potatoes

An experiment was conducted to compare the effect of diffuse light and dark storage of tuber seed on yield of patato by Sihombing (1986). This experiment was carried out at hight land Ciwidey at West-Java, (1080 m above sea level) on a farmer field in both dry and wet season. A randomized block design of non-factorial was applied with two storage method having 12 replicates on each season. The results indicated that the seed tubers stored in diffuse light storage showed better plant vigour and gave yielded increases from 16.9 to 26.9%, compared with tubers stored in the dark.

The effect of storage method and delay in planting of tuber seed on yield and quality of potato tubers was carried-out by Nainggolan (1993). A randomized block design with three replicates was used in this experiment. Two methods of storage used tuber seed (in dark and diffuse light) and planting periods (5 months of storage as control). The results showed that storage of tubers seed in diffuse light storage increased the yield up to 15.02% and the quality of tuber was better compared to dark storage. The delay in planting of tuber seed decreased the yield to 27.50 g/seed/plant and showed lower quality tuber.

Potato seed storage in mid-elevation area with diffuse light storage was done by Asgar and Asandhi (1994). The planting time of mid-elevation potato was in May to June, while the tuber seed used was harvested in December, long storage of potato seed affected poor quality of seed. One way to overcome the problem was by using diffuse light storage. Six varieties of potato used diffuse light storage. Six varieties of potato (Granola, Red Pontiac, Cosima, Monza, Cipanas, and Berolina) were stored in diffuse light storage and dark storage by using randomized completely block design with six replicates. The results showed that after four months storage the percentage of rotten tubers of Granola was 69%, although diffuse light storage was better than that in dark storage in terms of higher number of sprout, shorter, bigger and more healthy.

Storage of ware potatoes

Study on storage of ware potato and the loss due to storage method was conducted by Asgar and Asandhi (1990). Thirty farmers was sampled for interview. The results showed that some farmers stored their ware potato for several days until two months when the market price as low (Asgar, 1990). Two method of storage was used in the field (either covered delaying the harvest or pilled beside the field and covered with canvas) or in the storage which was usually seed storage.

The average loss was 7.1% for farmers having less than 1 ha land and 9.3% for farmers having 1 ha or more land. The highest loss was recorded at 25%.

Improvement of ware potatoes store method was carried-out by Asgar and Asandhi (1991). A two-layer of box was made in the storage. The distance between the two layers was 14 cm and filled up with rice husk. The box was divided into 12 parts to place the experimental unit consisted of four treatments and three replicates. The size of the experimental unit was 104 cm length x 77 cm width x 44 cm depth. The design used was randomized completely block design. The treatment was pilling potato tubers with: 1) vertical without ventilation, 2) horizontal without ventilation, 3) vertical with ventilation and 4) horizontal with ventilation. The ventilation made from proliferated tube was put vertically into the pile. The size of the tube was 74.5 cm of length, 8.5 cm of diameter, and the number of holes was 20. From this study could be concluded that ware potato storage using double layers of wall and filled up with rice straw could be introduced to farmers in the highland, since the temperature in the tubers piles was not significantly different with the room temperature. However, pilling the tubers horizontally was better than vertically as done by the farmers.

Experiments on the harvest time and storage period was done by Asgar and Marpaung (1997). Seed tubers with the size of 25 to 45 g/tuber collected from Pangalengan were planted at the experimental area at the Indonesian Vegetables Research Institute. The seed tubers were 72/plot. Fertilizing and maintanance at the field was conducted at the Indonesian Vegetables Research Institute recommendation. Manure was given side of planting with dosage of 30 tons/ha, ZA at 100 kg/ha, TSP at 250 kg/ha and KCl at 300 kg/ha. Seed tubers were planted with the sprout of 1 to 2 cm length and planting distance 80 x 30 cm on matches. A split plot design was used and plants were harvested on day 70, 80, 90, 100, and 110 days. Sub plot was storage period consisted of 1, 2, 3, 4, and 5 day. The result showed that harvest time of 100 after planting yielded good quality as evaluated by panelist. The best characteristic was found for tuber harvested on 100 days after planting and stored for two days.

Processing

The best variety for chips was Atlantic as investigated by Asgar and Kusdibyo (1997) using method as follows: seed tubers of 25–45 g/tuber collected from the farmers in Pangalengan and Wonosobo were planted at the experimental area of IVEGRI. The seed tubers were 72 per plot. Fertilizing and maintenance at the field used IVEGRI recommendation (1994). Manure was given side of planting with dosage of 30 tons/ha. Artificial fertilizer used at planting time were Urea at 200 kg/ha, ZA at 100 kg/ha, TSP at 250 kg/ha, and KCl at 300 kg/ha. Seed tubers were planted with the sprout of 1–2 cm in the length and planting distance was 80 x 30 cm on matches. A split plot design was used in this research and every treatment combination was repeated three times. A main plot was varieties consisted of Atlantic, Latif, and Granola. A sub-plot was harvest time consisted of 90, 100 and 110 days. The results showed that the quality of Atlantic variety and harvest time of 100 days were the best than the others in terms of the colour, brittleness, taste and appearance.

The chipping quality of 45 advanced potato clones was determined by Asgar and Chujoy (1999). The results showed that best clones with chipping scores ranged from 1.0 to 3.4 (light yellow to dark yellow) which were: I-853, ASN-691, Muziranzara, CFJ-691, CEW-691, Atlantic, ABZ-69-1, TS-2, VC24.16, Cruza-148, LT-5, Monsama, P-4, TS-13, 379706.34, 283232.11, CCN-69-1, MF-II, CFM-69-1, 3806010.14, 378501.3, Chiqita, Graso-28, I-1150, AGB-69-1, Desiree, I-11035, Cruza-27, and Precodepa.

Research on the evaluation of 12 potato processing clones was conducted by Basuki *et al*. (2003). Experimental area used was at 4 locations i.e: Pangalengan, Garut, Batur (Middle Java), and Tosari (East Java). Industry for potato chips modern scale, two home industries, and 42 farmers were interviewed to evaluate the production character, tuber quality, specific gravity, appearance and process product taste from 12 potato processing clones. The results showed that 380584.3 and FBA clones were preferred by farmers, home industry, and consumer. FBA-4 clone

coled be developed for modern industry with good handling. TS-2 and MF-II were received by modern industry processor as raw materials. These clones could be released as new variety for processing. The product of TS-2 was equal to Atlantic and Panda and product of MF-II was higher than that of the Atlantic and Panda.

Basuki (2005) showed that according to resistance of the leaf rotting diseases and swollen root nematodes, 380584.3, TS-2, FBA-4, I-1085 and MF-II clones were resistance. This figure was achieved from ten experimental areas which were 380584.3 resulted in 33.5 t/ha, TS-2 was 22.4 t/ha, FBA-4 was 28.1 t/ha, I-1085 was 25.3 t/ha and MF-II was 30.1 t/ha, respectively. Furthermore, he stated that the use of FBA-4, TS-2 and MF-II clones were suitable for raw materials of potatoes chips for modern industry, while 38058.4.3 and I-1085 were suitable for home industry and middle class.

Research on distribution of taste on potato slices was conducted by Kastaman *et al*. (2000). A completely randomized block design was used in this research. First factor was potato cultivar consisted of: Atlantic and Granola. Second factor was Sodium Metabisulfite concentration at 500, 750, and 1000 ppm. Third factor was ingridients consisted of: 1) salt, 2) salt and broth, 3) salt and chicken curry, 4) salt and meat simmered in spices and coconut milk, and 5) salt and pepper. The results showed that for Atlantic cultivar, dipping with sodium metabisulfite treatment was not significantly different on the colour, flavor, hardness and taste of potato chips. On Granola, there was a significantly different on organoleptic characters (colour, taste, appearance). Increasing in Indonesian spices such as *kaldu ayam* (chiken broth), *kari ayam* (chiken curry), *rendang* (meat simmersed in spices and coconut milk), and pepper gave significantly different to increase in consumer preference on organoleptic test. There was an interaction between cultivar and sodium metabisulfite concentration on the quality. Economic analysis for potato chips on the estimation of cost price production of Atlantic cultivar was Rp. 15.711/kg. The price of chips was Rp. 16.750/kg, whereas the price of raw materials was Rp. 4000/kg. For estimation of cost price of Granola cultivar was Rp. 13.211/kg and a sell price of chips was Rp. 14.240/kg with buying price of raw material at farmer level Rp. 1500/kg.

Quality assessment for several clones has already done by Asgar *et al*. (2010). The objective of this research was to test for clones/cultivars selected from the yield and quality. Quality test of 10 selective clones was determined. This research was conducted from July to September 2010. The research was arranged in randomized block design. The results showed that chips which having value between 2.00 and 2.36 (yellow uniform) for potato chips was clone 7 (391011.17 x 385524.9). Reduction sugar content from this clones were assumed to lower than reduction sugar content of the other potato clones having dark colour.

Conclusions

Considering the increasing demand for processed potato products, a systematic evaluation of cultivars and clones for processing quality would decrease high research priority. On-farm evaluation of promising storage technologies for seed and ware potatoes that have already been tested elsewhere should also be emphasized. Application of technologies to control postharvest losses due to pest and disease, transit, storage, and marketing were to improve the management control at pest and disease and in the process upgrade of participating of laboratories, stores and farmers practices. Research on improving of processing product is needed to increase in the quality including taste pass through giving spices (flavour). Research on packaging is mainly concentrated on the development of suitable packaging system for the product.

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Perception, Attitude and Factors Influencing Household's Acceptance to Transgenic Late Blight Resistant Potatoes

Asma Sembiring[*], Eri Sofiari

Indonesian Vegetable Research Institute, Lembang, Indonesia
**Corresponding author: Indonesian Vegetable Research Institute (BALITSA)*
Jl.Tangkuban Perahu No.517 Lembang, Bandung-40395, Indonesia
Tel : +62 22786245; Fax : 62 22786416
asma_h_1999@yahoo.com

Abstract

The objective of this study was to identify the perception, attitude and factors influencing the household's acceptance to the transgenic late blight resistant potatoes. The research used data taken from 101 respondents in Bandung West Java, Indonesia from September to November 2009 using stratified random sampling. The surveys showed that most of the respondents (99%) never heard about transgenic potatoes. About 36% respondents said that transgenic potatoes were safe to be consumed. At least, 55.4% respondents were willing to consume transgenic potato as long as there was no negative effect by consuming it. In addition, 44.6% respondents said that they were not willing to consume the potato because they did not know it. Result of Chi-square test showed that the most factors influencing household acceptance to transgenic potato were consumption frequency and consumers' potato criteria. Statistical analysis using logit binomial test showed that education and consumers' potato criteria were the most important factors for the household to accept the transgenic late blight resistant potatoes.

Keywords: consumers' acceptance, consumers' attitude, potato transgenic

Introduction

Potato is the fourth source of carbohydrate in the world and has a big chance as an alternative food diversification because it contains high nutrient. Potato production centers in Indonesia commonly locate in high land, with high rainfall and humidity. The condition is very suitable for late blight (*Phytopthora infestans*-Mont de Barry disease). Yield loss caused by diseases reached 60 to 80% (Wattimena, 1994). In severe attacked, the loss reached 100%.

The development of resistant potato varieties are one of the solution to reduce yield loss caused by late blight disease. Several sources of potato resistant to late blight have been found in some species of wild potatoes such as *S. demissum, S. bulbocastanum, S. stoloniferum,* and *S. microdontum* (Hawkes, 1994). Resistant genes to the late blight from *S. bulbocastanum* have been cloned and used to produce potato resistant late blight. The crossing between Katahdin Rb with Granola and Atlantik resulted in resistant filial to late blight (Song *et al*., 2003). In 2004, trial to Katahdin transgenic potatoes was conducted. The results showed that Katahdin could control late blight at the *early-season*. Field trail to examine potatoes resistant characteristic was held in 2007 and 2008 and several filials (F1) resistant to late blight infection.

Kotler and Susanto (2001) reported that several factors influence consumer behavior, including psychology. Psychology aspects involve perception, knowledge, and attitudes become important aspects in consumer acceptance to the product. Study on consumer behavior to the transgenic products has been conducted in some countries. The consumer acceptance to the transgenic products depends on their perception to the benefit and the risk of the transgenic (Byrne, 2006). Supporting of transgenic products come from United State of America and Japan consumers in agriculture and healthy sectors. The more information consumers get, the more positive response give to transgenic crops (Hoban, 1999). Other studies showed that respondents' knowledge related

to transgenic crops and labeling has a negative impact to consumer acceptance (Jill *et al*., 2001). In Korea, the consumers tend to reject transgenic food, especially women, people who get high education and persons who have enough information about transgenic (Onyango *et al*., 2004).

In Indonesia, the issue about transgenic crops is still in debates. Pros and cons emerge as the development of transgenic information and the willingness to plant the products with several reasons. Studies to observe factors influencing household acceptances' to transgenic crops has been done in three big cities in Indonesia, i.e. DKI Jakarta, East Java and North Sumatra concluded that the less information about transgenic crops consumers have, the easier for consumers to accept transgenic food. Meanwhile, in economic term, consumers from middle and higher economic strata are more willing to accept transgenic crops than the consumer from lower economic. Public acceptance to transgenic crops are also influenced by direct and indirect risk perception from transgenic food, benefit, risk and the credibility of policy institution in evaluating safety crops and the environment (Riska, 2009).

Issues on pros and cons about transgenic crops in Indonesia developing bigger than the product itself. The opponent was greater seen from media coverage rise out the negative effect of transgenic crops and it will affect consumer acceptances' to the transgenic potatoes. In fact, the product has not been released and sole in Indonesia. The commercialization of transgenic potatoes shall go through several trial tests in advance such as limited testing of varieties, limited testing laboratories (bio safety containment) and limited field test (bio safety confainment) based on Bio safety Commission regulation.

So far, there have been several studies conducted to determine perception, knowledge, attitude and consumer acceptances' to the transgenic cops/foods, yet specifically for *Phythopthora* resistant potato varieties. So, the study is expected contribute to increase knowledge related to consumer acceptances' to transgenic crops.

Materials and Methods

The survey was conducted on September to November in 2009. Household respondents were chosen as stratification of 101 persons in Bandung municipal, Indonesia. The stratification was based on income strata. Lower level with income was from IDR 1,000,000 to 2,000,000; middle level was from IDR 2,000,001 to 3,000,000 and high level was more that IDR 3,000,000.

The variables were characteristic of respondents, perception, attitude and respondent acceptances' to the potatoes transgenic. Data for perception and attitude were counted by qualitative statements on the ordinal scale (1 to 5) using Likert scale. Whereas, acceptance's data variable were measured by nominal scale (yes or no). Furthermore, the data were analyzed with Descriptive statistic method, Chi-square test and Logit binomial.

Results and Discussion

Demographic and Socioeconomic of the Respondents

The sums of respondents were 101, dominated by ages between 31 and 60 years, 78 respondents (78%), with average level of education were senior high school (34%). Most of the respondents were house wife (67%). Level of income per month were 1-2 millions rupiah (68%). The potatoes household average consumption renged from 1 to 2 times per week (64%) with number of consumption was 1,05 kg.

Consumer Perceptions' to the Potatoes

Descriptive statistic showed that more than 93% respondents stated that the potatoes was nutritious vegetable, made healthy, delicious and affordable by consumers, available in the market

and easy to be processed for any types of cuisines. As many as 88,1% respondents also said that the potatoes could be used as staple food (Table 1).

Table 1. Likert scale from household perception to the potatoes

Statements	Percentage (%)				
	1	2	3	4	5
The potatoes is nutritional food	6,9	91,1	2,0	0,0	0,0
The potatoes is a healthy food	11,9	87,1	0,0	1,0	0,0
The potatoes is delicious food	6,9	89,1	3,0	1,0	0,0
The potatoes price is affordable	6,9	88,1	3,0	2,0	0,0
The potatoes can be used as staple food	9,9	78,2	1,0	10,9	0,0
The potatoes is available in the market	31,7	68,3	0,0	0,0	0,0
The potatoes is easy to be processed	11,9	86,1	1,0	1,0	0,0

(1=strongly agree; 5=strongly not agree, N = 101)

The Consumers' Potatoes Criteria

Taste is the most important criteria for the consumers. The results were consistent with previous study concluded that the consumers noticed more to potatoes tastes (Ameriana *et al*., 1998). The color, taste and nutrition were also more important criteria's for the potatoes compared to the price, aroma and the safety to be consumed with value of 72,3; 24 and 50, 5%, respectively (Table 2).

Table 2. Likert scale from the consumer important criteria's' to the potatoes

Statements	Percentage (%)				
	1	2	3	4	5
Taste	4,0	89,1	2,0	5,0	0,0
Nutritional	9,9	83,2	5,0	2,0	0,0
Appearance	13,9	83,2	1,0	2,0	0,0
Price	5,0	67,3	4,0	23,8	0,0
Color	12,9	83,2	1,0	3,0	0,0
Aroma	2,0	21,8	4,0	72,3	0,0
Safety to be consumed	22,8	26,7	4,0	46,5	0,0

(1=strongly agree; 5=strongly not agree, N = 101)

Consumers' Perception and Attitude toward Transgenic Potatoes

Table 3 described that 70,3% of respondents always seeked the information of potatoes consumption. The information included the origin of the potatoes (respondents more likely the potatoes from Dieng-West Java), safety while be consumed, taste, size and the color.

Table 3. Likert scale on consumers' perception and attitude toward the potatoes

. Statements	Percentage (%)				
	1	2	3	4	5
Always seek information of potatoes consumption.	3,0	67,3	2,0	27,7	0,0
Knowing transgenic foods on the market	2,0	38,6	12,9	46,5	0,0
Knowing transgenic potatoes (raw/processed) on the market.	0,0	0,0	7,9	91,1	1,0
Transgenic potatoes are safe to be consumed	2,0	33,7	20,8	42,6	1,0
Transgenic labeling should be attached	7,9	80,2	3,0	7,9	1,0
Circulation of food made from transgenic potatoes need to be informed on community	8,9	78,2	6,9	5,9	0,0
Pros and cons related to transgenic including to potatoes are occurred because of lack of information on consumer socialization.	0,0	84,2	10,9	5,0	0,0
Transgenic potatoes need to be introduced on the consumers	6,9	90,1	1,0	2,0	0,0

(1=strongly agree; 5=strongly not agree, N = 101)

More or less 40,6% of respondents ever heard the circulating transgenic food on the market. Specifically for the potatoes, most of the consumers (99%) were not informed. Therefore,

definition of potatoes transgenic should have been explained to the respondents at the beginning. After that, questions related to transgenic were asked. After the explanation, 40 respondents (40%) said that they would be looking for more information associated with the transgenic potatoes, while 60% of respondents were not decided yet to seek transgenic potatoes information because they were unaware with the product, beside that, they have already familiar with the potatoes sole on the market. All consumers were not aware toward transgenic potatoes circulation in the market whether they were in raw or processed product.

The survey also indicated that fewer consumers (36%) stated that the transgenic potatoes were safe and edible. Transgenic potatoes were derived by placing a gene from naturally blight resistant wild potato, so the origin was still from the potatoes, less pesticide and guaranteed from the government (Curtis *et al*, 2004) become the reasons of these arguments. Reducing pesticide becomes essential factor for consumer acceptances' to transgenic food (Wen and Rickertsen., 2002; Hoban, 1999). The positive response from consumers are might because the respondent did not have enough information about transgenic products (Onyango *et al*, 2004; Arianti., 2009). Study conducted by Jill *et al* (2001) indicated that they had less knowledge about biotechnology, they though more positive about the use of biotechnology in food production. The rest repondents had no opinion regarding to the transgenic potatoes since they had no knowledge what transgenic was.

Respondent opinions to the importance of transgenic labeling placed 88%. Consumers also argued that transgenic potatoes information should be disseminated to the communities. Furthermore, regarding the potatoes transgenic pros and cons, most of respondents' response the issues emerged because the community awareness toward the issues were still low due to the lack of information. Therefore, almost all consumers agreed that information about the transgenic potatoes should be shared to the communities.

Consumer Acceptances' toward the Potatoes Transgenic

The consumer acceptances' to transgenic potatoes based on the consumer beliefs that the transgenic potatoes were safe to be eaten, consumer willingness to consume transgenic potatoes and the influence of debating transgenic potatoes to consumer decision resulted that 55.4% of consumers were willing to accept transgenic potatoes as long as no negative side effect. Moreover, the debate about transgenic crops does not affect consumer decisions to eat the product. About 44.6% of respondents were avoid to accept transgenic potatoes because they had no information, never heard and seen the potatoes, so that they were still reluctant to consume it.

Chi-square to test the connection between two variables among age, education, occupation, income, consumption frequency, perception, an important potato criteria's according to consumer and attitude showed that only consumption frequency and consumers' potato criteria significant was related to consumer acceptances' to transgenic potatoes with value 0,032 and 0,021 (table 4).

Table 4. Chi-Square test (significant in 5%, N=101)

No	Statements	Chi-square value
1	Age	0.781
2	Education	0.148
3	Occupation	0.687
4	Income	0.549
5	Consumption frequency	0.032*
6	Perception	0.226
7	Criteria's	0.021*
8	Attitude	0.303

Significant in 5%.

Meanwhile**,** logit binomial test indicated that the relationship between variables age, education, occupation, income, consumption frequency, perception, an important potato criteria's according to consumer and attitude overall to consumer acceptance's to transgenic potatoes resulted that age, occupation, income, consumption frequency and perception did not affect consumer acceptance's to transgenic potatoes. The variables affected consumer acceptance's were education and an important potato criteria's with value of 0,028 and 0,013, significant in 5%, respectively (Table 5).

Table 5. Logit binomial test

No	Statements	Logit value
1	Age	0.515
2	Occupation	0.367
3	Income	0.679
4	Consumption frequency	0.102
5	Perception	0.216
6	Attitude	0.315
7	Education	0.028*
8	Criteria's	0.013*

Significant in 5%.

Conclusions

About 55,4% of consumers were willing to eat transgenic potatoes as long as no negative side effect, while 46% of them were not willing to consume it because they did not have any information about the products. The most factors influencing household acceptance to transgenic potato were consumption frequency and consumers' potato criteria. Moreover, logit binomial test showed that education and consumers' potato criteria were the most important factors for the household to accept the transgenic late blight resistant potatoes.

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Relations Between the Amyloplast Sedimentation in Tubers and the Morphogenesis of Tubers in Yams

Michio Kawasaki [a*], Seito Hosokawa [a], Hayuru Yoshida [a], Kyoko Takahashi [a], Hiroki Suto [a]

[a] *Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan*
**Corresponding author: Faculty of Agriculture and Life Science, Hirosaki University, 3 Bunkyo-cho, Hirosaki, Aomori 036-8561, Japan*
Tel.: +81 172 39 3815; fax: +81 172 39 3815.
kawasaki@cc.hirosaki-u.ac.jp

Abstract

Tubers which have different forms in Japanese yam (Jinenjo) and Chinese yams were used in this study. In developing tubers of all materials, amyloplasts were locally formed at the part beneath the stele in tuber apexes. However, the numbers of total amyloplasts in each cell and settling amyloplasts in each cell at the parts of elongating tubers of Nagaimo and Jinenjo were greater than their numbers in the parts of thickening tubers of Genkotsujirou and Iseimo. When tubers of all materials matured, few amyloplasts were observed at the part. In this study, we discussed about the relations between the amyloplast sedimentation in tubers and the morphogenesis of tubers in yams.

Keywords: amyloplast, gravitropism, morphogenesis, starch granule, yam

Introduction

The tuber of *Dioscorea* is generally regarded as a tuber but it has some characteristics different from conventional tubers. Therefore the tubers of *Dioscorea* are also regarded as rhizophores. The tubers have various shapes. Even in a cultivar or a line, the form of tubers may be variable. The inconstant nature causes the quality of tubers and the work efficiency to decrease. Previously we reported that many amyloplasts were locally formed and settled down by gravity at the apexes of elongating tubers in Chinese yam (Kawasaki et al. 2008). In this study, relations between the amyloplast sedimentation in tubers and the tuber morphogenesis were investigated by using some Chinese yam (*Dioscorea opposite* Thunb.) and Japanese yam (*Dioscorea japonica* Thunb.).

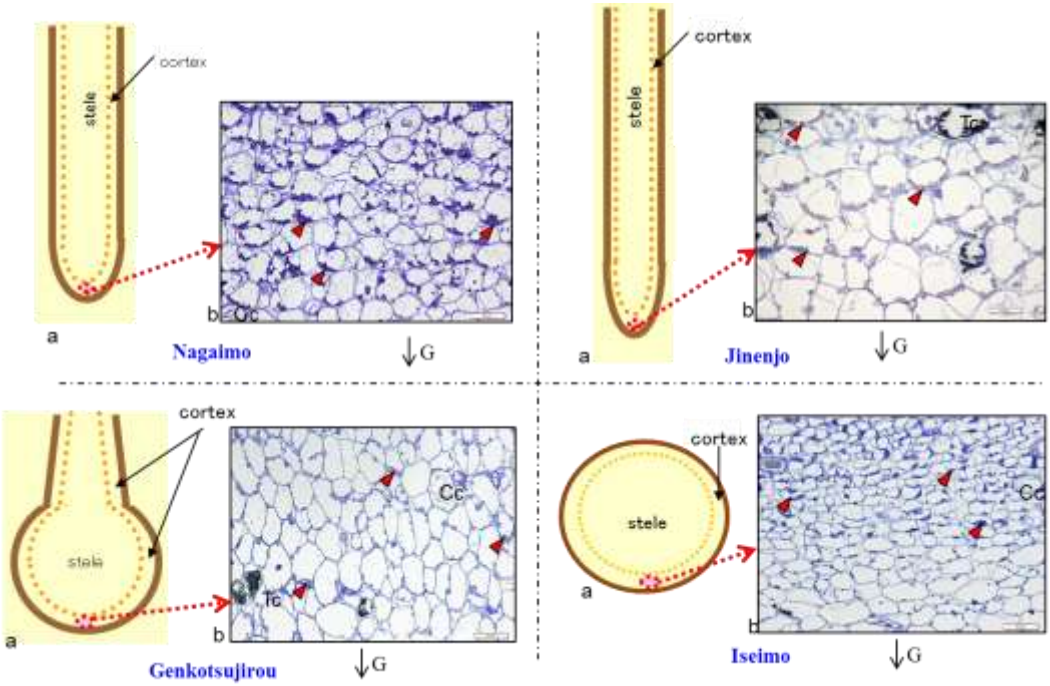
Materials and Methods

Japanese yam (Jinenjo) and Chinese yam, cv. Nagaimo which elongate until their harvest times to form long tubers, Chinese yam, cv. Iseimo which spherically thickens until harvest time, Chinese yam, cv. Genkotsujirou which elongates in the initial stage and then spherically thickens, were used. Developing and mature tubers were sampled and observed with a light microscope. Morphological characterization related with amyloplasts in tuber apexes was mainly investigated in this experiment.

Results and Discussion

In developing tubers of all materials, amyloplasts were locally formed at the part beneath the stele in tuber apexes. However, at the part beneath the stele in tuber apexes, the numbers of amyloplasts in each cell and amyloplasts which settled down in each cell were not the same among materials. The numbers of amyloplasts and settling amyloplasts at the parts in elongating tubers of Nagaimo and Jinenjo were greater than their numbers at the parts in thickening tubers of Genkotsujirou and Iseimo (Fig.1). In Genkotsujirou, the number of amyloplasts which settled down at the part of tubers in thickening stage more decreased than it in elongating stage. In tubers of Iseimo, both the amyloplasts in each cell and the amyloplasts which settled down in each cell at the part were lower level throughout growing period. When tubers of all materials matured, few amyloplasts were observed at the part. While amyloplasts had been accumulated in parenchyma of stele in tubers with growing, their directional bias was not observed in each cell in all materials. Crystal cells and tannin cells dispersed in the part beneath the stele in tuber apexes as in cortex surrounding the stele in all materials. The crystals and the tannin bodies were not also ubiquitous in each cell at the part in each material (Fig.1).

It was shown that the amyloplast sedimentation in tuber apexes was common structural characteristic in some *Dioscorea* materials. In root caps of plants, amyloplasts have been reported to act as gravisusceptors (Blancaflor *et al.,* 1998; Perbal, 1999) and it is known that they are related to morphogenesis of the roots. The results from this study indicated that the amyloplast sedimentation would be concerned in gravity perception and the characteristic of the sedimentation possibly was concerned in the morphogenesis of tuber in each material. We are also carrying out further research with a lot more cultivars and lines in *Dioscorea* by using electron microscopes.



(a) Diagram of longitudinal section of tubers. (b) Longitudinal sections of the part beneath the stele in tips of developing tubers. The sections were stained with toluidine blue O. Arrowheads indicate amyloplasts. Cc, crystal cell; G, the direction of gravity; Tc, tannin cell. Genkotsujirou was investigated at thickening stage.

Figure 1. Amyloplast distribution in tips of developing tubers.

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Optimalising Potato Productivity in Sembalun Highlands, Nusa Tenggara Barat – Indonesia

Baiq Nurul Hidayah[a*], Sudjudi[a], Lia Hadiawati[a], Dwi Praptomo Sudjatmiko[a], Peter Dawson[b], Terry Hill[b], Julie M. Warren[b]

[a]*Institute for Assessment of Agricultural Technology, Nusa Tenggara Barat Province, Indonesia*
[b]*Department of Agriculture and Food, Western Australia*
[*]*Corresponding author: Balai Pengkajian Teknologi Pertanian (BPTP) Nusa Tenggara Barat, Jalan Raya Peninjauan Narmada, Lombok Barat – NTB, Indonesia 83010*
nurul.hidayah@yahoo.com

Abstract

Potato cultivation in Nusa Tenggara Barat (NTB) Province of Indonesia locates in the Rinjani Valley highlands at 1,050 – 1,250 m above sea level. Farmers grow the Atlantic variety for a crisp processing company. The yield of Atlantic is low which is about 18.2 ton/ha, however, this is a profitable crop for farmers. Elsewhere in Indonesia the frequent cropping of potatoes has led to soil quality decline. To reduce the decline, potato farmers in NTB used compost and superphosphate to increase potato productivity. They also applied a new management technique. This is important because potatoes are a new crop in NTB and specific management suited to local conditions which has not yet been developed. The research was conducted at six farmers' fields and involving six Farmer Field School (FFS) groups. The compost treatments applied were local compost at 3,000 and 5,000 kg/ha. The super phosphate treatments applied were 300 and 600 kg/ha. Each treatment had six replicates by having six different farmer groups which had the same experimental design in their fields. The results showed that there was no significant difference in yields of super phosphate at 300 and 600 kg/ha which were 33.0 and 33.1 t/ha, respectively. Similarly there was not a significant difference in yield between the compost treatments with 3,000 kg/ha producing 33.0 t/ha, meanwhile with 5,000 kg/ha of compost produced 32.7 t/ha.

Keywords: productivity, potato, compost, farmer initiated learning, Nusa Tenggara Barat

Introduction

Potato (*Solanum tuberosum*) is an important vegetable commodity in Indonesia having a good market, both as a vegetable and raw material for food processing industries. Because of the high demands, potatoes are expected in the future for diversification of carbohydrate sources that may increase sustainability and overcome poverity in Indonesia. In Indonesia, potatoes are produced in 21 provinces, the biggest are in Sumatra and Java with the cropping area are around 64,148 ha. Demand for table and processing potatoes increases every year. The value of imported processing potatoes to Indonesia in 2007 was US$ 40 million whch was about 43,477 tonnes. In 2008 (from January to September) processing potato imports reached 29,187 tonnes with value of US$ 28 million. In 2009 Indonesia imported 48,000 tonnes with value of US$ 33 million. Indonesian domestic potato production has increased steadily at over 3% per annum since 1997 and reached 1,176,304 tonnes in 2009 (Badan Pusat Statistik, 2011).

Potato production is generally conventional and traditional or continuing from previous generations, so that yields are still low which is from 14.9 to16.4 tonnes/ha (average yield in Indonesia). Many problems are found to increase potato production in Indonesia including: (1) low quality and quantity of seed potatoes, which forms the main concern in the effort to increase potato production in Indonesia (Fuglie *et al.* 2006), (2) current conventional cultivation techniques (Kuntjoro, 2000), (3) topographic factors, where high areas with suitable temperatures for growing

potatoes are very limited (Kuntjoro, 2000), (4) the tropical conditions are optimum for the development of pests and disease of potato crops (Kuntjoro, 2000).

In NTB, potatoes are mainly cultivated in the Sembalun sub-district on the slopes of Mount Rinjani, from about 1,050 to 1,250 m asl. Potatoes are now the most important horticultural commodity for the Sembalun community. The potato variety grown by Sembalun farmers is Atlantic with a yield of 18.2 tonnes/ha (BPTP NTB, 2009) which is still relatively low because in several potato studies in Indonesia the yield has been 35 tonnes/ha (BPTP NTB, 2009). Sembalun farmers can grow potatoes in the dry season as well as the wet season. In the wet season potatoes are planted in dryland that has a potential area of more than 1,500 ha and in the dry season potatoes are planted in paddy fields after the rice harvest in the months of June and July with a potential area of 1,105 ha. In 2010, the percentage of the paddy area used to produced potatoes was just 15 %.

The main constraint to development of Atlantic potatoes in Sembalun is sub-optimal application of integrated crop management (ICM). Farmers still predominantly use chemical fertiliser and do not use organic fertiliser although there is the potential to produce and develop the local organic fertiliser. Farmers are also accustomed to control pests and diseases with chemical pesticides without observing threshold levels indicating whether control is required or not, also apply mixtures of various pesticides together without paying attention to the active ingredients causing an impact on the important natural predators. The dominant use of chemical fertiliser and pesticides is the reason why potato enterprises have high costs. In fact the constant use of chemical fertiliser will damage the soil structure and make the soil hard (Nurmayulis and Maryati, 2008). Furthermore, the excessive use of chemical pesticides will destroy the insect biodiversity and lead to the death of insects and other microorganisms antagonistic to pests and pathogens (Nurmayulis and Maryati, 2008).

The constant practising of conventional potato production systems will reduce the profitability of farmers (BPTP NTB, 2009), and in the long term will cause environmental damage and the loss of biodiversity in this region (BPTP NTB, 2009). Because of this, a sustainable and environmentally friendly plan to support agricultural development through the study of optimalising potato yield in the Sembalun highlands is needed. Sembalun is a small, isolated potato production area without specialist potato support services and so research into optimising potato production inputs will have to be carried out by the farmers themselves with local extension workers.

To overcome the contraint of sub-optimal crop management a technique was required that enabled farmers to become their own researchers. The technique used was a modification to the FFS methodology. The aim was to instigate demonstration plots that allowed the impact of single management changes to be measured by farmers. Previously the potato FFSs had compared an ICM plot versus a conventional plot. This resulted in a range of management changes between the plots which made it difficult to identify the cause of improvements in profits between the treatments. We call this improved methodology Farmer Initiated Learning (FIL).

This research aimed to help farmers develop and put into use a less costly potato production system that will significantly increase the ability of small farmers to take up potato production in the Sembalun highland.

Materials and Methods

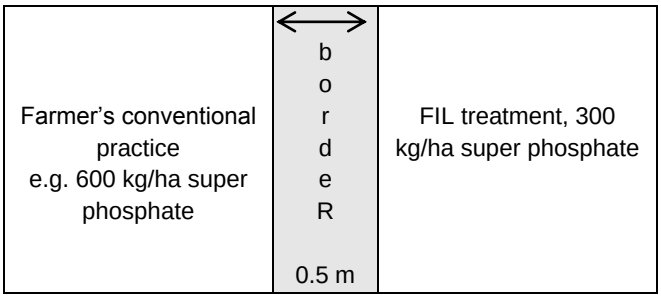
Experimental set-up

Simple FIL experiments to test one variable were introduced to Sembalun Farmer Field Schools in 2009. The research was conducted at six farmers' fields involving six Farmer Initiated Learning groups. At Sembalun six farmer groups compared the use of superphosphate with compost. The plots were around 1000 m^2 and all had a 50 m^2 yield assessment sub-plot pegged in the center. This meant that the yield measured by each farmer group came from the same size plot. The results from each farmer group were used as replicates. Results were presented on a t/ha

basis. The experiment was located at the Sembalun highland of NTB. The FIL groups were all members of the overarching farmer group Kelompok Horsela. The experiment was conducted at one growing season from June to November 2009.

Cultural practices and conditions

The compost treatments applied were local compost at 3,000 and 5,000 kg/ha. The superphosphate treatments applied were 300 kg/ha and 600 kg/ha. Other fertilisers applied were the same and included; NPK 600 kg/ha and sulphate of ammonia 300 kg/ha. The plot design is shown in Figure 1.



To the left the farmers' usual rate of superphosphate was applied. To the right a lower experimental rate of superphosphate was applied. Plots varied in size according to individual. Yield was determined by harvesting a 50 m^2 sub-plot in the centre of each treatment.

Figure 1. Example of experimental plot design.

Statistical analysis

The simple experiments were executed to plan by the six farmer groups. Yields were measured and recorded from the yield sub-plots. The results from each of the six farmer groups were used as replicates in an ANOVA. All the data were statistically analysed using analysis of variance (ANOVA). The significance of treatment effect was determined using F-test, and to determine the significance of the difference between the means of the two treatments, least significant differences (LSD) were estimated at the 5% probability level.

Results and Discussion

There was no significant difference in yields from superphosphate applied at 300 and 600 kg/ha. The respective yields were 33.0 and 33.1 t/ha (Table 1). Similarly there wasn't a significant difference in yield between the compost treeatments with 3,000 kg/ha producing 33.0 t/ha while 5,000 kg/ha produced 32.7 t/ha (Table 1). This indicated that farmers can improve their efficiency of phosphate and compost, therefore, it also meant to improve income through the reduction in the input costs.

Super phosphate cost was 2000 Rp/kg (BPTP NTB 2009) and the average farmer uses was 433 kg/ha. The finding that 300 kg of super phosphate is sufficient for potato production in the paddy areas of Sembalun means that they can save 133 kg of super phosphate or 266,000 Rp/ha which will improve farmers' income through reduced input costs.

Compost at 5,000 or 3,000 kg/ha did not significantly affect the yield of potatoes. Manure costs 497 Rp/kg (BPTP NTB 2009) and the average farmer uses 3,192 kg/ha. The finding that 3,000 kg of compost is sufficient for potato production in the paddy areas of Sembalun means that there can be a saving of 192 kg of compost or 95,425 Rp/ha for the average farmer which will also improve farmer income because of reduced input costs. Farmers who use above average organic

manure, the savings will be greater. For example, farmer who previously used 5,000 kg/ha of compost then he reduced to 3,000 kg/ha, the savings would be 994,000 Rp/ha.

Table 1. Results of Farmer Initiated learning-by-doing plots investigating the effect of lower super phosphate and compost rates – NTB 2009

Treatment	Description	Yield (t/ha)
Super phosphate		
300 kg/ha	experimental rate	33.0
600 kg/ha	farmers' usual application rate	33.1
Significance		ns
LSD		1.4
n		6.0
Compost		
3,000 kg/ha	experimental rate	33.0
5,000 kg/ha	farmer's usual application rate	32.7
Significance		ns
LSD		2.8
n		6.0

Conclusions

There was no significant difference in yields resulted from superphosphate at 300 and 600 kg/ha which produced 33.0 and 33.1 t/ha respectively. Similarly there wasn't a significant difference in yield between the compost treatments at 3,000 kg/ha producing 33.0 t/ha while at 5,000 kg/ha produced 32.7 t/ha. Therefore farmers can improve their efficiency of phosphate and compost inputs to improve income through reduced input costs. The six FIL groups within Kelompok Horsela demonstrated that these farmers and their agricultural extension workers now have the capacity to plan and coordinate a series of simple though specialized potato experiments. Sub-group results could be analyzed as replicates in an ANOVA of the combined results. This means that this isolated group of farmers can now undertake their own objective testing of new management techniques.

Acknowledgments

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Transformation and Transient Ekspresion Analysis of L-HBsAg DNA in Fruits of *Musa acuminata* Colla cultivar 'Ambon Lumut" and 'Mas' using *Agrobacterium tumefaciens*

Sony Suhandono*, **Lailatul B,** Ernawati Giri R, Putri DU, Dina RR, Ima MZ

School of Life and Sciences, Bandung Institute of Technology, Bandung, Indonesia
** Corresponding author: Bandung Institute of Technology. Jl. Ganesa No 10, Bandung 40132, West Java, Indonesia.*
Tel./fax.:+62-22-2534107, +62-22-2511575.
sony@sith.itb.ac.id

Abstract

Conventional HBsAg is still expensive for most Indonesia citizens. Recently, scientists developed plant-based vaccine which will give easy delivery, flexible production scale up, and produce cheaper vaccine. *Agrobacterium* transformation has been widely used in genetic engineering. This research was conducted to transform and analyze the L-HBsAg DNA expression in banana fruit using *Agrobacterium tumefaciens* and vacuum infiltration method. Unripe *Musa acuminata* Colla cultivars 'Ambon Lumut' (AAA) and 'Mas' (AA) were used in this transformation. The slices of banana were co-cultivated with *A. tumefaciens* strain AGL1 for Ambon lumut and GV3101 for Mas banana in three days at 25˚C±1˚C in the dark. Both strains contain a plasmid pCAMBIA 1390 with MeEF-1α promotor and L-HBsAg gene. Expression of L-HBsAg was confirmed by using RT-PCR with gene-specific primers of L-HBsAg. A 1,323 bp band of L-HBsAg was observed in gel electrophoresis as a result of RT-PCR. The sequence of recombinant plasmid was read with specific primers for L-HBsAg. These steps were performed using MACROGEN services. These results indicated that the cDNA sequences of samples and protein-coding DNA sequences of L-HBsAg were identical. Therefore, it can be concluded that the L-HBsAg DNA was inserted and expressed in unripe fruits of Ambon lumut and Mas banana.

Keywords : transformation, Agrobacterium tumefaciens*, L-HBsAg gene,* Musa acuminata*, ambon lumut banana, mas banana*

Introduction

Hepatitis B is infectious disease caused by Hepatitis B virus (HBV). It was estimated that more than 400 million people worldwide suffer from this disease (Piramanayagam *et al*., 2008). Effort to tackle this problem like vaccination has been available since 1980 (European Centre for Disease Prevention and Control, 2008). However, the vaccination program does not run well in developing countries like Indonesia because of the high price of the vaccine and syringe and inadequate facility for the storage and distribution of vaccines (Streatfield and Howard, 2003). One of the solutions developed to overcome these problems is the production of vaccine in a plant.

Research on the production of Hepatitis B vaccine in a plant is constantly being developed. Large hepatitis B surface antigen or L-HBsAg is the latest variant of HBV vaccine. It consists of pre-S2, pre-S1, and S antigen (Lou *et al*., 2007). It is expected that vaccination using the L-HBsAg may trigger immune responses effectively in preventing HBV infection.

Agrobacterium-mediated transformation is a method to be used to transform plant with foreign DNA fragments encoding L-HBsAg into the plant genome. A segment of *Agrobacterium tumefacines* Ti-plasmid called T-DNA is moved from a bacterium cell into the host plant chromosomal genome. The T-DNA region is bordered by two 25 bp direct repeat sequences, called left border and right border. After entering the plant host cells, the T-DNA sequence will express genes that incorporated into it. For this reason, the targeted recombinant gene usually is inserted in the T-DNA region (Taiz and Zeiger, 2002).

One of the plants that can be used to express the hepatitis B vaccine is banana. Banana trees can be grown in tropical regions and many people from various backgrounds and ages consumed the fruit. Bananas can be directly consumed so that the degradation process of the vaccine (protein) due to heating can be avoided (Radji, 2004). In this study expression analysis of the gene encoding L-HBsAg proteins in Ambon lumut and Mas banana fruit was made transiently because it requires less time and the result in transgene expression levels could be higher than stable expression in transgenic plant (Sheludko, 2008).

Materials and Methods

Plant material

Unripe banana used in this experiment was Ambon lumut and Mas fruits. The banana was specially ordered from a farmer. Age of the fruit was about three months from flower formation. The fruit skin color is green and gummy when peeled. While the fruit flesh is white and hard. The fruit was cut with a thickness of about 2-3 cm. Then the skin of the fruit was peeled off. The pieces were immersed in 1% sodium hypochloride for 10 min. After that, it was rinsed twice using sterile distilled water. The soaked pieces were cut again with a thickness of 2 mm. Each of the banana slices as thick as 2 mm was further divided into 8 sections.

Agrobacterium tumefaciens

Strain *A. tumefaciens* that used in transformation were AGL1 for Ambon lumut and GV3101 for Mas banana. Both strains contain a plasmid pCAMBIA 1390 with MeEF-1α promotor and L-HBsAg gene. The *Agrobacterium* strain with no binary plasmid was used as a control for the experiment.

Bacteria were activated on solid YEP medium with antibiotic for three days at 25˚C±1˚C, 250 rpm, in dark condition. Antibiotics for strain GV3101 were rifampicin at 50 ppm and kanamycin at 50 ppm. Antibiotics for AGL1 were carbenicilin at 100 ppm, rifampicin at 50 ppm and kanamycin at 50 ppm. While the bacteria lacking-plasmids were inoculated into the same medium, but without kanamycin.

Transferred of L-HBsAg gene into banana fruit

The vacuum infiltration method used in this experiment was modified from Matsumoto *et al* (2009). Single colony of bacteria was inoculated into 10 ml of liquid YEP medium containing antibiotics until the cell density has reached an OD_{600} of 0.5. An aliquot of 10% of the *Agrobacterium* cell suspension was again subcultured in 20 ml of same medium at same condition until the OD_{600} of 0.8. After that, the cells were collected by centrifugation at 2000 g for 5 min and suspended in infiltration medium on ½ MS (Murashige and Skoog) containing 2% of sucrose, 200 µM of acetosyringone, and 0.01% of *Silwet L-408*.

The banana slices cut and divided into eigth pieces, were put into 1.5 ml microtube containing 1 ml of the *Agrobacterium* cell suspension (OD_{600} = 0.8) in the infiltration medium. Then those were subjected to vacuum for 10 min, followed by co-cultivation for three days at 25˚C±1˚C in dark condition.

Expression analysis of L-HBsAg gene in banana fruit

To analyze the gene expression of L-HBsAg, the RNA isolation was performed using the method from Kansas *et al* (2008). The presence of RNA was confirmed by the protocol on agarose gel electrophoresis of RNA concentration of 2% (w/v) containing EtBr in 1x TAE buffer. DNase was added to the RNA preparation to eliminate genomic DNA isolation. Then the reverse transcriptase-PCR (RT-PCR) was made to convert total RNA into spesific cDNA of L-HBsAg gene in the banana fruit.

The iScript cDNA Synthesis Kit from Bio Rad Laboratories, Inc. were used to reverse transcript RNA into cDNA. PCR using specific primers of MeEF-1α promotor in 25 cycles was done to ensure the absence of genomic DNA from the total RNA. PCR was performed with Applied Biosystem 2720 Thermal Cycler. Primer sequences used were Me-EF1α forward *(*5' AAGCTTCCAGTGAATGGTCA 3') and Me-EF1α reverse (5'TGTGAACCTTCTCTCTAGACATTGTTAGT 3'). The PCR cycles consisted of 5 min at 94^{o}C, 30 sec at 94^{o}C, 30 sec at 50^{o}C, 1 min at 72^{o}C, and 7 min at 72^{o}C.

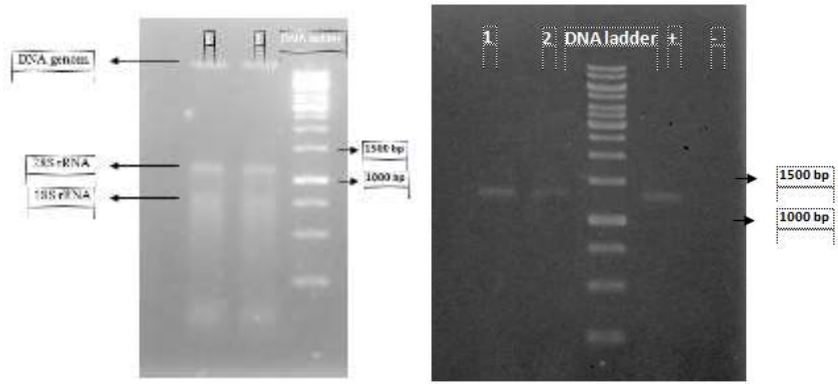
An order to amplify the expression of L-HBsAg from cDNA , the primers LHB-*forward* (5'GGATCCTGATGAAAATGAAGGTCCTTGTTTTCTTCGTTGCTACAATTTTGGTAGCATGGCAATGCCATGC GATGGGAGGTTGGTCCTCAAAACC-3') and LHB-*reverse* (5'GGTCACCTTAAATGTATACCCAAAGAC-3') were used. The series of cycles used were five min at 94^{o}C, 30 sec at 94^{o}C, 45 sec at 55^{o}C, 1 min and 30 sec at 72^{o}C, and 7 min at 72^{o}C.The PCR reaction composition were dNTP mix at 0,2 mM, Taq buffer 1x, $MgCl_2$ 1,5 mM, of primer forward at 1 µM, primer reverse at 1 µM, sample cDNA at 1 µg, DNA *polymerase* at 1 unit, and deion.

cDNA from the RT-PCR was cloned using the vector pGEM ®-T Easy vector system from Promega. Restriction analysis with EcoRI was made to confirm the clone in the plasmid. Restriction results were visualized on agarose gel electrophoresis. A nitrogen base sequence of cDNA was read as the last stage using specific forward and reverse primer L-HBsAg from MACROGEN.

Results and Discussion

Transformation of L-HBsAg gene into banana fruit

RNA isolation results indicated the presence of two RNA bands that dominant in the mid-agarose gel, which was ribosomal RNA (rRNA) 28S and 18S (Figure 1a). Quantification of total RNA with a spectrophotometer showed that the ratio λ A260/λ A280 in samples isolated RNA was 1.36 for Ambon lumut and 1.35 for Mas banana. The ratio obtained indicated that the purity of the isolated RNA was low because there may contain the impurities, such as genomic DNA and proteins. Electrophoresis results were also seen on the tape at the top indicating the presence of genomic DNA. Therefore, the isolation of total RNA were added DNase to eliminate genomic DNA so it was not interfere the next process.



a. b.

Figure 1. (a) Electrophoresis result of mRNA and (b) RT-PCR product from Ambon lumut (a) and Mas (b) banana

Confirmation the presence of L-HBsAg gene was made using PCR spesific primer of L-HBsAg. Electrophoresis results of RT-PCR products is shown in Figure 1b obtained after three consecutive PCR. This suggests that the gene encoding L-HBsAg was successfully inserted and expressed in unripe Ambon lumut and Mas banana.

Confirmation the presence of L-HBsAg gene was also made by restriction analysis and reading the sequence of nucleotide bases from cloned RT-PCR result. The results of restriction (Figure 2b) indicate that the presence of two bands of DNA size at 1300 and 3000 bp in Ambon lumut and Mas banana.

Final stages was reading the sequence of recombinant plasmid with specific primers for L-HBsAg. These steps were performed using MACROGEN services. The results from MACROGEN shows that the cDNA sequences of samples and protein-coding DNA sequences of L-HBsAg are identical. Therefore, it can be concluded that the cDNA sample was a cDNA of L-HBsAg. This means the L-HBsAg transgene successfully expressed at the mRNA level (transcription) in unripe ambon lumut and mas banana in transient system.

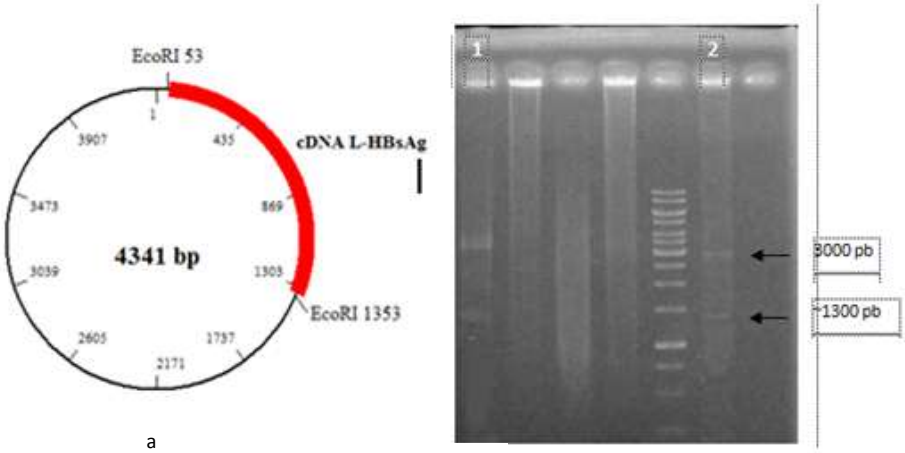


Figure 2. (a) restriction map of clone vector, (b) electrophoresis result from white colony that restricted using *Eco*RI. Sample 1 was ambon lumut while sample 2 was mas banana

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The Study and Early Evaluation of Resistance of Banana Accessions for Wilt Disease Caused by Fusarium oxyporum f.sp. cubense VCG 01213/16 (TR4)

Agus Sutanto[a,b,*], Sudarsono[a], Dewi Sukma[a], Catur Hermanto[b]

[a]*Department of Agronomy and Horticulture, Bogor Agricultural Institute, Bogor, Indonesia*
[b] *Indonesian Tropical Fruit Research Institute, Solok, Indonesia*
**Corresponding author: Indonesian Tropical Fruit Research Institute,*
Jl. Raya Solok-Aripan Km. 8, PO. Box 5 Solok, West Sumatera Indonesia,
Tel: +62 755 20137, Fax: +62 755 20592
bagusutanto_02@yahoo.com

Abstract

Fusarium wilt is one of main diseases of banana in Indonesia. This disease has destroyed banana plantation in almost all parts of Indonesia and it is difficult to be managed by agronomic and chemical controls. However, some species/cultivars show tolerance or resistance to *Fusarium* wilt. It indicates that those species/cultivars have resistance genes in their genomic DNA. The evaluation of banana plants for *Fusarium* wilt resistance can be carried out artificially using young plants from tissue culture. The objectives of this research were to evaluate young acclimatized tissue culture plants for *Fusarium* wilt resistance and to study the resistance mechanism of plant to *Fusarium* wilt disease. The experiment used five banana accessions; there were Calcuta-4 (AAw), Ketan (AAB), Klutuk (BB), Kepok (ABB) and Ambon Hijau (AAA), and double compartment for planting system. Before planting on the plastic cup containing sterile sand medium, roots of the plantlets were dipped in 10^6 conidia suspension of *Fusarium oxysporum* f.sp. *cubense* for five minutes. All of the cups containing inoculated plants were put on the plastic trays. The data were collected five weeks after planting. Base on the value of DSI (disease severity index) of RDI (rhizome discoloration index) and LSI (leaf symptom index), accessions will be categorized into highly susceptible, susceptible, tolerant and resistant. Result showed that Klutuk, Calcuta-4, Ketan and Kepok were categorized as tolerant, while Ambon Hijau was susceptible. All of tolerant accessions had symptom on leaves (LSI) and/or rhizome (RDI) at low level, and they still grew well.

Keywords: banana, resistance, fusarium wilt, early evaluation

Introduction

Banana is the most important fruit in Indonesia. Based on Ministry of Agriculture database (http://aplikasi.deptan.go.id/bdsp/hasil_kom.asp) banana contributed 31.10% of national fruit production in 2009, with the production reached 6.37 million ton. This potecy can support three main programmes of agricultural development; food security, development of agribussiness and prosperity. However, the successfull of those programmes are constrained by pests and diseases development that affect banana plantation with vary disease intensity rank from 0.08-100% (Hermanto *et al.*, 2011). One of destructive banana diseases in Indonesia is fusarium wilt disease caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*). Fusarium wilt control using practical cultures such as chemical, soil treatments, crop rotation, organic amendments may reduce the severity of the disease but relatively difficult to be adopted commercially (Pegg *et al.*,1993), therefore, the use of resistance cultivars is the best alternative for controlling this disease.

Field evaluation is the most reliable menthod for disease resistance selection, however, it requires high cost, manpower, space and facing the risk of environmental stress. Afforts to simplyfy the evaluation procedures have been carried out such as the use of young plant or *in vitro* calus as selected materials (Chand *et al.*, 2008), specific pathogen race or toxin as selective agent (Hadrami *et al.*, 2005), and screen house or *in vitro* condition as selection method (ŠVábová and Lebeda, 2005). The objectives of this

research were to evaluate young acclimatized tissue culture plants for fusarium wilt resistance and to study the resistance mechanism of plant to fusarium wilt disease

Materials and Methods

Planting Material, Foc Inoculation and Planting

The experiment used 10-15 cm in size of acclimatized *in vitro* plantlets of five banana accessions; Calcuta 4 (AAw), Ambon Hijau (AAA), Ketan (AAB), Kepok (ABB) and Klutuk Wulung (BB). Plantlets were gently uprooted and only those with healthy roots will be used for experiments by dipped in the *Foc* suspension (10^6 conidia/mL) for 5 min before replanted into cups containing sterile sand and placed in the trays for maintenance and observation. Plantlets were watered everyday and fertilized using nutrient solution (Hyponex®) every week.

Evaluation

Disease symptoms on leaves were recorded after the first two weeks, four weeks. Final evaluation was observed at fifth week based on the leaf symptom index (LSI) and rhizome discolouration index (RDI) (Mohamed *et al.*, 2001).

Scales for leaf symptom index (LSI) were:

1. No streaking or yellowing of leaves. Plant appeared healthy
2. Slight streaking and/or yellowing of lower leaves
3. Streaking and/or yellowing of most of the lower leaves.
4. Extensive streaking and/or yellowing on most or all of the leaves.
5. Dead plant.

Scales for rhizome discolouration index (RDI) were:

1. No discoloration of tissue of stellar region of rhizome or surrounding tissue.
2. No discoloration of stellar region of rhizome; discoloration at junction of root and rhizome.
3. Trace to 5% of stellar region discolored.
4. 6-20% of stellar region discolored.
5. 21-50% of stellar region discolored.
6. More than 50% of stellar region discolored.
7. Discoloration of the entire rhizome stele.
8. Dead plant.

After collecting data of LSI and RDI, the overall Disease Severity Index (DSI) for leaf symptoms and rhizome discoloration for each accession was calculated as follows:

$$DSI = \frac{\sum(Number\ on\ scale \times Number\ of\ seedlings\ in\ that\ scale)}{\sum(Number\ of\ treated\ seedlings)}$$

Furthermore DSI of LSI and RDI were translated into four categorize; resistant, tolerant, susceptible and highly susceptible (Table 1).

Table 1. Translation of DSI scales

DSI Scales for LSI	DSI Scales for RDI	Translation
1	1	Resistant
Between 1.1 and 2	Between 1.1 and 3	Tolerant
Between 2.1 and 3	Between 3.1 and 5	Susceptible
Between 3.1 and 4	Between 5.1 and 8	Highly susceptible

Results and Discussion

Early Evaluation of Banana Accessions toward *Fusarium* Wilt VCG 01213/16 (TR4)

Susceptible cultivar 'Ambon Hijau' produced disease symptom within two weeks after inoculation, with leaves chlorosis were started from older leaves to the younger leaves. Tolerant cultivar 'Klutuk Wulung' produced no symptom on leaves until five weeks after inoculation. The DSI of both LSI and RDI of five accessions and their susceptibility or tolerance status are shown in Table 2.

Table 2. The status of susceptibility or tolerance of five banana accessions

Samples	Calcuta-4		Ketan		Klutuk Wulung		Ambon Hijau		Kepok	
	LSI	RDI	LSI	RDI	LSI	RDI	LSI	RDI	LSI	RDI
1	1	1	1	1	1	1	2	4	2	2
2	1	1	1	1	1	1	2	2	1	1
3	1	1	1	1	1	1	1	1	1	1
4	1	1	3	3	1	4	4	5	1	1
5	1	1	2	3	1	1	2	4	1	1
6	1	1	2	2	1	1	2	5	1	1
7	3	3	2	3	1	1	3	5	1	1
8	1	1	1	1	1	1	2	4	1	1
9	2	3	2	3	1	3	2	5	1	1
10	1	1	1	1	1	1	2	4	1	1
11	1	1	1	1	1	1	2	3	1	1
12	1	1	1	1	1	1	2	3	2	2
DSI	1.25	1.33	1.50	1.75	1. 00	1.42	2.17	3.75	1.17	1.17
Status	Tolerant		Tolerant		Tolerant		Susceptible		Tolerant	

Notes: LSI = Leaf Symptom Index
RDI = Rhizome Discoloration Index
DSI = Disease Severity Index

Base on DSI of both LSI and RDI (Table 2), all accessions except Ambon Hijau were categorized as tolerant. Only one inoculated plant of Ambon Hijau had no symptom, while others showed symptom on leaves (LSI from 2 to 4) and rhizome (RDI from 2 to 5) (Figure 1B). Ambon Hijau is the member of Cavendish subgroup, which is naturally susceptible to *Foc* TR4. This finding was consistent with Hermanto *et al.* (2011), reported that 81% *Foc*-infected Ambon Hijau in Indonesia was caused by TR4 (VCG 01213/16). External symptom of infected leaves and internal symptom of Ambon Hijau were shown at Figure 1B. Meanwhile, only two out of twelve plants of Calcuta-4 showed *Foc* symptoms (DSI of LSI=1.25 and RDI=1.33, respectively) and the status of this accession was tolerant (Figure 1C). Calcuta-4 is wild-seeded species (*Musa acuminata* sp. *burmanica*) and they are often used for *Fusarium* wilt resistant breeding program (Tomekpe *et al.*, 2004).

Five out of twelve plants of Ketan showed symptom on leaf (LSI from 2 to 3) and rhizome (RDI from 2 to 3), however, DSI values of LSI and RDI were translated as tolerant (Figure 1E). Ketan also showed tolerant to Foc in some areas in Lampung, West Sumatera and West Java. Ketan had some synonyms in some places; Janten (Lampung), Jantan (West Sumatera), Uli (West Java), and Ketip (Nusa Tenggara Barat). This cultivar is popular as cooking banana. An interesting occurrence was shown by Klutuk Wulung. Two out of twelve plants produced symptom on rhizome (RDI=3), nevertheless, all plants showed no symptom on leaves (Figure 1A), therefore, DSI of LSI and RDI of this accession was translated as tolerant. Klutuk Wulung is seeded *Musa balbisiana* and scattered in Java, which usually people use leaves of it for wrapper and male bud flower for vegetable.

Another tolerant accession was Kepok, which two out of twelve plants showed symptom on leaves and rhizome (Figure 1D), and translation of DSI was tolerant. Kepok is very popular cooking banana and grows in whole part of Indonesia. Cases of infected plant of Kepok were found in West Java, Yogjakarta, Central Kalimantan and South Kalimantan. Kepok was not only infected by VCG 01213/16 (TR4), but also VCG 0120 and 01218 (Hermanto *et al.*, 2011).

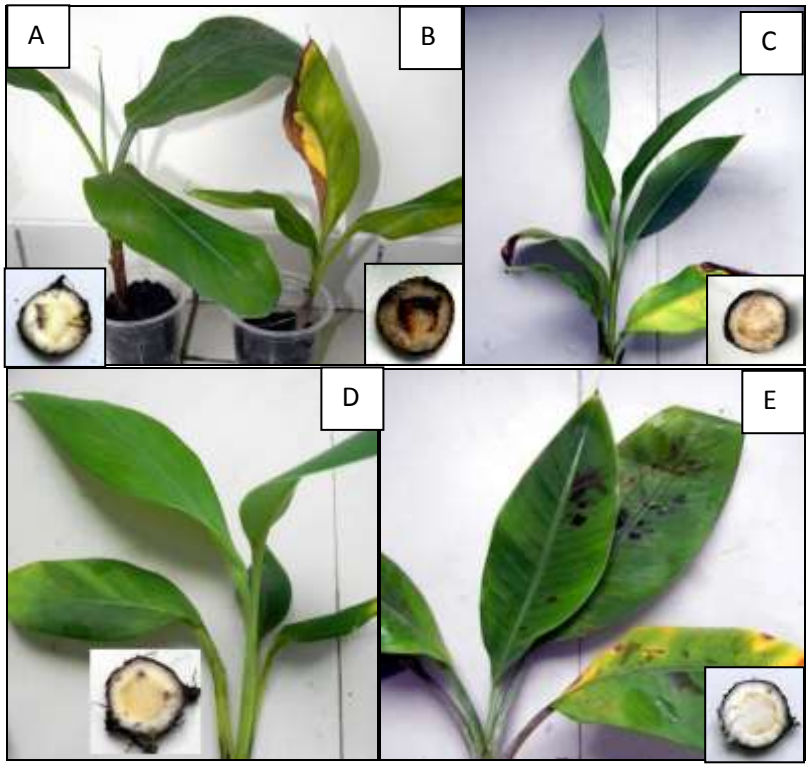

Figure 1. External (leaves) and internal (rhizome) symptoms of *Fusarium oxysporum* f.sp. *cubense* VCG 01213/16 on Klutuk Wulung (A), Ambon Hijau (B), Calcuta-4 (C), Kepok (D) and Ketan (E).

Resistance Mechanisms of Plant to *Fusarium* Wilt Disease

Resistance mechanism of plant against pathogen was started before the infection of pathogen into plant tissues. *Fusarium oxysporum* f.sp. *cubense* infects banana plant through root system. Since conidia attach hairy roots, they will germinate and penetrate into the epidermal cells of roots. Roots of resistant cultivar produce exudates that inhibit germination and growth of conidia; otherwise, exudates from roots of susceptible cultivar induce germination and growth of conidia (Li *et al.*, 2011).

Fungal pathogen is capable to penetrate plant roots through invading root epidermal cells directly, epidermal cell of root caps and elongation zone, natural wound in the lateral root base. During invasion, fungal hyphae produce cell wall degradation enzymes and penetrate to intercellular space, grow and develop branches and penetrate to other cells. Besides enzymes, fungal pathogen also produces micotoxins such as fusaric acid and beauvericin that affect trans-membrane electric potential, electrolyte leakage and respiration root cells (Pavlovkin 2006). Cell membrane damage causes the production of reactive oxygen species (ROS) and elicit the production of antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) (Kuzniak 2001), and transduction signal molecules that will trigger the production of pathogenesis related proteins such

as chitinase and β-1,3-glucanase. These enzymes will degrade fungal cell wall and inhibit growth and development of pathogen in the plant cells (Wu *et al*. 2008).

The case in Klutuk Wulung, which infection symptom appeared in the rhizome but no symptom on the leaves (Figure 1A) indicated that resistance mechanism of plant against pathogen occurred. The development of fungi was localized only in part of rhizome and blocked for further expansion.

This evaluation technique was adequate for screening of *Fusarium* wilt resistant cultivars and the expression of the disease can be obtained within 6-8 weeks. Using small plants for evaluation can reduce space requirement when compared to field evaluation.

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Response of Plant Roots to Drought Stress

Atsushi Kato, Saki Hoshiyasu, Akiho Yokota and Kinya Akashi*

Graduate School of Biologiccal Sciences, Nara Institute of Science and Technology
**Corresponding author: Graduate School of Biologiccal Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, 630-0192 Japan*
Tel.: +81 743 72 5561; fax: +81 743 72 5569.
akashi@bs.naist.jp

Abstract

Around the world, terrestrial plants are strongly affected by abiotic stresses such as drought, high or low temperatures, high light and salt stress. Among them, drought is one of the major factors limiting distribution and productivity of plants. Understanding the mechanisms for drought resistance in plants is important for breeding new crop varieties which can grow under water deficit conditions. In particular, plant root plays pivotal roles in water uptake and maintenance of water status of plants, thus is an important research target for plant physiologists and molecular biologists. Responses of plant roots to drought have been extensively investigated in model plants such as *Arabidopsis,* which have offered basic knowledge on the molecular mechanisms underlying the multilateral physiological responses in the roots. Moreover, recent physiological and molecular studies on drought-resistant plants have shed lights on how these 'xerophytes' cope with severe water deficit conditions by developing unique survival strategies.

Keywords: root development, drought stress, abscisic acid, xerophyte, wild watermelon

Morphological changes of roots in response to drought stress

Drought stress severely inhibits plant growth (Larcher, 1995). However, development of root is usually less affected than that of shoot under drought. In maize, it is reported that root growth is maintained at lower water potentials, while shoot growth is completely inhibited (Sharp *et al*., 2004). This report also showed that elongation activity is highly retained in root tip regions under water deficits. On the other hand, in the case of *Brassicaceae* species including *Arabidopsis*, inhibition of primary root elongation under drought is accompanied with morphogenetic differentiation process in the lateral roots. In this process, lateral roots remain short, hairless and often take a tuberized shape with concomitant accumulation of starch and proline (Vartanian *et al*., 1994; Xiong *et al*., 2006). This so-called 'short-roots' then switch to a dormant mode under prolonged drought periods, until the resume of growth upon rehydration conditions (Wasilewska *et al*., 2008). These observations therefore offer an illustrative example on the flexibility of root system architecture in plants under fluctuating water availability in the soils.

Abscisic acid (ABA) signaling and root morphogenesis

ABA is one of the major plant hormones which mediate adaptation of plants to environmental stresses. Many plants synthesize a large quantity of ABA in every tissue under stress (Shinozaki *et al.,* 2003). In the leaves, ABA is well known to stimulate stomatal closure and thus reduce transpirational loss of water under drought. In the roots, on the other hand, ABA inhibits elongation of primary roots (Leung *et al*., 1997; Bai *et al*., 2009) and decreases a number of lateral roots (Xiong *et al*., 2006). Moreover, ABA is shown to be implicated in the development of the 'short-roots' (Schnall and Quatrano, 1994). Furthermore, analysis of *Arabidopsis* mutants revealed that ABA-deficiency inhibits morphological changes of roots in response to the stress, which impaired stress resistance in these mutants. It is suggested that ABA and ABA signaling elements

are important factors for regulating morphological changes of the roots under drought stress (Wasilewska *et al*., 2008).

Compatible solutes in the roots

In many plant species, responses to drought stress involve changes in the fluxes of specific metabolic pathways, which lead to the accumulation of compatible solutes such as sugar derivatives and amino acids. Previous studies suggested that proline accumulation in the roots contributes to the osmotic adjustment and fortification of water uptake (Sharp *et al*., 1990; Voetberg and Sharp, 1991). In a recent study using *Arabidopsis* mutants impaired in proline metabolism, it is demonstrated that proline biosynthesis, transport and degradation are regulated in tissue- and stage-specific manners during drought (Sharma *et al*., 2011). Under drought condition, proline is mainly synthesized in photosynthetic tissue, and a part of the proline is transported to root meristems, which is further catabolized to various metabolites to support continued growth at low water potential. Arabidopsis mutants deficient in ABA biosynthesis show impaired proline metabolism and root growth elongation under water deficits, which is reversed by exogenous ABA (Sharma *et al*., 2011). These observations suggested that proline metabolism is partly regulated by ABA signaling pathway under drought, and these molecular networks coordinately contribute to the adaptation response of plants under stress conditions.

Root tropism under drought

In response to environmental signals, plants exhibit tropism to control the direction of their growth. Root system development is largely directed by the interaction of gravitropism and hydrotropism, which are the responses to gravity and moisture gradient, respectively (Gilroy and Masson, 2008). Gravitropism is dominantly orientated on primary roots, which is mediated by sedimentation of starch anchors in the amyloplasts of columella cells. The lateral gradient of auxin in the root cap is also known to be implicated in the root gravitropism. In water-stressed roots, hydrotropism is accompanied by the rapid degradation of amyloplasts in columella cells, which reduces responsiveness to gravity (Takahashi *et al.*, 2003; Eapen *et al*., 2005). Recent genetic analysis in *Arabidopsis* revealed that hydrotropism is mediated by novel factors such as MIZ1 and GNOM, the latter encoding guanine-nucleotide exchange factor for ADP-ribosylation factor-type G protein (Kobayashi *et al*., 2007; Miyazawa *et al.,* 2009), suggesting that GNOM-mediated vesicular trafficking plays a pivotal role in hydrotropism.

Root development in xerophytes

Plants which survive in water-deficit environments are called xerophytes, which develop unique drought resistance mechanisms (Wickens, 1998; Graham and Nobel, 1999; Akashi *et al*., 2008). Most xerophytes are characterized by their deep root system architecture. A global-scale investigation of 253 woody and herbaceous plant species reported that plants in the arid and semi-arid regions have more developed roots, with an average of 9.5±2.4 m for desert, and 15.0±5.4 m for tropical grassland/savanna, which are deeper than those for more humid regions such as temperate deciduous forest (2.9±0.2 m), temperate grassland (2.6±0.2 m) and tundra (0.5±0.1 m) (Canadell *et al*., 1996). Notably, maximum rooting depth was found to be 68 m for *Boscia albitruncas* in the central Kalahari Desert. These results illustrated that development of deep root system is advantageous for reaching to the deep water table in the soils, thereby enabling the survival in the water-limiting environments.

Interestingly, some xerophytes are characterized by their very shallow root system architecture (Graham and Nobel, 1999). For example, African baobab (*Adansonia digitata)* reaches to a height of 25 m with a trunk of up to 10 m diameter; nevertheless, roots of mature trees are

significantly shallow and rarely elongate beyond 2 m (Sidibe and Williams, 2002). Similarly, cactus (*Opuntia ficus-indica*) and agaves (*Agave deserti*) develop shallow and broad root system in which lateral roots are extensively produced. It has been discussed that these shallow roots are advantageous for capturing water derived from light rains, which are notorious for their infrequency in arid regions. These observations therefore suggest that contrasting strategies are employed by various xerophytes, to survive in the competitive environments for water resources.

Wild watermelon as a xerophyte model to study root response under drought

Deep root system observed in xerophytes is a favorable trait for future molecular breeding program towards water-saving agriculture. However, molecular mechanisms underlying this trait have not been fully elucidated. Wild watermelon is a xerophyte inhabiting the Kalahari Desert in Botswana, despite carrying out C_3-type photosynthesis (Kawasaki *et al.,* 2000; Miyake and Yokota, 2000), therefore offers an interesting model for how C_3 plants can cope with severe drought conditions. Previous physiological and molecular analyses demonstrated that root development of wild watermelon is significantly enhanced at the early stage of drought stress, which is accompanied with a dynamic change in the root proteome in this plant (Yoshimura *et al*., 2008). Drought-induced proteins include those for root morphogenesis such as actin and α-tubulin. Various enzymes for carbon/nitrogen metabolisms such as triosephosphate isomerase, malate dehydrogenase and methionine synthase are also induced under the stress, suggesting a global change in the cellular metabolism in the roots to support root growth at the early stage of drought stress. Interestingly, further changes in root proteome are observed at the later stage of drought stress, where factors for stress tolerance such as lignin synthesis-related proteins and molecular chaperones are preferentially induced at this stage. These results, therefore, suggested the presence of complex molecular networks for regulating abundance of respective proteins in this plant, in a temporally-programmed manner.

Towards understanding the molecular mechanisms for developing deep root system, experimental systems for exploring genetic resources in a given xerophyte should offer great opportunities to characterize genes involved in their root growth. A system for genetic engineering has been recently established in wild watermelon, where hairy root transformation technique enables to integrate a given foreign gene construct to both wild and domesticated varieties of watermelon (Kajikawa *et al*., 2010). Moreover, generation of a large-scale EST database, as well as transcriptome analysis are ongoing for wild watermelon, which should offer valuable platforms for screening and mining useful genes in this xerophyte. It is anticipated that these approaches would unravel the molecular mechanisms of root vigor in this xerophyte under drought.

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Growth and Development Characteristics of *Hoya multiflora* Blume

Sri Rahayu

Bogor Botanical Gardens, Indonesian Institute of Sciences
Jl. Ir. H. Juanda 13 Bogor, Indonesia
srirahayukrb@yahoo.com

Abstract

Hoya multiflora is one of the valuable germplasm in Indonesia utilized as ornamental and medicinal plants. This epiphytic plant faces problems in decreasing habitat, while the culture technique of this species has not established yet in Indonesia. It is important that all useful plant can be maintained to prevent the exploitation from the wild. Biological data on growth and development will provide a guideline to establish good culture techniques. The research aim was to investigate the growth and development of the seedlings of *Hoya multiflora* in two different conditions and growing media i.e. (1) in the natural habitat at the Bodogol Research Station of Gunung Gede Pangrango National Park, and (2) in a shade house of the Bogor Botanical Gardens as artificial habitat. Three different growing media was used in observation, i.e. (1) its phorophytes observed at natural habitat, (2) organic matter conducted at the shade house (3) tree fern log conducted at the shade house. The results showed that general, the growth and development of *Hoya multiflora* started by seed germination characterized by opened cotyledon, seedling establishment characterized by formation of alternate leaves arrangement, followed by growth of young plant characterized by formation of opposite leaves arrangement, growth and development of adult plant, flowering and fruiting. The critical point was at the seedling establishment phase, which best reached at the cocopeat medium. When the seedling established and developed to adult, there were no differences at the different growing media used. The best recruitment, growth and development of *Hoya multiflora* was at the cocopeat media located in the shade house.

Keywords: Gede Pangrango National Park, Hoya multiflora*, growth and development, Bogor Botanical Gardens*

Introduction

Hoyas (*Apocynaceae: Asclepiadoideae*) are becoming increasingly popular as ornamental plant, particularly in Europe, USA and Australia (Goyder, 2008). The international trade of this genus is increasing, but their existence in the nature obtains less attention, eventhough the conservation status was not state yet. In contrary, their habitat as epiphyte rapidly decrease mainly by the deforestation i.e. logging, burning, and forest conversion into plantation. The rapid increase on palm oil plantation especially prompted by the potential use as biofuel will multiply habitat lost for Hoyas as epiphyte.

The genus naturally originated from India, distributed to Australia and the Pacific Islands, with the greatest diversity is now found in South East Asia, particularly in Indonesia (Kleijn & Donkelaar, 2001; Wanntorp, 2006). There are about 150-200 Hoya species in the world (Hoffman *et all*, 2002), 50-60 of which are in Indonesia (Rahayu, 2001). *H. multiflora* widely distribute from India to New Guinea (Goyder, 2008) from 50-1500 m above sea level (Rahayu *et al*., 2010). This species is characterized by its short (non vein) plant, leathery (non succulent) oblong leaves and arrow head form white corona with yellow tip of the corolla. Flowers arranged in umbel appear at interpetiolar node. This plant produces white latex from all of its part.

Some indigenous people in the country have been utilizing some species of Hoyas as traditional medicine. The uses are for treat burns, cuts, convulsions, coughs, asthma, pneumonia, elephantiasis, encephalistis, fractures, gonorrhea, hemorrhoids, inflammation, insect bites/poisonous fish stings, orchitis, phthisis (tuberculosis), Pyroderma (skin disease caused by microorganisms), rashes, rheumatism/arthritis, swellings, traumatic injures/wounds, stomach and

intestinal ailments, and childbirth tonic (Zachos, 1998). A recent study shows their high potential as insecticide to the "Dengue" mosquito. *Hoya multiflora* Blume is one of the economic important as ornamental plant, and has been used traditionally as medicine, particularly to treat arthritis-rheumatism (Burkill, 2002) and stomach/intestinal ailments (Ambasta, 1986). The active compound of this plant unrevealed yet, but it could be Indomethacine like compound. Indomethacine, a common non-steroidal anti inflammatory drug (NSAID), has been used for more than 30 years to treat symptomatic pain of rheumatoid arthritis. Recently, this compound has been tested as a new class of anti HIV drug (Bourinbaiar & Lee-Huang, 1994) and seems to be specific since no toxicity at clinical doses.

Despite of their high economic importance, little is known about their cultivation especially in Indonesia. As this plant is loved as ornamental plant and has a medicinal property, the knowledge on its growth and development is needed. The knowledge will be useful for the cultivation and living collection management for *ex- situ* conservation purposes. This research aimed to observe the growth and development characteristics of *Hoya multiflora* Blume.

Materials and Methods

The observation on the growth and development characteristics of *H. multiflora* was conducted both at their own natural habitat at the Bodogol Research Station, Gunung Gede Pangrango National Park, West Java and in a shade house at the Bogor Botanical Gardens. There were 10 mature, 5 young and 3 clumps of seedling observed at Bodogol RS. Observation at the Bogor Botanical Gardens was focused on the flowering and fruiting time using 20 accession collected from the Bodogol RS (2004-2006); and seedling growth and development from the fruit produced (2005). The observation on growth and development conducted at the following parameters:

Germination

The experiment was conducted at the Bogor Botanical Gardens shade house. The shade house condition was under the paranet having 75% canopy. The seeds were shown on the 2 different kinds of growing media, namely (1) cocopeat at the plastic plate and (2) tree fern log. The seeds used were from the harvested mature fresh pod in the green house. Each of 20 seeds were shown on the media two times. The seedlings were watered daily. Parameters observed were seedling percentage, time of opened seed coat, time of hypocotyle, time of first leaf appearance, and time of opposite leaf formation.

Seedling establishment

Observation was conducted using the growing seedling taken from the seed growth germination experiment. The parameter observed was started at the established seedling growth to the young plant, recorded by the formation of opposite leaf arrangement.

Growth

The young plant stated as the seedling formed the first opposite leaves. The observation was done from the growth of shoot and leaf (number of leaf) to the plant formed the first inflorescence.

Flowering and fruiting

Observation was conducted to record the flower formation, time of flowering, and lower product. The process of flowering and fruiting were recorded. Observation also made on the fruit ratio to the number of flower.

Results and Discussion

Generally, the growth and development of *Hoya multiflora* started from (1) seed germination characterized by opened cotyledon, (2) seedling establishment characterized by formation of alternate leaves arrangement, (3) growth of young plant characterized by formation of opposite leaves arrangement, (4) growth and development of adult plant, (5) to flowering and fruiting. The process of growth and development at the different condition are as follows:

Germination (in shade house condition)

The germination process of *H. multiflora* started from seed coat opening, followed by cotyledone opening until the first leaf forming. *H. multiflora* evolve the epigeal germination, which cotyledone above soil surface. According to Copeland & McDonald (2001), there are two germination types based on location of storage reserves. The research showed that the seed of *H. multiflora* grew 2-3 days after showing and had the high viability up to 100% at the cocopeat media. The seed of *H. multiflora* was similar to *H. parasitica* without dormancy (Rahayu & Sutrisno, 2007). Showing seed at the tree fern media resulted in the lower viability (80%) and took longer time (7-30 days) to germatinate (Table 1). Overall, the germination process was faster at the cocopeat media than that at the fern tree fern log. There was microclimate different condition between cocopeat media and tree fern log, especially on the media humidity and water capacity. Cocopeat media was reported to be able to absorb more water and keep wet for a longer time than the log. The time of germination is faster at the cocopeat media, as more water stimulate the seed imbibitions. Besides that, the cocopeat media also provided ready to use nutrient. Media humidity and water content are the main factors for seed germination. Germination of seed involves the imbibitions of water, a rapid increase in respiratory, mobilization of nutrient reserves and initiation of growth in the embryo (Fenner & Thompson, 2006). Water is a basic requirement for germination as it is essential for enzyme activation, breakdown, translocation and use of reserve storage material (Copeland & McDonald, 2001).

Table 1. Seed germination of *H. multiflora* at the two different kinds of media

Media	Seed coat opening (days)	Cotyledone opening (days)	First Leaf Formation (days)	Seed viability
Tree fern log	7-30	14-45	41-60	80
Cocopeat	2-3	5-7	13-20	100

Seedling establishment

The seedling is characterized by alternate leaf formation. Seedling establishment was reached 12 month after seed germination. Seed on the cocopeat media had sorter time to grow (Table 2) and the highest establishment (80%). In contrary, at the tree fern log resulted the lowest growth and establishment (30%). Natural phorophyte was better to support seedling establishment of *H. multiflora* compared with the tree fern log media. Tree fern log had the lowest water holding capacity and nutrient availability compared with the cocopeat media and natural phorophyte. At the natural phorophyte, the seedling of *H. multiflora* grew on the ant nest (Rahayu *et al*, 2010), which provided nutrient and water holding capacity for the requirement of the *H. multiflora* to grow. During the seedling establishment, nutrient requirement increase from the former phase (germination). The tree fern log had the most limiting factor for seedling establishment of *H. multiflora*. According to Fenner & Thompson (2006), factor limiting establishment are competition between seedlings and abiotic hazards i.e. occurance of physical damage and lack of moisture. The limiting factor at tree fern log was dominantly by lack of moisture which caused seedling desiccation. Seedling desiccation is a particularly acute hazard in the branches of trees (epiphytes) and the main cause of

seedling mortality (Fenner & Thompson, 2006). While the limiting factor at the natural phorophyte was dominantly by competition because many seeds were germinated at one hole (ant nest).

Table 2. Time of seedling growth of *H. multiflora* at different media

Media	Number of alternate leaves	Time of first opposite leaf formation (months)	Plant height at the time of first opposite leaf formation (cm)	Successful seedling percentage
Tree fern log	12-13	14 ± 2,0	18,4 ± 1,2	30
Cocopeat	12-13	12 ± 1,0	25,6 ± 0,7	80
Natural phorophyte	13-17	13 ± 1,5	19,8 ± 1,5	50

Young to adult

At this phase, plant growth was determined after seedling establishment, characterized by opposite leaf formation. At this phase, growth and development of plant were dominated by the increase in the node number followed by the increase in leaf number. There are two leaves (opposite formation) at each node. The new node emerged at about every one month. When 12 nodes completed (after one year), new branch developed at the shoot base and followed by the emergence of peduncle. The time at this phase was quite similar to the occurance at different growing media (Table 3). This condition indicated the stability of the plant after seedling establishment phase.

Table 3. Growth and development of *H. multiflora* at different growing media

Media	Time of node growth (days)	Number of opposite leaves' node at the first flowering	Time of first flowering (Months after germination)	Plant height at the first flowering (cm)	Number of branch at the first flowering
Tree fern log	30 ± 9	12±1	20-28	30±5	2
Cocopeat	28 ± 7	12±1	18-30	30±2,5	0-2
Natural phorophyte	29 ± 8	12 ±1	18-30	40±10	0-2

Flowering and fruiting

All plant at the different growing media and location developed and produced flowers and fruit. The time of flower and fruit development was quite similar (Table 4). This condition indicated the time for seedling establishment and development to adult plant, when the reproduction process occured, i.e. plant produced flower and fruit. It means all growing media provided nutrient and basic requirement for the plant development. While fruiting process occurred at both different environments i.e. at the shade house condition and at the natural habitat. The pollination process in *H. multiflora* needs the presence of pollinator (Rahayu *et all*, 2010) and Chasanah (2010) has been identified that Vespidae was the pollinator of *H. multiflora* at the Bodogol forest.

Table 4. Flower and fruit development of *H. multiflora* at different condition

Media	Peduncle development (days)	Bud development (days)	Anthesis (days)	Percentage of fruit set	Fruit development (days)
Tree fern log	30 ± 10	28 ± 5	14 ± 1	0.01	35 ± 4
Cocopeat	29 ± 9	27 ± 4	14 ± 1	0.01	34 ± 5
Natural phorophyte	30 ± 11	29 ± 5	14 ± 1	0.01	36 ± 5

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Effects of High Water Table and Waterlogging on Sunflower Growth, Yield and Seed Quality

Satoko Yasumoto[a,*], Yukari Terakado[b], Morio Matsuzaki[a]

[a] *Biomass production and processing team, National Agricultural Research Center, Tsukuba, Japan,*
[b] *Ibaraki Prefectural Agricultural Experimental station, Daitoku, Ryugasaki, 301-0816, Japan*
** Corresponding author: Institute of National Agricultural Research Center, 3-1-1 Kannondai, Tsukuba, Japan, Tel./fax: +81 298 838 7038*
ysatoko@affrc.go.jp

Abstract

Understanding the response of sunflower (*Helianthus annuus* L.) to high water table and waterlogging is important for successful cultivation in monsoon Asia. We examined the physiological responses of sunflower cultivars to these conditions. In the rotational paddy field, where the water table depth was shallow, growth of the plant was suppressed, seed yield was decreased and harvest quality was deteriorated. In the artificially inclined field, the ditch surrounding the sloped plot was constantly filled with water, the growth of plant and oil concentration were decreased significantly when the water table was shallower than about 30 cm. In the pot experiment, where water was logged at different growth stages, the relationship between seed yield and the underground dry weight was significant ($r = 0.87$, $p < 0.01$). In cultivars with vigorous growth of underground part, many adventitious roots were appeared. Screening for the appearance of these roots may be an effective way to select sunflower cultivars that are capable of tolerating high water table and tolerating waterlogged conditions and avoiding decreases in seed and oil yield.

Keywords: sunflower, water table, waterlogging, seed quality, adventitious roots

Introduction

Regarding the effects of short-term waterlogging on sunflower, there were some reports about that to the growth, yield and quality. Orchard and Jessop (1984) reported on sunflower and sorghum that growth stages were greater importance than the duration of waterlogging. And Orchard and So (1985) reported that waterlogging at the vegetative and floral initiation stages reduced the root growth and it reduced the water use and nutrient uptake (Orchard *et al*. 1986). Grassini *et al*. (2007) reported waterlogging during grain filling determines direct physiological responses that decrease grain yield. Regarding the fatty acid, there were some reports on the factors changing their compositions. The temperature condition was widely reported as one of the factors. Nagao *et al.* (1984) and Sobrino *et al.* (2003) reported that the oleic/linoleic acid ratio was increased with higher temperature during grain filling. But there are few reports about the effects of shallow water table to sunflower growth and quality.

We are studying the cultivation of sunflower on rotational paddy field for the purpose of human consumption, and for the use of bio-diesel fuel. In the field, the shortening of maturing periods and reductions of stem length, leaf number and disk diameter were observed in many cultivars. And the decrease of seed yield and oil content, oleic acid composition and the increase of linoleic acid composition were also observed. The objective of this paper is to elucidate the effects of shallow water table on the growth, yield and quality and to elucidate the effects of waterlogging in different stages and in different cultivars of sunflower.

Materials and Methods

Field and pot experiments

Experiments I was conducted in 2007 and 2008 on an artificially sloped plot at Ibaraki Agriculture Institute (Ryuugasaki, N: 35°54', E:140°12')The slope had 8.3 m in length and 0.86 m in height at one end. It could set of 10 rows with different water table. Their water table depths were from 0 cm to 86 cm in 2007, and from 3.7 cm to 78 cm in 2008. The row spacing was 0.95 m in 2007 and 0.90 m in 2008 and the distance between plants was 0.2 m in both years. The ditch surrounding the sloped plot was constantly filled with water after establishment of seedling until the finish of the experiment. Soil moisture content was measured by a soil moisture probe (Profile Probe PR2, Daiki Rika Kogyo Co. Ltd., Tokyo). Two hybrid varieties were used. One was traditional type; the name was Hybrid sunflower (Hy.) (Kaneko Seeds Ltd. Gunma), and the other was mid-oleic type, 63M80 (Pioneer Hi-Bred International, Inc., USA). They were sown on 7 June in 2007 and on 5 June in 2008 as early sowing and on 26 June in 2007 and in 2008 as late sowing. Before planting, a chemical fertilizer was applied at the rate of $N\text{-}P_2O_5\text{-}K_2O$ = 8.4-8.4-8.4 g m^{-2}, in 2007 and 2008. Root growth of the two cultivars at flowering (22 Aug.) was examined by excavating the whole root systems of three plants in late sowing block in 2008 at two water depths (3.7 cm and 20.2 cm). Stem length, leaf nitrogen, number of seeds per a flower disk, flowering date, and maturing date were measured. Soil moisture content was measured by a soil moisture probe (Profile Probe PR2, Daiki Rika Kogyo Co. Ltd., Tokyo). Yield of each plot was determined using the same method as reported by Izquierdo et al. (2002). Thousand kernel weight, oil content and fatty acid composition were measured by the same method as reported by Yasumoto et al. (2011). All treatments were replicated two or three times depending on the varieties.

Experiments II was conducted in 2010. It was examined effects of waterlogging on sunflower at different developmental stages by a pot experiment. The pots were 50 cm long, 65 cm wide and 45 cm deep. Seven hybrid varieties were used. In the cultivars, 3 were traditional type, 4 were mid-oleic type. Before planting, a chemical fertilizer was applied at the same rate as in Experiment I. Waterlogging treatments were imposed at different developmental stages, establishment and flower bud visible. The control was irrigated according to the necessary. Because the flowering time was delayed by waterlogging at the stage of establishment, the seeds of the plants for this treatment were sown on 25 May, while the others were sown on 4 June in 2010. The duration of the waterlogging was the same as in the experiments reported by Wample and Davis (1975), namely 4 days in each treatment. The parameters as in Exp. I were measured. And root dry weight and total dry weight were also measured. Yield and seed quality were measured as in Exp. I. The sampled roots were carefully washed using a colander. The roots and other samples were dried at 60 °C until constant weight (for about 48 hr.) in a forced-air circulation oven, to determine the dry weight.

Sample and data analysis

Sampled seeds were air-dried. Two g seeds from each sample were crushed and the oil was extracted with *n*-butyl alcohol. The measurement of oil concentration and fatty acids composition in total fatty acid were determined by the method of Caviezel (Pendl et al., 1998) using a gas chromatograph (B-820,NihonBüchCo.Ltd.,Tokyo). The concentrations of fatty acids were calculated from their peak areas. And the percentage of the total fatty acid content was calculated from their peak areas.

Statistical analysis

The results were analyzed by ANOVA. All statistical analyses were performed with SPSS 11.0 for Windows (SPSS, 2001). All values are expressed as mean values. Significant differences were established by the Tukey's test at $P<0.05$. A correlation was calculated and the significance levels ($P<0.01$) are based on the Pearson coefficients.

Results

Exp. I. Effects of water table depth on growth, yield and quality

In both years, stem length, disk diameter and seed and oil yield were significantly reduced with shallower water table. Even in the condition that the depth to water table was 3.7 cm, Hy. grew more vigorous roots near the ground surface than 63M80 (Fig. 1a-b). Fig. 2 showed the decrease of leaf nitrogen, number of seeds per a disk, thousand kernel weight, seed yield. That was clearer upper than about 30 cm of the depth to water table. Oil concentration was also decreased upper than about 30 cm of the depth to water table. And oil concentration was some higher in early sowing block. The decrease in oleic acid and the increase in linoleic acid with rising water table were also somewhat clearer in the plants sown on early sowing block. Their results were shown about same tendency in 2007 and in 2008.

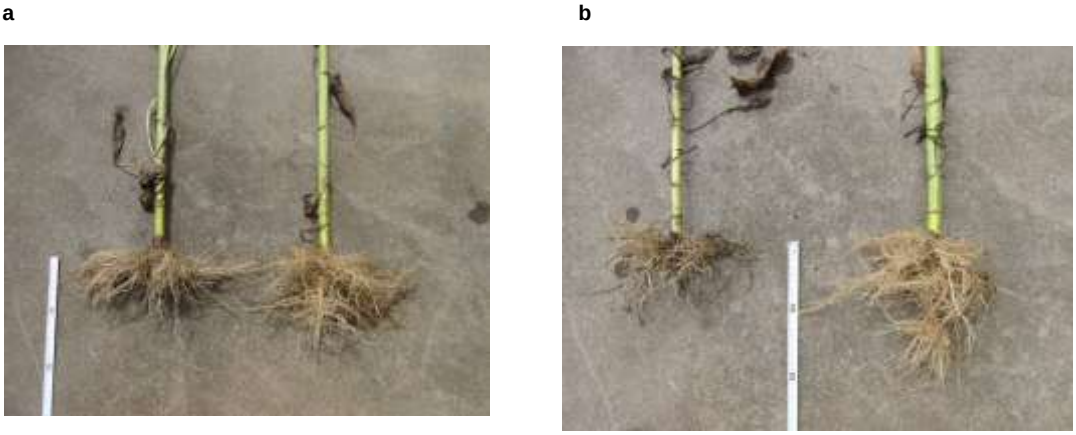
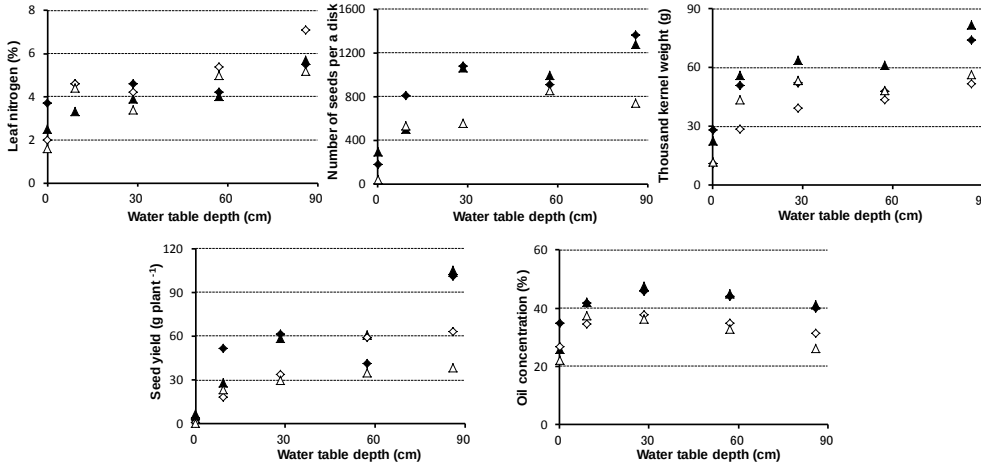


Figure 1. Root growth of sunflower grown at water table depths of 3.7 cm (left) and 20.2 cm (right). (a) Hy. (b) 63M80.



◆: Hy., ▲: 63M80 in early sowing, ◇:Hy., △: 63M80 in late sowing.

Fig. 2. Growth, Yield and seed quality in the inclined plot in 2007.

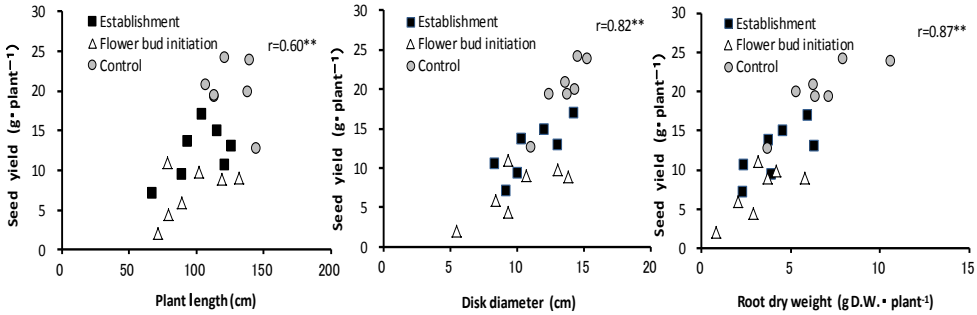
Exp. II Effects of waterlogging on sunflower at different developmental stages

There were significant differences between the stages of waterlogging (Table1). The sunflower was affected by waterlogging (Table1). In many cultivars, seed yield was decreased by waterlogging at the flower bud initiation stage. The response of waterlogging to root dry weight was different between cultivars. In many cultivars, their root weight was decreased by waterlogging at the flower bud initiation stage. In some cultivars, adventitious roots were formed after waterlogging treatment. The high significant relationship between seed yield and plant characters as the disk diameter and the root dry weight were observed through the different stages of water logging treatments (Fig.3).

Table1. Effects of waterlogging treatment on the growth, yield and harvest quality in 2010

Growth stage at waterlogging	Type	Cultivar	Flowering date	Maturing date	Ripening period (days)	Sum. Temp. (℃)	Plant height (cm)	Disk diameter (cm)	Yield ($g\ plant^{-1}$)	Thousand kernel weight (g)	Oil concentration (%)	Oleic acid (%)	Linoleic acid (%)	Shoot dry weight (g)	Root dry weight (g)
Establishment	Trad.	Hybrid sunflower	July 28	Sep. 2	36	1143	126	13	13.0	24.6	38.7	36.1	50.7	39.5	6.3
		North Queen	July 28	Aug.29	33	984	121	8	10.6	27.2	25.5	42.1	42.9	28.2	2.4
		IS3011	July 28	Aug. 30	34	1009	104	14	17.0	36.7	35.2	40.2	47.7	47.4	6.0
	Nusun	63M80	July 28	Sep. 2	37	1170	67	9	7.1	19.5	12.2	68.5	19.0	13.9	2.3
		Hysun521	July 26	Aug.26	32	935	89	10	9.4	27.6	30.3	58.0	27.9	29.4	3.9
		Hysun 511	July 26	Aug.26	32	945	93	10	13.7	32.9	31.5	67.5	18.9	29.3	3.8
		Hysun530	July 25	Aug.26	33	992	115	12	14.9	26.0	34.6	69.3	17.3	34.7	4.6
Flower bud initiation	Trad.	Hybrid sunflower	July 31	Sep.1	33	1039	102	13	9.8	25.0	33.4	35.1	49.7	41.6	4.2
		North Queen	July 28	Aug.26	30	871	132	11	9.0	24.6	30.8	43.6	42.1	50.6	3.7
		IS3011	July 30	Aug.28	30	864	79	9	4.4	18.1	28.8	37.9	48.3	33.4	2.9
	Nusun	63M80	July 30	Sep.2	35	1124	119	14	8.9	36.6	40.5	72.6	15.1	47.2	5.8
		Hysun521	July 29	Aug.28	31	982	89	8	5.9	21.1	29.3	65.0	21.8	26.0	2.1
		Hysun 511	July 25	Aug.26	33	962	78	9	11.0	19.5	30.5	67.8	18.3	33.0	3.2
		Hysun530	July 30	Aug.25	27	908	72	6	2.1	17.8	29.1	70.5	15.7	8.6	0.8
Control	Trad.	Hybrid sunflower	July 31	Sep.1	34	1080	138	14	19.9	34.2	34.7	38.7	47.1	39.7	5.3
		North Queen	July 30	Aug.26	28	826	144	11	12.7	27.4	29.6	43.6	41.3	57.0	3.7
		IS3011	July 30	Sep.1	34	1048	121	15	24.2	31.6	37.9	35.2	52.9	54.9	7.9
	Nusun	63M80	July 29	Sep.2	36	1141	139	15	23.9	35.8	43.0	72.3	17.6	62.8	10.6
		Hysun521	July 29	Aug.31	34	967	114	14	19.3	28.8	37.1	63.2	24.1	62.1	7.1
		Hysun 511	July 26	Aug.26	32	761	107	14	20.8	24.6	32.6	61.0	25.9	62.7	6.3
		Hysun530	Aug. 1	Aug.31	32	876	113	12	19.4	26.5	30.2	74.2	11.0	61.3	6.4
		Stage			**	**	**	**	**	**	*	ns	ns	**	**
		Cultivar			**	**	**	ns	*	**	**	**	**	ns	ns
		Stage×Cultivar			**	**	**	ns	ns	ns	ns	ns	ns	ns	*

ns, not significant. **, * indicated statistically significant at P<0.01 and 0.05.
ns, not significant. ** indicated statistically significant at P<0.01.



** indicated statistically significant at P<0.01.

Figure 3. Relationship seed yield and plant characters after each water logging treatment.

Discussion

In Exp. I**,** the plant growth and seed yield were reduced with shallower water table while the oil concentration was highest in the plot with a water table depth of 30 cm. It was quite shallow. And from this result, it was thought that the growth, yield, and seed quality of sunflower were affected by soil moisture conditions. In Exp. II, there were differences in the response to waterlogging between cultivars. In some cultivars, adventitious roots were formed after waterlogging. Kramer (1951) reported the adventitious roots contributed to plant survival during flooding. Wample and Davis (1975) reported that adventitious roots formation was one of the responses to flooding. And Jackson (1955) reported that when the original root system was flooded, adventitious root developed to prevent injury to the shoot and to promote shoot recovery from flooding. And in Exp. I**,** the differences of root growth between cultivars was also observed. Even in the condition that the depth to water table was 3.7 cm, Hy. grew more vigorous roots near the ground surface than 63M80. So this difference of appearance of the root was thought to be related to differences in the ability of these cultivars to tolerate flooding and high water table.

These results of this study suggested that the water management was important for improving the growth and quality of sunflower. Further research is needed to identify the physiological mechanisms responsible of sunflower to excess soil moisture.

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Population Genetics of *Hoya multiflora* at Sukamantri of Gunung Salak, West Java, Indonesia Based on Isozyme Analysis

Sri Rahayu[a]* and Rochadi Abdulhadi[b]

[a]*Bogor Botanical Gardens, Indonesian Institute of Sciences (LIPI), Jl Ir. H. Juanda 13 Bogor.Indonesia*
[b]*Research Centre for Biology, LIPI, Cibinong Science Centre. Jl Raya Cibinong Bogor, Indonesia*
**Corresponding author: Bogor Botanical Gardens, Indonesian Institute of Sciences (LIPI),*
Jl Ir. H. Juanda 13 Bogor. Indonesia. Telp/fax 0251-8322187.
srirahayukrb@yahoo.com

Abstract

Hoya multiflora Blume is tropical epiphytic plant reported having some medicinal properties in the rural communities in Asiatic counties i.e India and Malaysia. In Europe and USA, this plant has been internationally traded as exotic ornamental plant. The traded plant was directly extracted from the wild habitat, which imply on the population equilibrium. Population genetic study on epiphytic *Hoya multiflora* plant has been conducted using the plant population at Sukamantri at gunung Salak, West Java Indonesia. The study was based on the isozyme analyses by using PER, EST, MDH, ADH, AAT. The results showed that analyses using POPGENE software indicated that the genetic differentiation was low, but the estimation on gene flow was high.

Keywords: Hoya multiflora*, isozyme, population genetic*

Introduction

Hoya multiflora Blume (*Apocynanceae:Asclepiadoideae*) is one of the economic important species as ornamental plant, and has been used traditionally as medicine, particularly to treat arthritis-rheumatism (Burkill, 2002) and stomach/intestinal ailments (Ambasta, 1986). The active compound of this plant is unrevealed yet, but it is predicted to be Indomethacine like compound. Indomethacine, a common non-steroidal anti-inflammatory drug (NSAID), has been used for more than 30 years to treat symptomatic pain of rheumatoid arthritis. Recently, this compound has been tested as a new class of anti HIV drug (Bourinbaiar & Lee-Huang, 1994) and it seems to be specific since no toxicity at clinical doses.

The distribution of *H. multiflora* ranges from India to New Guinea (Goyder, 2008) from 50 to1500 m above sea level (Rahayu *et al*., 2010). This species is characterized by its short (non vein) plant, leathery (non succulent) oblong leaves and arrow head form white corona with yellow tip of the corolla. Flowers is arranged in umbel appear at interpetiolar node. This plant produces white latex from all of its part (Rahayu 2006). In Europe and USA, this plant has been internationaly traded as exotic ornamental plant. The traded plant was directly extracted from the wild habitat, which imply on the population equilibrium.

All of *Hoya* plant species including *H. multiflora* are epiphyte. In the natural habitat, the presence of this plant depends on the presence of host plant, which defined as phorophyte (Benzing 2008). So this species faces problems in decreasing habitat due to the increase of deforestation. The decrease on the number of forest tree will imply on the population decrease of this species, which in turn will imply on the genetic erosion toward extinction. Little is known about population genetic of this species. The study on population genetic will provide data to support conservation strategies and development of this species as economic plant. Genetic markers generally have contributed to the study of plant biology by providing methods for detecting genetic differences among individuals. There are some important ecological topics which often use allozymes as powerful markers, especially to assess the genetic differentiation among populations

(Zeidler, 2000). This research was aimed to assess the genetic differentiation of *Hoya multiflora* populations at Sukamantri of gunung Salak, West Java Indonesia based on the isozyme analysis.

Materials and Methods

Sample source

A total 50 samples were obtained from three populations of *H. multiflora* from Sukamantri. All populations were located at 700 – 800 m above sea level. Population 1 was from Buper Sukamantri with the total sample of 16, population 2 was from Tapos with the total sample of 16 and population of 3 were from Bobojong with the total sample of 18. All of sample were collected as stem cutting and planted at the greenhouse at Bogor Botanical Gardens as pot plants. After 2 months of acclimation, the plant produced new shoot and young leaves. Young leaves with the size less than 3 cm long were used as enzyme source.

Isozyme extraction

The method of enzyme extraction followed Soltis and Soltis (1989). Leaves were processed at the same day they were collected from the plants. Approximately 400 mg of samples were placed in a mortar, extract buffer were added than it was grinded well. The liquid of extract was adsorbed by a 0.5 x 0.5 cm filter paper and ready to placed in to the prepared gel.

Electrophoreses

Starch was prepared as follows: equal amounts of the two starch types (16.5 + 16.5 g) were mixed well and fully suspended in 100 mL the appropriate gel buffer in a flask. The remaining 200 mL of this buffer were brought to a boil before being added to the suspension. The buffer systems used depended on the enzyme activities to be assayed, and included the following: AAT (Aminoacid transferase), ADH (Alcohol dehidrogenase), EST (Esterase), PER (Peroksidase), MDH (Malate dehidrogenase), ACP (Acid phosphatase). The gel were mold to become firm. The wells (0.5 x 0.5 cm) were prepared and fill with the samples and indicator (bromphenol blue). The electrophoresis was run in a refrigerator at 4oC for 3 h at 100 V at the beginning and increase to 200V.

Staining and visualization

After electrophoresis the gels were sliced producing three replicas that were placed in different enzyme staining solutions. Gels were placed on a white light transilluminator to improve the identification of bands colocalizing with the specific enzyme activities.

Data Analysis

The polymorphic bands were transformed into numeric data as co-dominant data, and run by using POPGENE software (Yeh *et all*, 1999). The genetic variation intra and inter population were measure with assumption population at Hardy–Weinberg's equilibrium, $p2+2pq+q2 = 1$. Genetic variation (Ht) in a population was measure by Nei's (1978), while genetic variation inter population (genetic diferentiation=Gst) was measure as fixation index (Fst) of (Wright 1978); $Gst = Fst = (Ht-Hs)/Ht$.

Results and Discussion

There were four alleles found with the various distributions. The common two allele was A and B which express at the all isozymes. Four alleles were found at the EST, three alleles at the ADH, ACP, MDH, and two alleles at PER and AAT (Table 1). Among the four alleles, A was the

most frequent in EST, ADH, and ACP, and express more than 50% (dominant). The allele C was only found at EST with the lowest frequency (0.06) and categorized as a rare allele. Allele O express in EST, ADH, ACP and MDH in low frequency (below 0,3).

Table 1. Allele frequency from total populations

Allele \ Locus	EST	PER	ADH	ACP	MDH	AAT
Allele A	0.5600	0.4400	0.8400	0.5600	0.3000	0.3800
Allele B	0.1400	0.5600	0.1600	0.1600	0.6600	0.3800
Allele C	0.0600	-	-	-	-	-
Allele O	0.2400	-	0.2800	0.0400	0.2400	-

Allele heterozygocity as shown at Table 2, was range from 0.2303 to 0.5631 with the mean was 0.4197 (under 50%). The highest was at EST (0.5631), and the lowest was at ADH (0.2303). The result express the low heterozygocity wich mean its low genetic diversity.

Table 2. Allele heterozygocity

Locus	Sample size	Obs-Hom	Obs-het	Exp-hom*	Exp Het*	Nei**	Ave Het
EST	50	0.6000	0.4000	0.3820	0.6180	0.6056	0.5631
PER	50	0.7600	0.2400	0.4971	0.5029	0.4928	0.3307
ADH	50	0.6800	0.3200	0.7257	0.2743	0.2688	0.2303
ACP	50	0.8400	0.1600	0.4057	0.5943	0.5824	0.4220
MDH	50	0.4800	0.5200	0.5176	0.4824	0.4728	0.4641
AAT	50	0.7200	0.2800	0.3331	0.6669	0.6536	0.5083
Mean	50	0.6800	0.3200	0.4769	0.5231	0.5127	0.4197
St. Dev		0.1265	0.1265	0.1405	0.1405	0.1377	0.1218

* Homozygocity and heterozygocity estimation (Levene ,1949)

**Number of polymorphic loci = 6; Proportion of polymorphic loci = 100.00 %

The genetic differentiation (Gst = Fst) as shown at Table 3, was very large, which performed by the Fst = 1.1485. A very large genetic differentiation was performed by Fst more than 0.25.

Table 3. F statistic and gene flow estimation

Locus	Sample size	Fis	Fit	Fst	Nm*
EST	50	0.2847	0.3339	0.0688	3.3863
PER	50	0.2581	0.5015	0.3280	0.5121
ADH	50	-0.4273	-0.1967	0.1616	1.2970
ACP	50	0.6161	0.7165	0.2617	0.7053
MDH	50	-0.1472	-0.1293	0.0156	15.7817
AAT	50	0.4444	0.5663	0.2194	0.8896
Mean	50	0.2268	0.3650	0.1788	1.1485

*Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

According to Wright (1978), genetic differentiation classified as follows:

Fst = 0 – 0,05 indicated low genetic diffrentiation

Fst = 0,05 – 0,15 indicated moderate genetic differentiation

Fst = 0,15 – 0,25 indicated large genetic differentiation

Fst > 0,25 indicated very large genetic differentiation

Gene flow (Nm) was estimated from genetic differentiation (Gst) as follow:

Nm = 0.5(1 - Gst)/Gst

A very large genetic differentiation, mean there were very large differentiation between (inter) populations, and indicated low genetic diversity at intra populations. This is also indicated the very low of gene flow. At this condition, conservation strategy was need to conserve of all population, and sampling was need from representative of all population.

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Endophytic Bacteria as an Alternative Agent for the Biological Control of Plant Parasitic Nematodes on Black Pepper

Abdul Munif [a,*], Rita Harni, Siswanto[b]

[a] *Department of Plant Protection, Bogor Agricultural University ,Bogor, Indonesia*
[b] *Research and Development Agency, Ministry of Agriculture, Indonesia*
**Corresponding author: Department of Plant Protection, Bogor Agricultural University, Jl. Kamper Kampus IPB Darmaga, Bogor, Indonesia 16680.*
abdulmunif@ipb.ac.id

Abstract

Plant parasitic nematodes cause damage and losses significantly to black pepper in Indonesia. Control of plant parasitic nematodes with pesticides is often restricted due to their high toxicity and negative impact on the environment. The need for environmentally safe control strategies has increased interest in developing biological control measures. Endophytic bacteria are ubiquitous in most plant species and reside within healthy plant tissue without producing symptoms of damage. The internal plant habitat provides several advantages for endophytic bacteria as biological control agents: 1) colonization of an ecological niche also used by plant pathogens, 2) less competition with other microorganisms, 3) sufficient supply wirth nutrients, 4) less exposure to environmental stress factors, and 5) better translocation of bacterial metabolites throughout the host plant. The objective of this work was to evaluate the effect of endophytic bacteria isolated from black pepper to control *Meloidogyne incognita* on black pepper. The results showed that some endophytic bacteria were able to reduce the root galls caused by *Meloidogyne incognita* and juveniles of nematodes in the soil up to 70% as well as to promote the growth of pepper seedlings.

Keywords: endophytic bacteria, Meloidogyne incognita*, black pepper, root gall*

Introduction

Black pepper (*piper nigrum* L) is one of the important export commodities in Indonesia. However, the production of black pepper is now threatened by pests and diseases. One of the main disease on black peper is yellow diseasae caused by plant parasitic nematodes i.e. *Meloidogyne* sp. and *Radopholus similis*. Several control methods have been developed to combat the nematodes, but they are still a serious problem of black pepper especially in Bangka Island, Indonesia. The use of chemical pesticides with persistent pesticides can result in negative impacts to the environment, pathogens become more resistant, disruptive presence of beneficial microbes in the soil, and human health. Use of pesticides also provides residual effects on pepper which is currently an one obstacle for consumers, mainly for export purposes that are very concerned about the health and environmental aspects. In connection with the need to develop a system of agricultural production, including plant disease control systems are environmentally sound one of them by optimizing the use of biological agents.

Endopyitic bacteria are bacteria living inside plant tissues without doing symptoms on these plants. As the internal plant habitat, endophytic bacteria provide several advantages as biological control agents: 1) colonization of an ecological niche also used by plant pathogens, 2) less competition with other microorganisms, 3) sufficient supply with nutrients, 4) less exposure to environmental stress factors, and 5) better translocation of bacterial metabolites throughout the host plant. Several studies have shown that endophytic bacteria isolated from various plant tissues are able to suppress plant parasitic nematodes *Meloidogyne incognita* on cotton and tomato plants (Hallmann 2001) as well as to control plant nematode *Pratylenchus* sp on patchouli (Harni 2010). In addition, some bacterial isolates endophytes have been reported to enhance plant growth because

it can increase the availability of nutrients to induce plant resistance. The objective of this research was to determine the potential of biological agents bacterial endophytes isolated from pepper for controlling *Meloidogyne incognita* on pepper and their effects on the plant growth.

Materials and Methods

Isolation of Endophytic Bacteria

A total of 10 samples of healthy pepper plants were taken at random each of pepper plants and were cultivated in Bangka, Bogor and Sukabumi. The pepper plant roots were transported to the laboratory for immediate processing. The roots were washed with running tap water to remove adherent soil particles then blotted dry on tissue paper. The root material was weighed and surface sterilized with alcohol at 70% for 30 sec and in 2% of sodium hypochlorite (NaOCl) containing 0.01% of Tween 20 for 3 min, followed by four rinses with sterile 0.01 M of potassium phosphate buffer (PB) at pH of 7.0 (80 g NaCl, 2 g KCl, 11.5 g Na_2HPO_4, 2 g KH_2PO_4). To confirm complete surface sterilization (sterility check), the surface disinfected roots were imprinted on Tryptic Soy Agar (TSA). If bacterial growth occurred within 48 h, samples were discarded. The pepper roots were then macerated with a sterile mortar and pistil in three times PB (w/v). The macerate was decanted into sterile conical flasks and shaken for 30 sec. A dilution series was made and 100 µl of each dilution was plated onto 1/10 strength of TSA on petri disk. Petri plates were incubated at 24°C for 2-3 days and colony forming units (cfu) were determined. Three replicates were made for each dilution. On each petri plate containing approximately 10 bacterial strains was marked and all bacterial strains from this zone were transferred and purified on full strength of TSA. The bacterial strains were stored in Tryptic Soy Broth (TSB) plus 20% of glycerol at -20°C. Two isolates collection of endophytic bacteria were used in this experiment.

Inoculum of Parasitic Nematodes

Inoculum of nematode *Meloidogyne incognita* was isolated from the infected roots pepper of nematodes in Central Bangka, Province Bangka-Belitung Indonesia. Subsequently, the nematodes were cultured and propagated on susceptible tomato plants (cv. Ratna) for 2 months. After that plant was uprooted and the nematodes was extracted and used as a source of inoculum.

Effect of Endophytic Bacteria on Meloidogyne incognita

The effectiveness of biological agents against *M. incognita* on pepper seedlings was done in a greenhouse. Two months pepper cuttings with one segment were treated with isolates of endophytic bacteria. Eight isolates of selected bacterial endophytes isolated from root pepper and patchouli isolate MER7, AA2, HEN1, HEN3, MER9, ANIC, TT2 and EH11 were used in this study. The bacterial isolates were grown on TSA medium 100% for 24-48 h at room temperature. A single colony of the bacterial isolate was transferred into 100 ml of liquid TSB medium and shaken for 2 days with a speed of 150 rpm at a room temperature. Furthermore, the bacterial suspension was centrifuged at 11.000 rpm for 15 min at -4^oC to separate the supernatant/culture filtrate with a bacterial cell culture. Suspension of bacteria was made by diluted the bacterial cell with sterile water. Roots of 3 months old of pepper seedlings were soaken for 1 h in bacterial suspension with a population density of 10^9-10^{10} CFU. The treated pepper was planted subsequently in pots. One week after the bacterial treatment, the plants were inoculated with 1000 larvae of the nematode *M. incognita* per plant. Each treatment was repeated for 5 times and arranged in a completely randomized design, with positive control was the plant only nematode inoculation, while negative control was plants no inoculation with nematodes. Three months after inoculation the plants were harvested and the number of galls and the population of nematodes in the roots and the soil were observed as well as the plant height and weight, root weight, number of branches and number of leaves.

Results and Discussion

Effect of Endophytic Bacteria against *M. incognita* on Black Pepper

In this study, eight isolates of endophytic bacteria isolated from root pepper and patchouli were tested on seedling of black pepper against *M. incognita* in the greenhouse. All of isolates of bacterial endophyte were able to reduce gall and juveniles of *M. incognita*. Four out of eight isolates, i.e EH11, HEN1, HEN3 and TT2 significantly reduced gall nematodes compared to the control. Isolate EH11 showed the highest in reducing gall nematodes compared to other isolates (Table1). The isolates of bacteria were also able to suppress juveniles of nematode in the soil compared to control. Six isolates of EH11, AA2, HEN1, MER9, ANIC and TT2 significantly reduced number of juveniles compared to control. The highest influence in suppressing number of juveniles of *M.incognita* was showed by isolate HEN1 and followed by EH11. Five Isolates of HEN1, EH11, TT2, ANIC, and AA2 were able to reduce the population of *M. incognita* in the soil at more than 75%.

Table 1. Effect of isolates of biological agents bacterial endophyte against the number of galls and the population of nematodes larvae

Treatments	Number of galls	Population of the juveniles	Population of reduction (%)
Isolate MER 7	70,4 ab	1212 b	15,36
Isolate EH11	9,0 c	23 ef	96.97
Isolate ANIC	66,0 ab	312 cde	78,21
Isolate MER 9	57,4 ab	504 c	64,80
Isolate AA2	48,8 bc	352 cd	75,41
Isolate HEN1	42,2 bc	13 f	99,00
Isolate HEN3	38,4 bc	1176 b	17,87
Isolate TT2	32,6 bc	50 ef	96,50
Control + (with nematode)	101,0 a	1432 ab	-

Values followed by same small letters on the same column are not significantly different at 5% by DMRT.

Biological agents can suppress the development of plant diseases through a mechanism of competition, predation and the resulting antibiotics (Kloepper *et al*. 1991). Some research indicates that the use of biological agents endophytic bacteria through seed treatment reduce 30-50% of the amount of gall of *M. incognita* on cotton plants (Hallmann *et al.* 1997). Some bacterial endophytes isolated from the roots patchouli suppress populations of *Pratylenchus brachyurus* at 73.9% on patchouli plants in the greenhouse (Harni *et al*. 2007). Biological agents, including endophytic microbial may protect plants against plant-parasitic nematodes through various ways such as by producing toxic compounds that are nematicidals (Sikora *et al*. 2007; Yang *et al*. 2011). In addition, certain bacteria can also suppress the development of plant diseases due to the ability of bacterial endophytes in binding Fe (III) and to produce HCN (Keel *et al*. 1992). A treatment of culture filtrate of endophytic bacterial isolate isolated from patchouli was also able to reduce *Pratylenchus barchyurus* larvae up to 100%, 24 h after the treatment (Harni 2007).

Application of several bacterial endophytes can increase plant growth of black pepper. Isolate MER7 increased plant hight of pepper, number of branches and number of leaves (Table 2). Isolates EH11, MER9, TT2 increased plant height compared to control, while isolate HEN1 increased the number of brances compared to control. Vetrivelkalai *et al*. (2010) reported that bacterial endophytes suppressed the gall number of nematodes on bhendi, but not all of bacterial isolates increased the plant growth.

Table 2. The influence of endophytic bacteria on the growth of pepper plants inoculated with *M. incognita* 3 months after treatment

Treatments	Plant height addition (cm)	Numbers of branches increment	Numbers of leaves increment
Isolat MER7	24,20 a	4,6 a	12,6 ab
Isolat EH11	21,60 ab	2,0 bcd	9,4 abc
Isolat MER9	20,40 ab	2,0 bcd	10,0 abc
Isolat TT2	20,40 ab	1,6 bcd	7,8 bc
Isolat AA2	18,80 ab	2,0 bcd	7,8 bc
Isolat HEN1	17,60 ab	2,8 abc	9,2 abc
Isolat HEN3	16,40 ab	2,2 bcd	9,2 abc
Isolat ANIC	14,76 b	1,2 cd	5,2 c
Control + (with nematode)	16,00 b	0,4 d	6,4 c

Values followed by same small letters on the same column are not significantly different at 5% Duncan test.

Inoculation with nematodes caused damage from the stabbing stylet and secretion of enzymes released nematodes when the nematodes feed. Hallmann *et al*. (1997) reported that nematodes are taking root cells can reduce the ability of plants to absorb water and nutrients from the soil and cause symptoms such as lack of water and nutrients. Besides, the reduced concentration of plant growth regulators such as auxins, cytokinins and gibberellins can occur because the nematode secretes enzymes cellulose and pectinase that may degrade the cell up to the root tip injuries and broken, this leads to auxin is not active then growth of plant will be stunted.

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The Effect of Organic and Inorganic Fertilizers on Growth and Yield of Red Ginger (*Zingiber officinale* Rosc.)

Rudiyanto* and Tri Muji Ermayanti

Research Center For Biotechnology LIPI
Jl. Raya Bogor Km. 46 Cibinong Bogor 16911, Indonesia
Phone: +62-21-8754587, Fax: +62-21-8754588
**Corresponding author: rudidinasty@yahoo.com*

Abstract

Ginger (*Zingiber officinale* Rosc.) has been used for medicinal purposes in Southeast Asia since ancient times. Yield of ginger is affected by soil where the crop grows, climatic conditions and its cultivation management. Similar to other tuber crops, ginger needs high nutrients on soil. The objective of this experiment was to investigate the growth and yield of red ginger plants grown in media suplemented with organic and inorganic fertilizers. The research was conducted in a glasshouse in the Research Center for Biotechnology LIPI. The treatments tested were factorial between the medium and fertilizers used, with the total of treatments was 16 with 5 replicates of each treatment. Treatment M1 was soil : manure : grit (1:1:1); M2 was soil : manure : chaff (1:1:1); M3 was soil : manure : grit (1:2:1); M4 was soil : manure : chaff (1: 2:1); P0 was no addition of fertilizer control treatment); P1 was NPK granule (18:9:10) at 5 g/plant; P2 was NPK (20:20:20) at 1 g/l given once a week; P3 was NPK (32:10:10) at 1 g/lt giving once a week. Growth and yield parameters measured were height, number of leaves, number of shoots, stem diameter (from 0 to 24 weeks), leaves fresh and dry weight, stem fresh and dry weight, root fresh and dry weight, and leaf area recorded at month 4 and 6. After 9 months, red ginger was harvested to determine its yield. The results indicated that treatment with M2P2 was the best treatment for height, number of leaves and stem diameter, of red ginger, meanwhile M4P3 resulted in the highest number of shoots among the others (11.25). The highest production of rhizome of red ginger plant after 9 month growth was achieved by M1P2 treatment (174.058 gr fresh weight/ polybag and 19.75 dry weight/ polybag). Physiological parameters showed that the highest Leaf Area Index (LAI) value was from M2P2 treatment (3.83 after 4 months of growth), and from M4P3 (6.415448, after 6 month of growth); M3P2 treatment produced the highest net assimilation rate (NAR) at 0.01, M3P1 had the crop growth rate (CGR) value at 0.031548. The best value of Harvest index (HI) was reached by M3P0 treatment at 2.038.

*Keywords :*Zingiber officinale *Rosc., red ginger, organic and inorganic fertilizers, growth, yield*

Introduction

Ginger (*Zingiber officinale* Rosc.), a monocotyledon belonging to family Zingiberaceae and in the natural order Scitamineae, is herbaceous perennial, usually grown as annually for its pungent rhizome. It is native of Southeast Asia and one of the earliest oriental spices known to Europe (Parthasarathy *et al*., 2003; Ravindran and Babu, 2005; Kandiannan *et al*., 2009). Ginger normally propagates by its rhizome. Ginger has been used for medicine purposes in Asia since the ancient times. For example, it is used as folk medicine, as a carminative, stimulant of the gastro-intestinal tract, and counter-irritant. The rhizome is believed to have diaphoretic and diuretic effects, and anti-inflammatory. Extracted ginger rhizome contains gingerol inhibits the growth of *Helicobacter pylori* CagA+ strains *in vitro*, and may contribute to chemopreventative effects (Mahady *et al*., 2003). Ginger is also widely used as a spice in forms of fresh ginger, dried whole or powdered ginger, and preserved ginger.

Crop yields is a function of three major factors; namely the soil where the crop is grown, climatic conditions and management practices. Like other root and tuber crops, ginger has a high

nutrient demand on soils. It does well in farmlands newly opened from long fallows, making use of nutrients reserves accumulated during the fallow period (Attoe *et al*., 2009)

Management practices used in agriculture will influence soil properties, nutrient use efficiency and crop production. Conventional crop management systems that rely on inorganic fertilizers and agrochemicals have, in recent years, increased agricultural productivity, albeit at a high environmental cost (Pimentel, 2005). Organic farming systems which depends on organic sources of nutrients (i.e., animal manure, crop residues, green manure crops and catch crops) may sustain productivity at reduced environmental cost by enhancing microbial nutrient turnover (Watson *et al*., 2002).

The objective of our study was to investigate the growth and yield of red ginger plants grown in media suplemented with organic compared with inorganic fertilizers.

Materials and Methods

The research was conducted in a glasshouse in the Research Center for Biotechnology LIPI. The treatments tested were factorial between the medium and fertilizers used, with the total of treatments was 16 with 5 replicates of each treatment. Table 1 showed the combination treatment of inorganic and organic fertilizers used in this experiment.

Table 1. Treatment of Combination between organic and inorganic fertilizer

Code	P0	P1	P2	P3
M1	M1P0	M1P1	M1P2	M1P3
M2	M2P0	M2P1	M2P2	M2P3
M3	M3P0	M3P1	M3P2	M3P3
M4	M4P0	M4P1	M4P2	M4P3

Note: M1: soil : manure : grit (1:1:1) M2: soil : manure : chaff (1:1:1)
M3: soil : manure : grit (1:2:1) M4: soil : manure : chaff (1: 2:1)
P0: no addition of fertilizer (control treatment) P1: NPK granule (Decastar 18:9:10) at 5 g/plant
P2: NPK (Hyponex 20:20:20) at 1 g/l given once a week P3: NPK (Growmore 32:10:10) at 1 g/l giving once a week

Seedlings of 1 month old were used in this experiments. Seedlings were grown in polybags of 25 x 30 cm. Each polybag contained growth medium with different trreatment of fertilizers as shown at Table 1. Growth and yield parameters measured were height, number of leaves, number of shoots, stem diameter recorded from 0 to 24 weeks. However, since the data were plenty, only data at week 24 was presented. After 9 months, red ginger was harvested to determine its yield as fresh and dry weight of the rhizomes.

Results and Discussion

Growth of red ginger after 24 weeks planting on different combinations of fertilizer types and concentratitons was shown at Table 2. The results indicated that treatment with M2P2 was the best treatment for height, number of leaves and stem diameter, of red ginger (Table 2), meanwhile M4P3 resulted the highest number of shoots among other treatments. This indicated that the best medium for growing red ginger until 24 weeks was a combination between M2; soil: manure: chaff (1:1:1) and NPK fertilizer containing NPK (20:20:20) at 1 g/l that was given once a week.

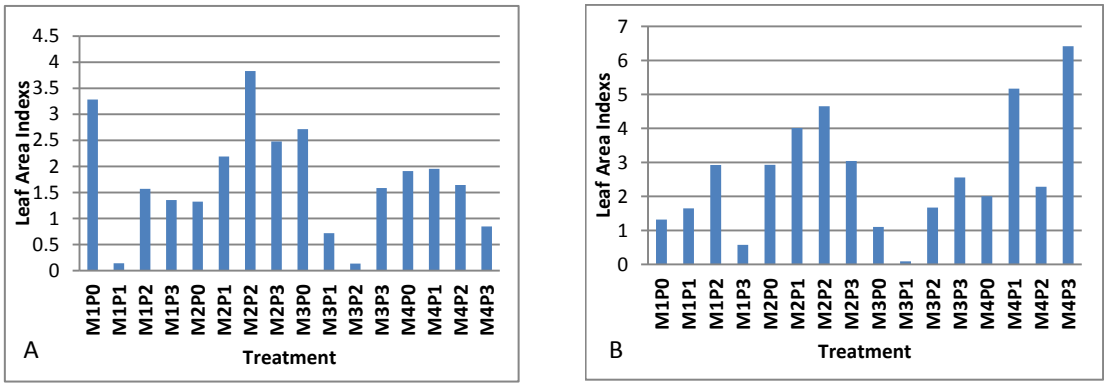
Unlike the case with other treatments, Slow Release fertilizer (P1: NPK granule (18:9:10) at 5 g/plant) actually had no significant effect on shoot growth performance, which were indicated by the low values of plant height, leaves number, shoots number and the plant diameter. Ginger is an herbaceous plant that needs nutrient appropriately. When it was fertilized with the low supply of nutrients from the granular fertilizer, the optimum vegetative growth could not be achieved.

Nutrient management is always an important consideration for ginger since large quantities of nutrients is required for optimal growth, especially for N, P and K nutrients. However, farmers generally overuse N, P and K and ignore manure fertilization. They are not used to apply appropriate fertilization in many crops, therefore they need knowledge about balanced fertilization between inorganic and organic fertilization (Li *et al*., 2010). Organic and conventional farming practices differ in the use of several agriculture management strategies, including the use of cover crops, green manure, and fertilization, which may influence soil properties, greenhouse gas emissions and productivity of agroecosystems.

Table 2. Growth of red ginger after 24 weeks of planting treated with different combinations of fertilizer type and concentration

Treatment code*	Height of plant (cm)	Number of leaves	Number of shoots	Stem diameter (cm)
M1P0	70.33	36.00	5.67	0.50
M1P1	54.07	42.00	4.33	0.37
M1P2	82.38	94.75	9.00	0.58
M1P3	74.83	63.75	6.00	0.48
M2P0	82.33	50.33	5.33	0.50
M2P1	99.13	111.00	10.00	0.55
M2P2	100.63	104.25	9.00	0.63
M2P3	80.025	99.00	9.50	0.55
M3P0	79.23	66.67	7.33	0.57
M3P1	34.00	18.00	4.00	0.30
M3P2	75.63	53.00	6.75	0.48
M3P3	63.38	42.75	7.50	0.48
M4P0	81.83	84.00	8.00	0.57
M4P1	82.13	98.25	9.50	0.63
M4P2	94.50	116.25	9.00	0.63
M4P3	93.25	123.50	11.25	0.53

Note : *description of each code of treatment is shown at Table 1.



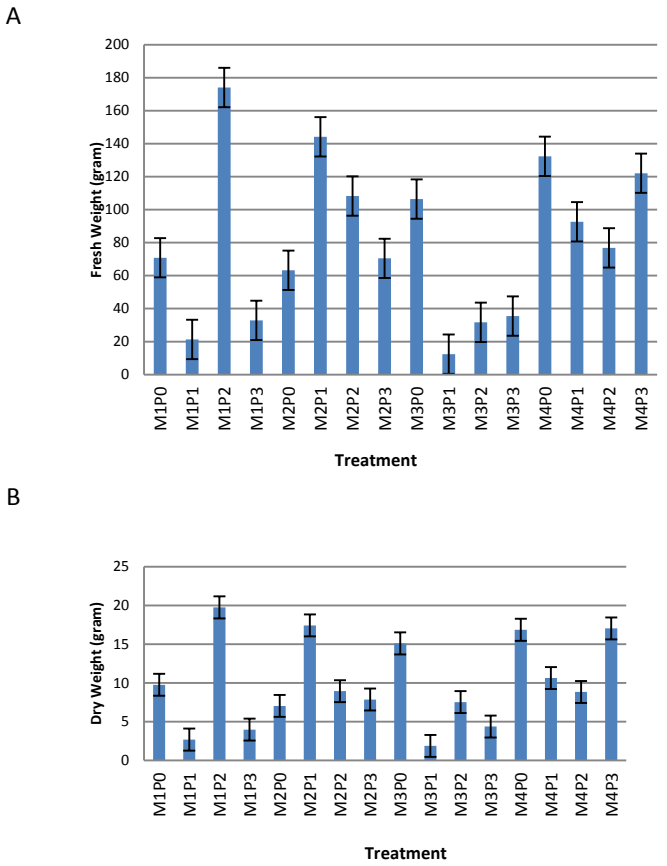
Note : each code of treatment is presented on Table 1.

Figure 1.Leaf Area Index (LAI) of red gingeron 4 (A) and 6 month (B) of age.

Leaves are important organs of the plant. Leaf area (LA) is a variable key for most agronomic and physiological studies involving plant growth, light interception, photosynthetic efficiency, evaporation, and responses to fertilizers and irrigation (Blanco & Folegatti, 2005). Therefore, LA strongly influences growth and productivity. Estimating LA is a fundamental component of crop growth models (Lizaso *et al*., 2003). Figure 1 showed that the M4P2 treatment to

ginger resulted the highest value of leaf area index (LAI) at 4 months after planting. However, at 6 month old plant, the highest LAI was achieved when treated by M4P3 treatment combination.

The perennial rhizome of the ginger plant is a specialized segmented stem structure that grows horizontally just under the soil surface. Upright-growing shoots are produced from the tips of lateral rhizome branches. Adventitious roots and lateral growing points emerge from the nodes of the rhizome stem. In ginger, the roots emerge from the lower rhizome sections. For commercial purposes, ginger is grown as an annual crop, the rhizomes are harvested after seven to nine months when the rhizome is physiologically ripened (Wilson &Ovid, 1993).



Note : each code of treatment is presented on Table 1.

Figure 2. Fresh weight (A) and Dry Weight (B) of red ginger rhizomes after 9 months old grown on various fertilizer application.

Figure 2 showed that the highest fresh and dry weight of rhizomes produced by treatment M1P2. M1 was soil: manure: grit (1:1:1) medium with the addition of NPK fertilizer supplement (Hyponex 20:20:20) at 1g/l given once a week (P2). While the lowest weight of rhizomes was produced on the M3P1 treatment, where M3 was soil: manure: grit (1:2:1) medium and P1 was a slow release NPK granules (with N:P:K ratio of 18:9:10) with the dose of 5 g/plant. This result indicated that the slow release fertilizer (P1) provided less amount of nutrient than what ginger plant required during the maximum vegetative growth and generative phase.

Ginger plants, that was grown in soil containing more manures increased ginger plant growth and rhizome production. The use of humic acid as a fertilizer could directly affect physiological processes in plants. Besides its role as an auxin, humic acids directly influence the

metabolic processes of plants such as respiration, synthesis of nucleic acids, and ion absorption. The highest rhizome production of red ginger plant after 9 month growth was achieved by M1P2 treatment (174.058 gr fresh weight/ polybag and 19.75 dry weight/ polybag). In addition to that, the best value of Harvest index (2.038) was reached by M3P0 treatment.

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Growth Analysis of Superior Clones of Temulawak (*Curcuma xanthorrhiza* Roxb.) Grown with Organic Fertilizers

Bambang Pujiasmanto* and Samanhudi

Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University
Jl. Ir. Sutami 36A, Surakarta, Indonesia 57126
** Corresponding author: Tel./Fax: +62 271 637457;*
bpmanto@yahoo.com

Abstract

Curcuma xanthorrhiza as a medicinal plant needs to be preserved and improved for its yield. The curcumin is very useful for human health. This species is useful to improve the body immune system. Curcumin of many genotypes of *C. xanthorrhiza* has been successfully evaluated for their potentials and its potential yield from genotypes throughout Java and islands for selection of superior clones. The research was done based on the Randomized Completely Block Design (RCBD), using superior clone types as the single factor obtained from Sragen, Jember, Sumenep, Pasuruan, Blitar, and Malang. The data were analyzed using F-test at significance level of 5% followed by Duncan Multiple Range Test (DMRT) at significance level of 5%. The results showed that Malang clone had the best vegetative growth and maximum yield compared with other clones. The plant height, leaves number, leaf width, leave area, leaf area index (LAI), dry weight of straw, fresh rhizome weight variables of Malang clone was the highes among other clones. The maximum vegetative growth pattern of plant height obtained by Malang clone that reached 155.13 cm, the highest fresh rhizome production of Malang clone was 22.31 tons/ha.

Keywords: growth analysis, temulawak, Curcuma xanthorrhiza*, organic fertilizer*

Introduction

Temulawak (*Curcuma xanthorrhiza* Roxb.) is one species of medicinal plants belonging to family Zingiberaceae is potential to be developed, and is one of nine types of seed plants from the Directorate General of POM as an ingredient. Utilization of this plant is varied. It is useful for maintenance and improvement of health and treatment of disease as well as raw materials for traditional medicines and cosmetics (Nurjannah *et al*., 1994). Temulawak rhizome can be used as hepatoprotector, increase the body's immune system, anti-bacterial, anti-diabetic, anti-hepatotoxic, anti-inflammatory, anti-oxidant, anti-tumor, diuretic, depressant, hipolipodemik, and as beverage as well as for natural dyes (Purnomowati and Yoganingrum, 1997; Raharjo and Rostiana, 2006).

All potentials should be evaluated to determine the type of temulawak with the most potential to be developed in certain areas. Several types of temulawak has been successfully evaluated its potential based on curcumin content and gowth characteristics for selection of all types of temulawak throughout Java. Kuswanto has been elected six best types of temulawak, including Sragen, Pasuruan, Blitar, Sumenep, Malang, and Jember.

Temulawak selected cultivars need to be tested in various production areas, in order to investigate their genetic capabilities grown in various areas. One of the evaluation was done by planting selected temulawak, the superior clones was then elected to be cultivated at the production centers of temulawak in the district of Sragen, Central Java.

Materials and Methods

This study was started in May 2010, at Karang Pong, Kali Jambe, Sragen, Cetral Java, located at 7° 30' LS and 110° 50' BT and altitude of 180 m above sea level. The soil type is vertisol. The materials used in this study were temulawak seeds, consisting of six clones derived from the superior selection of Java.

The design of the experiment was Randomized Completely Block Design (RCBD). Origin of clones was used as the single factor. The experiment had 6 standard of excellence e.i. 6 clones obtained from Java. Each treaatment had 3 replicates. Addition of organic fertilizer was of 15 tons/ha manure.

Observation variables included plant height, number of leaves per clump, number of tillers per hill, leaf width, leaf area, leaf area index, fresh weight of rhizomes per plant, dry weight of straw, specific leaf area, and relative growth rate. Data were analyzed using the F test at 5% level, if there was a significant difference, then it was followed by DMRT at the level of 5%.

Results and Discussion

Height of Plant

Malang clone temulawak observed at intervals of two weeks showed high growth rate of plants significant different compared with other clones (Figure 1). Malang clone growth at 5 WAP seemed to have the fastest plant height compared with five other clones. This shows the level of adaptation of Malang clone was very high, especially during the process of adding plant height.

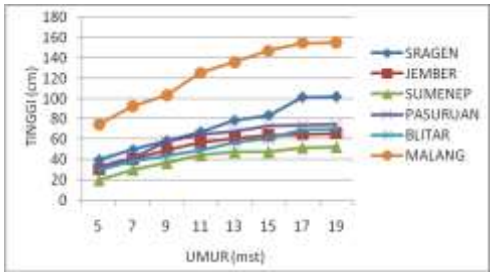


Figure 1. Plant height of six superior clones of temulawak (*Curcuma xanthorrhiza*).

Table 1. The average plant height of six superior clones of temulawak (*Curcuma xanthorriza*) at 19 WAP

Clones	Plant height (cm)				
	I	II	III	Total	Average
Sragen	108.80	98.13	99.50	306.43	102.13 c
Jember	68.38	60.63	68.25	197.26	65.75 ab
Sumenep	45.00	59.38	53.75	158.13	52.71 a
Pasuruan	67.75	69.25	87.75	224.75	74.92 b
Blitar	86.50	61.75	61.25	209.50	69.83 ab
Malang	151.00	146.60	167.80	465.40	155.13 d

Note: Values followed by the same letter showed no significantly difference according to DMRT at 5%.

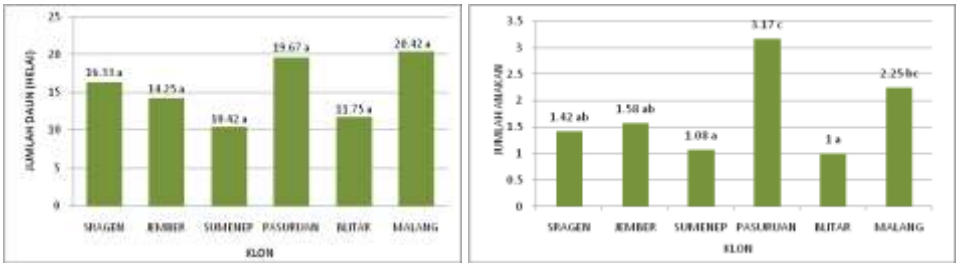
Increased plant height of Malang clone during vegetative growth was the highest among the clones. Sragen clone was the second having the plant height similar to Malang clone. At the late

vegetative phase (19 WAP) height of Sragen clone was only 102.13 cm, which was much lower than that of Malang clone (155.13 cm) (Table 1). This would indicate that vertisol soil type is suitable for temulawak. Plant height among the clones was significantly different at 5% with Malang clone was the best.

Number of Leaves and Number of Tillers per Clumps

The number of leaves of the temulawak represents its during the vegetative phase. When the plants reach the generative phase, the existing leaves turns to dry. The increased of leaf number may also indicate the increase of rhozome production of temulawak. The more number of leaves formed, the better rhizome formation during the vegetative phase of the plants. The number of leaves produced by temulawak tuber is influenced by the number of tillers formed in a single clump of plants. The leaves formed by the temulawak having a lot of puppies will increase the number of leaves corresponded to the number of puppies available. Tillers per clump of temulawak are capable of producing 6-9 leaves which affects the increasing number of leaves in one plant.

Statistical analysis that there was no significant different among clones of temulawak in terms of leaf number. Figure 2A shows that the highest number of leaves found on the Malang clone, while the Sumenep clone produced the lowest of number of leaves. The number of tillers per hill shows that the Pasuruan and Malang clones had the highest number of tillers (Fig. 2B) the average number of tillers per hill reached 3.17. However, this amount was not significantly different with other clones



A B

Note: Values followed by the same letter showed no significantly difference according to DMRT at 5%.

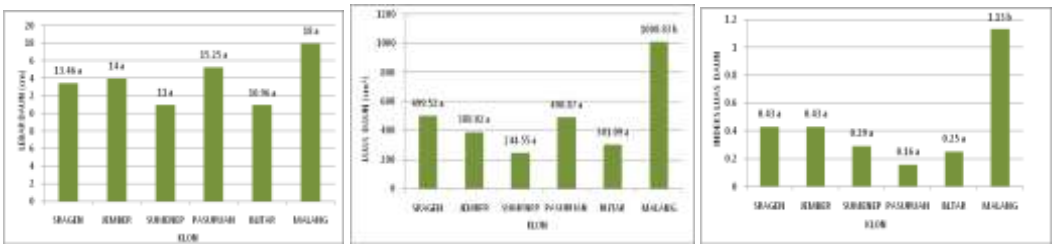
Figure 2. Number of leaves (A) and of six clones of temulawak (*Curcuma xanthorrhiza*) at 19 WAP.

Pasuruan clones into clone with the highest number of suckers, is due to the number of seedlings have buds more when compared with the five other clones. This will impact on the growth of temulawak, number of shoots that emerged after the primary buds more so it appears as a leafy plant with a number of seedlings in a clump of more than one. The number of chicks that many of the temulawak will have a negative effect form of competition for the acquisition of nutrients and light. For the competition in terms of food reserves during early growth, the number of chicks that more will tend to have stronger competition because it has only one rhizome pieces that serve as the seed.

Leaf Width, Leaf Area and Leaf Area Index

Leaf width of all clones were not significantly difference (Figure 3A), and there was no difference on their leaf morphology. The width of the leaf ranged from 10.96 cm to 18.00 cm. The leaf area was significantly difference among the clones with the Malang clones was the best. Malang clones had the highest leaf area with an area of 1,008.83 cm2, while for the lowest on Sumenep clones with only 244.55 cm^2 leaf area (Figure 3B). The leaf area index is related to photosynthetic ability of plants. Widiastuti *et al*., (2004) reported that at low light intensity plants produce larger leaves, thinner with a thin layer of epidermis. It has slight palisade tissue, wider

spaces between cells, and more number of stomata. Meanwhile, plants that received high-light intensity produces smaller leaves, thick, compact with fewer number of stomata, the cuticle layer and a thicker cell wall with space between cells is smaller and hard texture of leaves.



A B C

Note: Values followed by the same letter showed no significantly difference according to DMRT at 5%.

Figure 3. Leaf width (A), leaf area (B) and leaf area index (C) of six clones of temulawak (*Curcuma xanthorrhiza*) at 19 WAP.

Greater leaf area may be harmful, it can also be beneficial to plants. Large leaf area would be advantageous for plants because it will enhance the ongoing process of photosynthesis. Optimal photosynthesis caused photosynthesis product formed also increased, so plant growth will be optimal. Irwanto (2009) revealed that the products of photosynthesis are proportional to the total active leaf area that can perform photosynthesis. Widiastuti et al. (2004) reported that with increased leaf area, assimilates generated will also be greater. Narrow leaf area beneficial for plants because it can reduce water loss through excessive transpiration.

Leaf area index obtained after the analysis was performed to Malang clone has a real difference when compared with leaf area index of the other five clones. Temulawak was obtained from leaf area index is not too large. Of the six clones of temulawak only five clones are at rates below one. Malang clones became the most extensive high-leaf area index reached 1.13 (Figure 3C). This shows that for the Malang clones had interaction especially among the leaves of shade plants. Meanwhile, other five clones had a smaller leaf area index, which was about 0.5. No interactions occur among the clones because of wide spacing used in the experiment was higher than the leaf area produced of temulawak.

The higher of leaf area index (LAI) was beneficial. Sitompul and Guritno (1995) reveal that the density of the leaves is closely related populations of plants or plant spacing. The closer the distance between plants, the higher the density between the leaves and fewer quanta of radiation (light) is to layers of lower leaves.

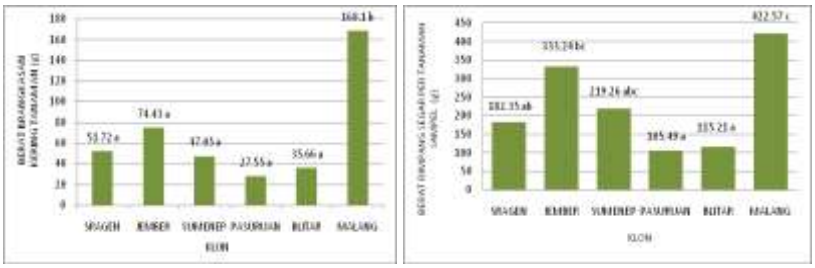
Biomass Dry Weight and Fresh Rhizome Weight per Plant

The statistical analysis showed that Malang clone had the highest biomass production significantly different with other clones (Figure 4A). Dry weight of Malang clones was 168.1 g, the lowest was Pasuruan clone having 27.55 g. This indicated that with increasing leaf area of temulawak was total plant dry weight increase corresponded to the crop yields increase.

When leaf area was higher then its dry weight, this will correspond directly to the total dry weight of the plants. Similarly, the weight of rhizomes that produced was also high. High weight of biomasss showed that the assimilation process may also rearchs to maximum.

Statistical analysis of rhizome fresh weight showed that there was significantly difference among the clones. Malang clone obtained the highest rhizome fresh weight per plant (422.57 g) (Figure 4B), meanwhile Pasuruan clones was the lowest, only reached 105.49 g. Conversion into yield per hectare Malang clones was 22.31 tons. Other temulawak clones was reported to have less

then 20 tons per hectare. Therefore, Malang clone was potential to be promoted as the best clone. This clone is best when it grows intensively on the vertisol soil.



A B

Note: Values followed by the same letter showed no significantly difference according to DMRT at 5%.

Figure 4. Dry weight of biomass (A) and rhizome fresh weight (B) of six clones of temulawak (*Curcuma xanthorrhiza*) at 19 WAP

Specific Leaf Area

Specific leaf area of six different clones showed no significantly different when they were observed until 17-23 WAP. At 17 WAP, Pasuruan was the best, significantly different compared with other clones, but only Sragen clone was significantly different compared with Sumenep clone at 23 WAP (Table 2).

Table 2. Specific leaf area of temulawak (cm^2)

Clones	Age (WAP)			
	17	19	21	23
Sragen	4,033.3 a	3,136.7 a	8,143.3 a	8,033.3 b
Jember	3,806.7 a	1,930.0 a	3,760.0 a	2,710.0 ab
Sumenep	2,556.7 a	886.7 a	1,620.0 a	1,556.7 a
Pasuruan	9,533.3 b	7,083.3 a	24,360.0 a	6,656.7 ab
Blitar	4,033.3 a	1,736.7 b	1,780.0 a	1,993.7 ab
Malang	4,526.7 a	2,863.3 a	4,150.0 a	3,336.7 ab

Note: Numbers followed by same letter on the same column are not significant at different levels of multiple range tests of Duncan's 5%.

Relative Growth Rate (RGR)

RGR on almost all clones of temulawak at the age of 19-21 weeks after planting tend to reduce. The high reduction occurred when plants were 21 weeks old, except for Pasuruan and Blitar clones lowest RGR occurred at the age of 23 weeks after planting (Table 3). This occurs because the plant entered generative phase. After entering the generative phase of growth, clones Malang, Jember, Sumenep, and Sragen increased in RGR. This showed that the clones were ble to produce a new dry matter per unit at initial dry material. Meanwhile, Pasuruan and Blitar clones was not able to produce new dry matter per unit at initial dry material.

Table 3 shows that clones of temulawak from Malang has the highest RGR than the other clones both at the beginning of growth or to close to maximum vegetative growth.

Decline in RGR indicated the differences in rates of photosynthesis. The process of photosynthesis will be disrupted if water is not enough to plants. A low rate of photosynthesis may decrease to growth of vegetative organs, especially to the plant height. The decrease in photosynthesis may be due to increase of stomata diffusion and non-stomata barrier. Decrease of

RGR may also due to the slow growth of vegetative organs (leaf area, plant height, number of tillers, dry weight, and root dry weight of plants).

Table 3. Relative growth rate (RGR) of temulawak (g/week)

Clones	Age (WAP)		
	19	21	23
Sragen	2.17 ab	1.37 a	1.40 a
Jember	2.40 ab	1.52 ab	1.61 ab
Sumenep	2.46 ab	1.60 ab	1.68 b
Pasuruan	1.75 a	1.77 ab	1.65 b
Blitar	2.09 ab	1.78 ab	1.55 ab
Malang	2.83 b	2.21 b	2.44 c

Note: Numbers followed by same letter on the same column are not significant at different levels of multiple range test of Duncan's 5%.

Conclusions

Malang superior clone had the maximum vegetative growth and yield of its fresh rhizome. This clone also had the highest of the plant height, leaf number, leaf width, leaf area, leaf area index (LAI), dry weight of straw, rhizome fresh weight, specific leaf area, and RGR. The maximum plant height of Malang clone was 155.13 cm, the fresh rhizomes of temulawak was 22.31 tons/ha.

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Effect of Drought and Inoculation of Arbuscular Mycorrhizal Fungi in Enhancing Productivity and Tolerance Mechanism of Grasses

PDMH Karti, [a*] L Abdullah[a], J Nulik[b], Nurhayati[c], OR Nahak[d] , S Nofyangtri[a]

[a]*Faculty of Animal Science, Bogor Agricultural University, Bogor, West Java. Indonesia*
[b]*BPTP, Naibonat, Kupang,NTT, Indonesia*
[c]*BPT, Ciawi, West Java, Indonesia*
[d]*University of Timor, NTT, Indonesia*
[*]*Corresponding author: Faculty of Animal Science, Bogor Agricultural University, Bogor, West Java. Indonesia.*
Hp :+6281314227713
pancadewi_fapetipb@yahoo.com

Abstract

Drought stress considered to be one of the most important limiting abiotic factors of plant growth and yield in many areas, and Arbuscula Mycorrhizal Fungi (AMF) symbiosis can protect host plants against its detrimental effect. This research was conducted to study the effect of drought and inoculation of Arbuscula Mycorrhizal Fungi on the productivity and tolerance mechanisms of *Stenotaphrum secundatum* and *Ischaemum timurensis* grasses. This research used a completely randomized design with four treatments: D_0(Control), D_1 (with AMF), D_2 (drought), and D_3 (drought and with AMF). Parameters observed were the soil moisture content, leaf water potential, leaf relative water content, shoot and root dry weight, root length, proline, and soluble sugar. The data were analyzed with analysis of variance (ANOVA) and the differences between treatments were analyzed with Duncan range test. The results showed that drought stress significantly ($P<0.05$) decreased soil water content, leaf water potential, leaf relative water content, shoot dry weight in *Stenotaphrum secundatum*. Drought stress significantly ($P<0.05$) decreased soil water content and leaf water potential, but enhanced proline and soluble sugar content in *Ischaemum timurensis*. AMF inoculation did not affect productivity of *Stenotaphrum secundatum*, but in *Ischaemum timurensis* enhanced proline and soluble sugar content but decreased leaf water potential on drought stress. One of the tolerance mechanism in *Ischaemum timuriensis* is by increasing the proline and soluble sugar contents.

Keywords: drought, arbuscular mycorrhizal fungi, grasses, Stenotaphrum secundatum, Ischaemum timurensis

Introduction

Plants in nature are continuously exposed to several biotic and abiotic stresses, water deprivation being one of the commonest. Soils too dry for crop production have been estimated to cover 28% of the earth's land surface (Bray, 2004). Nevertheless, plants have developed several physiological, biochemical, and molecular mechanisms in order to cope with drought stress. Besides the natural responses of plants against drought, it must be considered that most terrestrial plants can establish a symbiotic association with the arbuscular mycorrhizal fungi (AMF). When the AMF symbiosis is established the fungus receives carbon molecules from the plants, and the plants receive nutrients (especially phosphorus) and water from the fungus (Harrison, 2005; Gosling *et al.*, 2006). In this way, AM plants are usually more tolerant to several stresses, including drought, than non-AM plants (Augé, 2001, 2004; Ruiz-Lozano, 2003; Ruiz-Lozano *et al.*, 2006). This research was conducted to study the effect of drought and inoculation of AMF to the productivity and tolerance mechanisms of *Stenotaphrum secundatum* and *Ischaemum timurensis* Grasses

Materials and Methods

The materials used in this study were two types of grasses namely *Stenotaphrum secundatum,* and *Ischaemum timurensis*. The two types of grasses are the result of selection about 30 forage species on preliminary research (Karti, 2010). Other materials used were fiber pots as many as 32 units (diameter = 20, high = 100 cm), Mycofer, growing media in the form of soil and manure, tools WP4 potentiometer, coolbox, and others.

This research used a completely randomized design with four treatments: D_0 (control), D_1 (with AMF), D_2 (drought), and D_3 (drought and with AMF) and four replications. Parameters observed were soil moisture content, leaf water potential, leaf relative water content, shoot and root dry weight, root length, proline (Bates, 1973), and soluble sugar (Dubois *et al.,* 1956 modified by Buysse & Merckx, 1993). The data were analyzed with analysis of variance (ANOVA) and the differences between treatments were analyzed with Duncan range test.

Results and Discussion

Drought stress significantly ($P<0.05$) decreased soil water content, leaf water potential, leaf relative water content, shoot dry weight in *Stenotaphrum secundatum* (Table 1). AMF without drought treatment did not show significant differences, but it showed significant different ($P<0.05$) in drought treatment. Drought stress reduced levels of the soil led to a decrease in water absorption. The low uptake of water caused the shoot dry weight decreased because of declining photosynthesis process. The addition of AMF increased significantly ($P<0.05$) the shoot dry weight, this is due to the ability of AMF in increasing absorption of water so that improving the photosynthesis process.

Table 1. Effect of drought and the addition of AMF to soil moisture, leaf water content, relative water content, shoot dry weight, and root dry weight of *Stenotaphrum secundatum*

Variables	Treatments			
	Do	D1	D2	D3
Soil moisture	38.45 ± 0.46 a	39.14 ± 0.59 a	23.76 ± 1.04 b	22.98 ± 0.79 b
Leaf water potential (-Mpa)	-0.68 ± 0.2 b	-0.51 ± 0.6 b	-6.79 ± 1.6 a	-7.00 ± 1.7 a
Relatif water content (%)	82.28 ± 2.8 a	87.2 ± 6.4 a	29.69 ± 6.4 b	34.51 ± 10.2 b
Shoot dry weight (g/pot)	27.6 ± 3.5 a	29.0 ± 4.7 a	14.7 ± 2.1 c	22.3 ± 2.3 ab
Root dry weight (g/pot)	2.5 ± 1.9	4.8 ± 3.5	2.9 ± 0.7	3.2 ± 1.0

Description: D_0 (control), D_1 (with AMF), D_2 (drought), and D_3 (drought and with AMF).

Table 2. Effect of drought and the addition of AMF to soil moisture, leaf water potential, leaf relative water content, root length, shoot dry weight, and root dry weight, proline and soluble sugar of *Ischaemum timuriensis*

Variables	Treatments			
	Do	D1	D2	D3
Soil moisture (%)	$35,52 \pm 0,58^{a}$	$35,33 \pm 1,97^{a}$	$23,15 \pm 1,28^{b}$	$24,32 \pm 2,01^{b}$
Leaf water potential (-Mpa)	$-1,02 \pm 0,19^{b}$	$-1,36 \pm 0,11^{a}$	$-1,46 \pm 0,17^{a}$	$-1,19 \pm 0,27^{ab}$
Leaf relative water content (%)	$88,25 \pm 3,50^{a}$	88,48 ± 0,90	86,10 ± 1,71	86,78 ± 0,72
Root lenght (cm)	128,0 ± 6,38	128,50 ± 5,80	128,25 ± 4,92	127,75 ± 11,76
Shoot dry weight (g/pot)	38,87 ± 9,21	41,70 ± 18,36	29,10 ± 12,35	30,70 ± 5,88
Root dry weight (g/pot)	5,30 ± 3,77	6,65 ± 4,20	8,30 ± 3,76	10,23 ± 2,99
Proline	$51,68 \pm 7,63^{H}$	$43,36 \pm 7,04^{H}$	$67,48 \pm 7,43^{H}$	$59,05 \pm 17,73^{H}$
Soluble sugar	$6,64 \pm 2,43^{LM}$	$4,78 \pm 1,36^{M}$	$23,09 \pm 2,71^{EF}$	$17,24 \pm 1,88^{GH}$

Description: D_0 (control), D_1 (with AMF), D_2 (drought), and D_3 (drought and with AMF)

In *Ischaemum timuriensis* drought stress significantly (P<0.05) decreased the soil moisture content, leaf water potential, and significantly increased (P<0.05) the proline and dissolved sugar, but did not show significant effect in leaf relative water content, root and shoot dry weight. In drought conditions, plants decreased leaf water potential, but with the addition of AMF the decrease could be minimized compared to that with non-AMF. In these plants, drought treatment did not cause a change in relative leaf water content, root and shoot dry weight. This suggests that these plants include drought resistant crops. Tolerant plants that can increase levels of proline and soluble sugar to maintain water absorption so that the growth process can run well.

Drought resistance mechanisms in *Ischaemum timuriensis* is by secreting proline and soluble sugar. In Figure 1 shows that drought stress increased proline levels in both treatments with the addition of AMF or not. In *Ischaemum timuriensis* a sharp increase in proline was seen after 40th day.

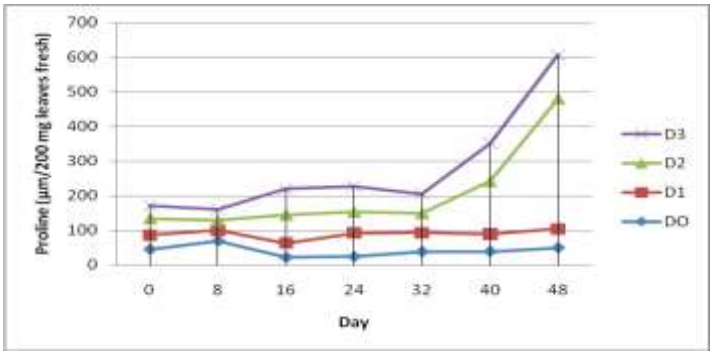

Figure 1. Proline Content of Ischaemum timuriensis.

In *Ischaemum timuriensis* (Figure 2) shows that drought stress showed the highest elevated levels of dissolved sugar. The addition of AMF on drought stress decreased levels of dissolved sugar, it means that the AMF can increase the absorption of water through the hyphae.

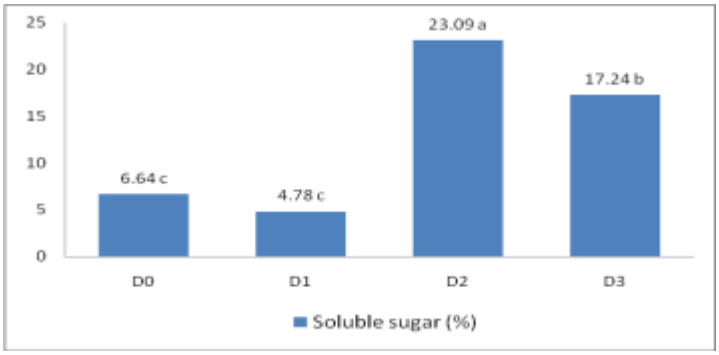

Figure 2. Soluble Sugar of Ischaemum timuriensis.

Conclusion

Drought stress can reduce soil moisture, leaf water potential, leaf relative water content, shoot dry weight in *Stenotaphrum secundatum*. AMF inoculation on drought stress can increase the shoot dry weight. On *Ischaemum timuriensis* drought stress can reduce soil moisture, leaf water potential, and increasing proline and sugar dissolved. *Ischaemum timuriensis* is included in drought-resistant plants with resistance mechanisms through the increased secretion of proline and soluble sugar.

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The Cooling Effect of Forage Crop, Kudzu (*Pueraria lobata*) Vine Covering over Livestock Buildings

Takuya Koyama[a], Mika Yoshinaga[b], Kei-ichiro Maeda[a] and Akira Yamauchi[a*]

[a] *Department of Biosphere Resources Science, Nagoya University, Nagoya, Japan*
[b] *Department of Architecture, Meijo University, Nagoya, Japan*
[*] *Corresponding author: Nagoya University, Chikusa, Nagoya 464-8601, Japan*
ayama@agr.nagoya-u.ac.jp

Abstract

Heat stress during summer season can decrease livestock productivity, and cause high economic losses to livestock farmers. Although many cooling techniques (e.g. sprinklers, fans, etc.) are available to improve the thermal environment of livestock buildings, they require high construction and energy costs. This research aims to investigate an economical livestock buildings covering method with kudzu (*Pueraria lobata*), which can be used as a cooling technique during summer season and as forage for livestock after use. Kudzu is a fast-growing, climbing and perennial vine, and its shoot can be utilized as high nutritious feed for livestock. In this study, the cooling effect of the covering method with kudzu was investigated by comparing the room temperatures (RTs) between the covered (Covered) and the non-covered fabricated house (height 2.6 m, width 3.65 m, depth 1.83 m) (Non-covered). Two-year-old kudzu seedlings were transplanted beside the south side of the houses in May 2009 and 2010 and induced to climb the plastic nets placed over the south walls and roofs of the houses. RTs of Covered and Non-covered and global solar radiation were measured every 5 minutes during summer season in 2009. The covering rate and the leaf number in 18 frames (0.4 m × 0.4 m each) that were set on the south wall and roof of Covered and stem length were measured every 2 weeks in 2009. The covering rate and the dry weight in 120 frames were measured during autumn 2010. The stem length reached around 4 m long by July 2009. In general, the height of a standard livestock building is around 4 m. These facts showed that two-year-old kudzu seedlings could cover the walls of a standard livestock building during summer season. The estimated average covering rate and leaf number among 18 frames, and the RT differences between Covered and Non-covered showed positively significant relationships ($P<.001$). The maximum RT difference was observed to be 3.44 °C, when the estimated average covering rate was 43.9 % and the estimated average leaf number was 529 leaves m-2. The covering rate and dry weight in the frame showed a positively significant relationship ($P<.001$). The maximum dry weight of 253.4 g m-2 was observed when the covering rate was 80.8%. We conclude that the kudzu covering is an effective cooling technique for livestock buildings during summer season and is potentially capable of producing biomass to provide feed for livestock after use.

Keywords: kudzu, green wall, heat stress, livestock productivity, forage crop.

Introduction

The global average surface warming in the 21st century will range from 1.1 to 6.4 °C (IPCC, 2007). The global warming has the potential to occur adverse thermal conditions in the livestock building, which can ultimately reduce productive performance or possibly cause deaths of livestock (Hahn, 1995). Thus, some techniques for environmental modifications such as water misting with spray, forced ventilation with fans or air-conditioning, shading with insulated roof or reflective coated roof are needed to maintain animal production (West, 2003). On the other hand, the employment of these techniques causes higher energy consumption, worsens global warming, and increases general costs for animal production (Nardone, 2010).

The ultimate goal of this study was to establish the economical livestock buildings covering method with the use of kudzu (*Pueraria lobata* (Willd.) Ohwi) vine. That is to cover the walls and roofs of livestock buildings with kudzu vine, which can be used as a cooling technique during summer seasons and as feed for livestock after use.

Kudzu is a fast-growing, climbing and perennial vine. Their vines can grow 10 to 30 m in one growing season (up to 30 cm a day), and create broad canopies (Mitich, 2000). Farmers in the USA cultivated kudzu over a terrace to provide shade in the early 1900s (Tsugawa, 1986). Moreover, kudzu has the wide range of utilization as forage because it is able to provide feed for sheep, swine and chickens as well as cattle (Kristen, 2011). Chemical composition and digestibility characteristics of kudzu were equivalent to other commonly fed forage (Corley *et al*., 1997)

The use of external vegetation can be a useful tool for thermal regulation for buildings. Holm (1989) showed using dynamic computer model that the leaf cover produces a constant 5 °C cooling effect in room temperature in summer. The vegetation consistently lowered the wall surface temperature by about 17 °C, and saved air-conditioning energy as high as 80% (Meier, 1990). However, these studies placed little or no emphasis on the conditions of the plants.

Ip *et al.* (2010) analyzed the shading performance of a vertical climbing plant canopy by measuring number of leaf layers, area of canopy with each leaf layer and solar transmissivity, and established the thermal model that enables to present the shading performance of the climbing plant canopy over its annual growing and wilting cycle. Unlike non-biological devices, the conditions of plant sare affected by not only the characteristics of plants such as leaf area, leaf number, leaf thickness, leaf angle, leaf movement and moisture content of leaves but also the response to the environmental conditions such as solar radiation, ambient temperature, humidity and soil moisture content, which affect the shading performance, the thermal insulation and evaporative cooling produced by transpiration.

The objectives of this paper were to investigate the cooling effect of the dynamic changes of kudzu vine covering by comparing the room temperatures (RTs) between the covered (Covered) and the non-covered container house (Non-covered), and to evaluate the dry matter production of kudzu vine that was used for the economical livestock buildings covering method. The leaf number and covering rate in each frame that was set on the south wall and roof of Covered, the room temperatures of Covered and Non-covered, and environmental conditions around the houses were determined at regular intervals during summer season. At harvest, the covering rate and dry matter weight in 120 frames were measured.

Materials and Methods

Plant materials

Hard-stems (lignified) were collected from kudzu natural stands in the Graduate School of Bioagricultural Sciences, Nagoya University (35°09'N, 135°58'E) from April to May in 2008 and 2009. Then, the hard-stems were cut into around 12 cm with one node and grown in the commercial and artificial soil that is well-drained and poorly nutrient in plastic container (585mm long, 185mm wide and 145mm deep). After cuttings were rooted and sprouted, they were transplanted to plastic pots (25 cm in diameter and 30 cm in height) containing air-dried paddy soil and raised in a glasshouse for one year. In winter 2008 and 2009, all stems were cut at 5cm from the soil surface, and afterwards left undisturbed. Next spring, some buds were sprouted from the rooted cuttings. Sprouted nursery stocks (plant materials) were only used for this study.

Experimental design

Four container houses (2600mm high, 3650mm wide and 1830mm long) were set in series from east to west on the Togo field of the Graduate School of Bioagricultural Sciences, Nagoya University (35°06'N, 137°04'E) in 2009, and 5 container houses were set in 2010. One container house excluding both ends was not covered with kudzu vine (Non-covered), and plant materials were transplanted beside the other container houses in May 25, 2009 and May 24, 2010, and induced to climb the plastic nets (mesh size: 120mm×120mm) placed on the south wall and the roof of the houses.

Measurements

Only two container houses excluding both ends (Non-covered and Covered) were used for evaluating the effect of the dynamic changes of kudzu vine covering on the room temperature reduction in 2009. The houses on both ends were excluded because they received more solar radiation than the inner houses. The stem length, the leaf number and covering rate in each frame (400mm×400mm), which was set on different heights of the south wall (650mm, 1300mm and 1950mm above the soil surface) and on different locations of the roof (465mm, 915mm and 1365mm from the south edge of the roof), were measured on the 3 plants except both ends every 2 weeks from June 10 to September 29, 2009.The estimated average leaf number and covering rate among 18 frames were calculated from the inclination of averages of these data.The room temperatures (RTs) of Covered and Non-covered were recorded every 5 minutes with thermo recorder (TR-72U: A&D Co. LTD.) and global solar radiation was recorded every 5 minutes with pyranometer (MS-801: EKO INSTRUMENT CO., LTD.) from July 15 to September 22, 2009.To evaluate the dry matter production of the economical livestock buildings covering method with use of kudzu vine, the covering rate and the dry weight in 120 frames, which were located on the south wall and the roof of 4 containers, were measured during autumn in 2010.

Image analysis

Before taking digital images of Covered, the white-colored frames (400mm×400mm) were set behind the plants and in front of the south walls and roofs to facilitate processing images. Digital images, composed of 12 megapixel, were obtained with the digital camera (μ-7040: OLYMPUS corporation). The backgrounds except the plants in the frames were cleared manually, and plants were converted into binary image with photoshop (Adobe Photoshop CS2: Adobe). The pixels of the processed image were calculated with imageJ (Public domain software for image processing and analysis in Java). Calculated pixels of the plants and the frames were used for computing the covering rate.

Results and discussion

The stem length reached around 4 m long by July, and some vines exceeded 10 m at the end of August in 2009. In general, the height of a standard livestock building is around 4 m. These facts showed that two-year-old kudzu seedlings could cover the walls of a standard livestock building during summer season.

Fig. 2 shows that the changes in estimated average leaf number and covering rate among 18 frames. The estimated average leaf number gradually increased until the middle of July in 2009. Afterwards, the estimated average leaf number increased rapidly and reached the peak of 530 leaves m-2 on September 10, 2009.The estimated average covering rate showed similar trend with the estimated average leaf number, and it peaked at 45% at the final measurement on September 25.

The RTs of Non-covered were clearly influenced by ambient temperature and global solar radiation. The RT differences between Non-covered and Covered were also influenced by ambient temperature and global solar radiation. At the same time, the RT differences tended to gradually increase. Therefore, the data of the RT differences were sorted and averaged out daily according to the global solar radiation; every 0.2 kWM-2; and the processed data were used to highlight the effect of the covering method with kudzu.

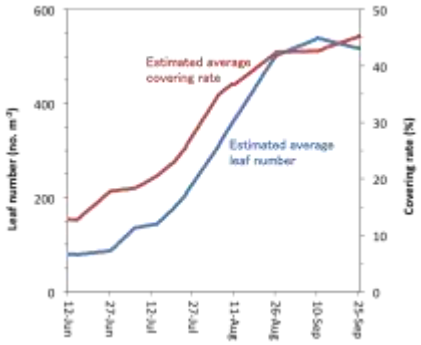
Under the global solar radiation conditions less than 0.2 kW m-2, there was a significantly negative relationship between the RT differences, and the estimated average leaf number ($P<.001$) and the estimated average covering rate ($P<.001$). However under the global solar radiation conditions between 0.2 kW m-2 and 1.0 kW m-2, there were significantly positive relationships between the RT differences, and the estimated average leaf number ($P<.001$) and the estimated

average covering rate (P<.001). The inclinations of each regression equations increased as the global solar radiation increased under the global solar radiation conditions between 0.2 kW m-2 and 1.0 kW m-2. The maximum RT difference was observed to be 3.44 °C, when the estimated average covering rate was 43.9 % and the estimated average leaf number was 529 leaves m-2. The covering rate and dry matter weight in the frame showed a positively significant relationship (P<.001). The maximum dry weight of 253.4 g m-2 was observed when the covering rate was 80.8%.

We conclude that the kudzu covering is a possible cooling technique for livestock buildings during summer season and is potentially capable of producing biomass to provide feed for livestock after use.

This photo was taken on September 18, 2009.

Figure 1. Two container houses(2600mm high, 3650mm wide and 1830mm long) excluding houses on both ends (*Non-covered* and *Covered*) were used for evaluating the effect of the dynamic changes of kudzu vine covering on the room temperature reduction in 2009.



The estimated average leaf number and covering rate were calculated from the inclination of averages of leaf number and covering rate among18 frames (400mm×400mm), which were located on the south wall and roof of *Covered*.

Figure 2. Changes in the estimated average leaf number and covering rate among 18 frames in 2009.

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Effect of Weed Control Management on Herbage Yield and Quality in the Established Dwarf Napiergrass (*Pennisetum purpureum* Schumach)

Renny Fatmyah Utamy [a], Yasuyuki Ishii [b,*], Sachiko Idota [b], Lizah Khairani [c]

[a] *Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Japan*
[b] *Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan*
[c] *Graduate School of Agriculture, University of Miyazaki, Miyazaki, Japan*
**Corresponding author: Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen-kibanadai Nishi, Miyazaki 889-2192, Japan. Tel.: +81 985 58 7251; fax: +81 985 58 7251.*
yishii@cc.miyazaki-u.ac.jp

Abstract

In our previous extension activity of dwarf variety of late-heading type (DL) napiergrass (*Pennisetum purpureum* Schumach) to southern Kyushu, weed control management is found to be a crucial factor for obtaining good establishment and considerable herbage dry matter (DM) yield in an established year. The objective of this study was to examine the effect of several weed control practices, *i.e.* mixed sowing of annual setaria (abbreviated as S), which has no regrowth ability in stem-elongated tillers, paper-mulching (as P) and hand-weeding (as W), compared with no-weeding (as –W) on DM yield and quality of this species for two years (Trial-1,2). Weed control practices had a significantly ($P < 0.05$) positive effect on plant height, tiller density, percentage of leaf blade and leaf area index in DL napiergrass, compared with no-weeding (S-W or –W), and paper-mulching (P or P+S-W) had the highest yields in both trials. Setaria-sowing had a partially mitigating effect of weed damage on growth of DL napiergrass, while additive DM gain from setaria could compensate the yield decrease in DL napiergrass and reduce herbicide cost. Neither IVDMD nor CP content was affected by any weed control in either trial. Thus, paper-mulching and annual setaria-sowing could be a good proposal in weed control of this species.

Key words: annual setaria, dwarf napiergrass, herbage yield, paper mulch, weed control

Introduction

Based on extension activity of dwarf variety of late-heading type (DL) napiergrass (*Pennisetum purpureum* Schumach) to southern Kyushu, weed control management is found to be a crucial factor to obtain good establishment of this grass and achieve considerable herbage yield in the established year (Utamy *et al*., 2011). Hand-weeding was a sole weed control practice, which caused physical and spiritual burden for farmers before the launch of herbicides. Therefore, invention of easy and environmentally effective weed control technology has been strongly desired.

Weed invasion into the established forage crop fields is a visible sign of management problems. Damages to forage crop production by weeds are mediated principally from loss in growth rate and yield, and secondarily from decline in forage quality. However, in forage crop production, use of herbicide should be avoidable because of the negative effect on livestocks and increase in production cost (Sakai & Kawanabe, 1981).

Weeds in the inter-row space of DL napiergrass are normally controlled by hand-mowing machine 2–3 times before the first defoliation of this grass. Repeated weed control is essential until the leaf canopy is well established at the establishment. Even though close spacing is desirable from the weed control point of view, weeds do invade even at 50 × 50 cm of plant spacing.

Mulching at the inter- and intra-row spaces reduces weed problems by preventing the seed germination and suppressing growth of emerged weed seedlings, resulting in facilitating soil fertility and plant productivity (Wilson *et al.*, 1987; Obiefuna, 1991; Salau *et al.*, 1992). Mulching is a well-known method for the establishment of horticulture crop such as lettuce (Moniruzzaman, 2006) and

tomato (Anzalone *et al*., 2010) and also in paddy rice field (Won *et al*., 2011). In the grass cultivation, mulching is often used as living mulch or cover crop such as white clover (Deguchi *et al*., 2005), legume (Hiltbrunner *et al*., 2007) and hairy vetch (Mohammadi, 2010). However, paper-mulching has not been applied to DL napiergrass as weed control management.

The other way for weed control management is the oversowing of annual grass species to compete weed at the early growth of perennial forage crops. DL napiergrass was oversown with temperate Italian ryegrass (*Lolium multiflorum* Lam.) to get herbage in the spring-early summer season (Ishii *et al*., 2005). In the present study, tropical annual setaria (*Setaria italica* cv. Natsukanso), released from Yukijirushi Seed Co. Ltd., is utilized as once-cutting herbage with no regrowth ability if it starts stem elongation at the harvest, gives early summer growth, and should be also ideal to suppress summer weeds at the early growth of perennial forage species (Wakamatsu, 2004).

Therefore, the objectives of this study was to examine the effect of weed control management on dry matter yield and herbage quality in the established DL napiergrass by paper-mulching, oversowing of annual setaria and several time of weeding practices, compared with no weeding control in two years.

Materials and Methods

In 2008, DL napiergrass transplanted at 2 plants m^{-2} was imposed by three treatments (P, S+W, S-W) with three replications by Latin square design in Sumiyoshi Livestock Science Station (31°98´N, 131°46´E), University of Miyazaki (Trial-1). In 2010, DL napiergrass transplanted at 2 plants m^{-2} was imposed by four treatments (P+S-W, S+W, S-W, -W) with three replications by a randomized block design in Kibana Agricultural Science Station (31°83´N, 131°41´E), University of Miyazaki (Trial-2). Growth attributes including yield, *in vitro* DM digestibility (IVDMD) and crude protein (CP) content as herbage quality were determined in both trials. Efficiency of weed control practices in plant parameters, such DM yield, IVDMD and CP content, was evaluated by the percentage of plant parameter value in each weed control practice to that in no weed control.

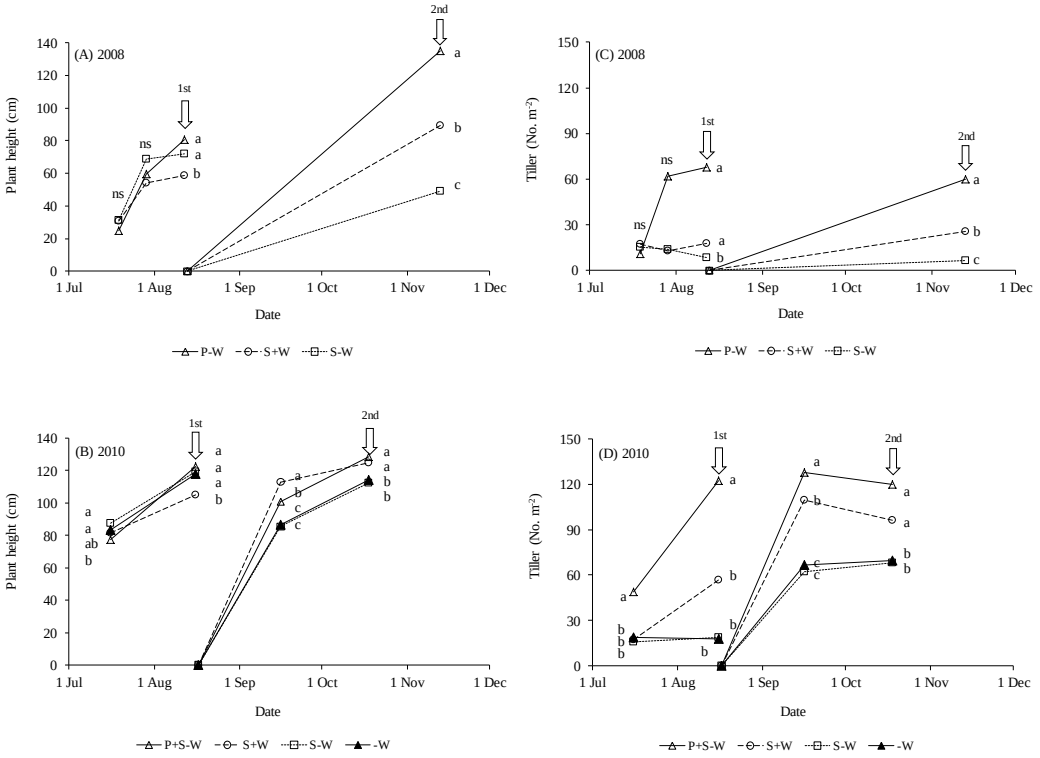
Results and Discussion

Weed control management had significantly ($P < 0.05$) positive effects on plant density, tiller density, percentage of leaf blade and leaf area index in DL napiergrass, compared with no-weeding (S-W or –W), and paper-mulching (P or P+S-W) had the highest yields in both trials (Figure 1).

Adoption of several weeding practices such as weeding, paper-mulching and setaria-sowing can be assessed by the percentage of gain or loss in attributes under the particular practice relative to those under no adoption of the practice (Table 1). Adoption of paper-mulching facilitated to obtain largest positive gain of DM yield in DL napiergrass at two defoliations in both Trials 1 and 2, respectively. Situation was similar for the positive gain by adoption of hand-weeding, while the degree of gain was reduced from paper-mulching (Table 1). The advantage of paper-mulching in DM yield was closely corresponded with positive gain in plant height, tiller density and leaf area index through improvement in light penetration (Peltzer & Köchy, 2001) and prevention of plant damage from weeds (TruGreen Tree and Shurub Field Guide, 2008). Positive effect of paper-mulching on DM yield matched with several crops such as paddy rice (Won *et al*., 2011), lettuce (Moniruzzaman, 2006), tomato (Anzalone *et al*., 2010) and turmeric (Sanyal & Dhar, 2006).

Weed control management by sowing setaria had no significant ($P > 0.05$) effect to suppress weed DM yield in Trial-2 (data not shown). However, 1 g m^{-2} DM production from setaria reduced 4 g m^{-2} of DM production from weeds ($y = 104.553 – 0.247x$, $r = 0.52$, $P > 0.05$). In several

stand establishments of perennial species, annual species are used to suppress weeds and mitigate the competition of crops from weeds. In the present study, annual setaria has characteristics to give good growth in early summer and suppress summer weeds (Wakamatsu, 2004).



Arrows indicate the time for the first (1st) and second (2nd) defoliation. P, interrow space was covered by paper mulch; S, oversown with setaria; W, weeding and –W, no weed control. Symbols with different letters are significantly different among weed control managements at each date by LSD method at 5% level. ns: $P > 0.05$.

Figure 1. Changes in plant height and tiller density of DL napiergrass under several weed control management in 2008 (Trial-1) and 2010 (Trial-2).

Neither IVDMD nor CP content was affected by any weed control in either trial. Consistently positive effect of any weed control practice on quality attributes was hardly obtained in either trial, while decline in CP content under weeding and paper-mulching practices was common at the second and first defoliation in Trial 1 and 2, respectively (Table 1). Living mulch with white clover improved plant nutrition by enhancing phosphorus uptake in maize (Deguchi *et al*., 2005). The present paper-mulching and hand-weeding could not contribute to herbage quality of DL napiergrass, possibly due to the negative correlation of DM yield with quality attributes under similar fertilization in this species. Dwarf napiergrass cv. Mott, which has almost equivalent plant attributes to DL napiergrass, had IVDMD and CP content at 67.5 and 13.2%, respectively (Sollenburger *et al*., 1988), almost corresponded with the present IVDMD at 74 and 60% at the first and second defoliation, respectively, in Trial 1.

Table 1. Effect of weed control practices on efficiency in several parameters of plant total in DL napiergrass at each defoliation in 2008 and 2010

Year	Defoliation	Parameter	Practice		
			Weeding	Paper-mulching	Setaria-sowing
2008	1st	DM Yield	248	2466	—
(Trial 1)		IVDMD	4	1	—
		CP content	2	-9	—
	2nd	DM Yield	7554	54091	—
		IVDMD	14	-6	—
		CP content	-16	-24	—
2010	1st	DM Yield	74	275	-4
(Trial 2)		IVDMD	6	7	1
		CP content	-19	-6	11
	2nd	DM Yield	73	73	36
		IVDMD	-1	-1	0
		CP content	-3	9	3

DM yield, dry matter yield; IVDMD, *in vitro* dry matter digestibility; CP content, crude protein content.

Conclusion

Paper mulch is not common to use in DL napiergrass cultivation, while it proved to be effective to avoid weed damage and facilitate good growth with high DM yield of this species. Cost of paper mulch is 50 yen/meter (Sanyo Seishi Co. Ltd., Tottori) and setting of paper mulch is a labor-extensive weed control practice. Thus, based on the amount of natural seed bank of weeds, paper mulch or other degradable mulching material can be applied to DL napiergrass, so as to reduce weed competition at the establishment. Annual setaria-sowing gave advantage to get herbage yield at the first defoliation of DL napiergrass, although prompt harvest time of annual setaria should be examined in the mixed cropping with DL napiergrass.

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Effect of Density of Mother Plants on Efficiency of Nursery Production in Dwarf Napiergrass (*Pennisetum purpureum* Schumach)

Asuka Yamano[a], **Yasuyuki Ishii[b,*]**, Koutarou Mori[a], Renny F Utamy[c], Sachiko Idota[b]

[a] *Graduate School of Agriculture, University of Miyazaki, Miyazaki, Japan*
[b] *Faculty of Agriculture, University of Miyazaki, Miyazaki, Japan*
[c] *Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Japan*
** Corresponding author: Faculty of Agriculture, University of Miyazaki, 1-1 Gakuen-Kibanadai Nishi, Miyazaki 889-2192, Japan.*
Tel.: +81 985 58 7251; fax: +81 985 58 7251.
yishii@cc.miyazaki-u.ac.jp

Abstract

Recently, dwarf napiergrass (*Pennisetum purpureum* Schumach) of late-heading type (named as DL) was introduced widely for multi-purpose uses into Kyushu Island, Japan. Napiergrass needs to be propagated vegetatively, while nursery production was not efficiently established yet. We examined to obtain an optimal plant density of mother plants for the efficient nursery production (per both unit land area and labor time bases). Dwarf napiergrass was transplanted at three levels (1, 4 and 8 plants m^{-2}) from cell-tray plants by Latin square method in the University of Miyazaki (31°83´N, 131°41´E) on 2 July, 2010. Compound fertilizer containing 14% of N, P_2O_5 and K_2O was applied monthly at 5 g each m^{-2} $time^{-1}$ (annual total 20 g) and growth attributes of plant height and tiller density were monitored monthly. Dry matter yield (DMY) of mother plants and efficiency of nursery production using single-node stem cutting were determined per both land area and labor time on 8 December, 2010 and wintering ability was determined on 17 May, 2011 (Trial-1). Nursery plant was grown at cell-tray bed in glasshouse during wintering from December to April and trimming effect on tillering ability of nursery plants was checked on 13 April, 2011 (Trial-2). In Trial 1, plant height and tiller density increased with increasing plant density and tiller density saturated earlier at 4 and 8 plants m^{-2} than at 1 plant m^{-2}. Although DMY and number of nursery plants per mother plant was significantly higher ($P < 0.05$) at 1 plant m^{-2}, those per m^2 were significantly higher ($P < 0.05$) and labor time for nursery production per cutting, significantly lower ($P < 0.05$) at 4 and 8 plants m^{-2} than at 1 plant m^{-2}. Wintering ability was considerably high above 93% among 3 densities. In Trial-2, emerged percentage of nursery plants was consistent around 75% among 3 densities and trimming increased tiller number to promote quicker establishment of nursery plants in the field. Thus, 4 and 8 plants m^{-2} were concluded to be optimal for nursery production in the established year.

Keywords: dwarf napiergrass, nursery production, plant density, trimming, wintering ability.

Introduction

Napiergrass (*Pennisetum purpureum* Schumach) is a tropical C_4 grasses and its dry matter yield was higher than other crops. Napiergrass can be used for multi-purpose uses such as feeding to herbivores (Fukagawa *et al*., 2010; Utamy *et al*., 2011), rotational grazing use of dwarf variety (Ishii *et al*., 2009), feedstock for bioethanol production (Khairani *et al*., 2010) and phytoremediation activity (Hamano *et al*., 2011) in Kyushu, Japan. Napiergrass needs to be propagated vegetatively, while efficient nursery production is not so established that the extension of this grass species is hardly progressed. Therefore, in the present study, we examined the effect of mother plant density on the efficiency of nursery production per both land area and labor time bases (Trial-1). In Trial-2, we examined the effect of trimming on growth attributes of nursery plants, to obtain faster establishment of this grass species.

Materials and Methods

Dwarf variety of late-heading type (DL) napiergrass, which was overwintered on a cell-tray bed in a glasshouse from December 2009 to April 2010, was transplanted into the field at three densities (1, 4 and 8 plants m^{-2}) by Latin square method in University of Miyazaki (31°83´N, 131°41´E) on 2 July, 2010. Compound fertilizer containing 14% of N, P_2O_5 and K_2O was applied monthly at 5 g each m^{-2} $time^{-1}$ (annual total 20 g) and growth attributes of plant height and tiller density were monitored monthly. Dry matter yield (DMY) of mother plants and efficiency of nursery production using single-node stem cutting were determined per both land area and labor time on 8 December, 2010. Wintering ability, such as percentage of overwintered plants (POP) and regrown tiller number (RTN), was determined on 17 May, 2011 (Trial-1). Nursery plant was grown at cell-tray bed in a glasshouse during wintering from December to April and the effect of trimming at 5 cm above the ground on tillering ability of nursery plants was checked to determine plant height, tiller number and dry matter weight on 13 April, 2011. Additional fertilizer was supplied at 9.8 g N m^{-2} on 16 April, 2010 (Trial-2).

Results and Discussion

Effect of mother plant density on growth rates of several plant attributes, dry matter production and nursery plant production

With the increase in mother plant density, the growth rate of plant height and especially number of tillers used for nursery production tended to increase and reached the stage of maximum tiller density earlier in early August than at the lowest density of 1 plant m^{-2} (Figure 1). Thereafter, it occurred self-thinning to diminish less vigorous tillers and to decrease the tiller density at 4 and 8 plants m^{-2}.

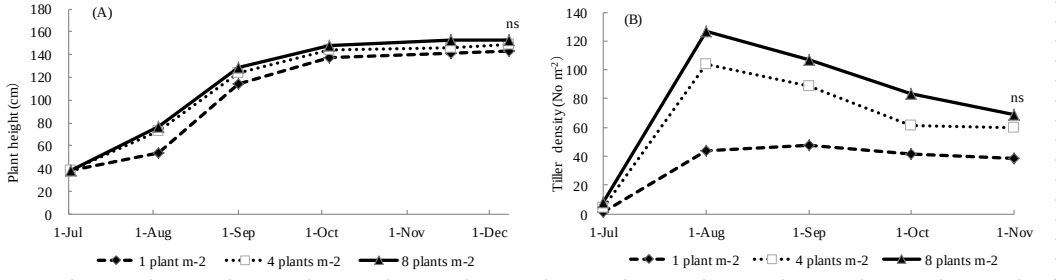

Figure 1. Changes in plant height (A) and tiller density (B) among three densities of mother plants.

Dry matter weight per plant was the highest at 1 plant m^{-2}, followed by 4 and 8 plants m^{-2}, while dry matter yield per unit land area (m^2) increased with the increase in mother plant density to reach the highest yield ($P < 0.05$) at 8 plants m^{-2} (Figure 2).

While number of nursery plants per mother plant was significantly higher ($P < 0.05$) at 1 plant m^{-2}, those per m^2 were significantly higher ($P < 0.05$) and labor time for nursery production per cutting, significantly lower ($P < 0.05$) at 4 and 8 plants m^{-2} than at 1 plant m^{-2} (Figure 3). Even though plant leaf area was not determined in the present study, it is estimated that optimum leaf area index (LAI) was attained at earlier stage to enhance dry matter yield in early December with the increase in plant density. It is suggested that the period to make internode and node with tiller buds matured would be proportional to periods from tiller emergence as the basis for matured tiller buds suitable for nursery production. It is suggested that even delayed tillers emerged in early September could be used for nursery production at 1 plant m^{-2}, which did not have enough time for tiller buds to matured (Figure 1 (B)).

These tiller productions also need more tillers to produce nursery plants, resulted in extending time for nursery production and increasing cost of labor power at lower densities.

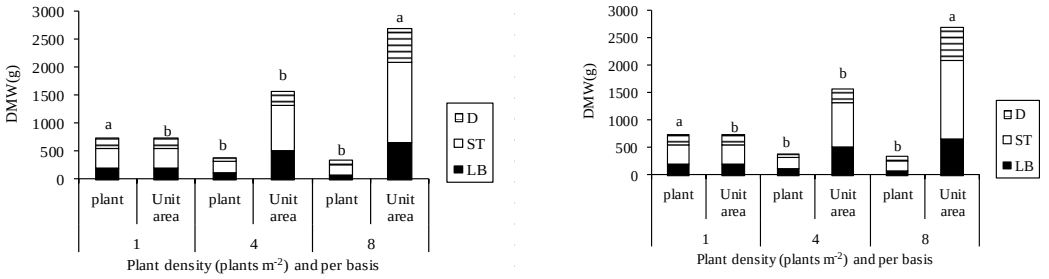

Figure 2. Dry matter weight (DMW) of plant organs per both plant and unit area bases among three densities of mother plants on 8 December 2010.

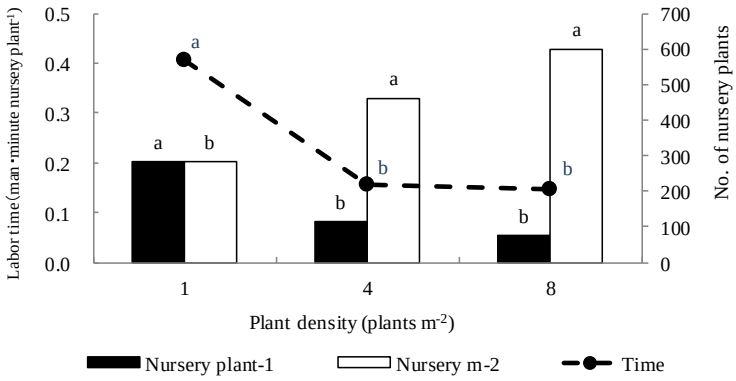

Figure 3. Nursery plant number and labor time for production per mother plant and per unit area.

Relationship between plant density of mother plants and sustainability of plants

In the present study, POP was hardly affected by plant density of mother plants and POP overpassed 93% in all densities to show sustainability of this grass species. However, RTN per unit land area (m^2) tended to decrease consistently with the increase in mother plant density (Figure 4). Since this phenomenon would affect negatively on maintaining tiller number for vegetative nursery production and sustainability of mother plants, it is essential to continue examining the potential of nursery production by these mother plants.

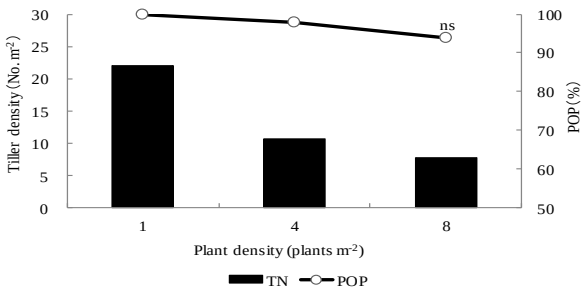

Figure 4. Percentage of overwintered plants (POP, ○) and regrown tiller density (■) on 17 May.

Effect of trimming on nursery plants

In Trial-2, emerged percentage of nursery plants was consistently 75% among 3 densities at 2 months after planting (Figure 5). Trimming on nursery plants affected to increase tiller number so as to promote quicker establishment of nursery plants in the field, even though plant height and dry matter weight of nursery plants decreased by the trimming practice (data not shown).

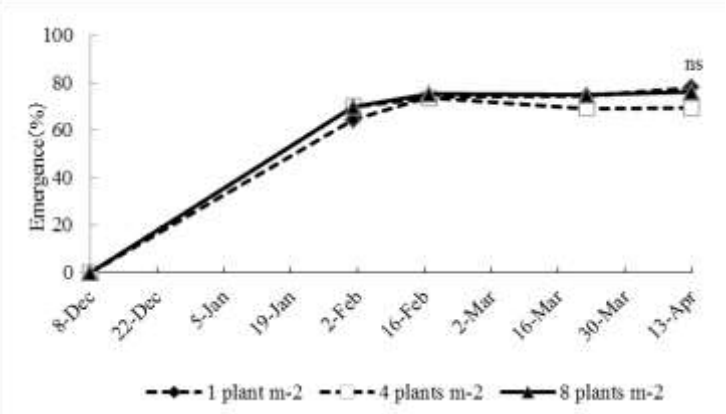

Figure 5. Changes in emerged percentage of nursery plants among three densities of mother plants during wintering period from early December 2010 to mid-May 2011.

In conclusion, 4 and 8 plants m^{-2} were suggested to be optimal for nursery production in the established year of DL napiergrass in southern Kyushu, Japan.

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Analysis of the Major Seed Storage Protein, 13S Globulin, in Common Buckwheat (*Fagopyrum esculentum* Moench)

Tomoyuki Katsube-Tanaka [a,*], Nadar Khan [a], Yusuke Takahashi [a], Mariko Nakagawa[a]

[a] *Graduate School of Agriculture, Kyoto University, Kitashirakawa, Kyoto 606-8502, Japan*
** Corresponding author: Graduate School of Agriculture, Kyoto University, Kitashirakawa, Kyoto 606-8502, Japan.*
Tel.: +81-75-7536043; fax: +81-75-7536065.
tanakato@kais.kyoto-u.ac.jp

Abstract

Common buckwheat seed proteins are valuable proteins with high nutritional and biological values. The proteins are also health-promoting proteins with many physiological functions, such as the ability to lower blood cholesterol and to prevent accumulation of fat, constipation, mammary carcinogenesis and colon carcinogenesis. However, the proteins are allergenic to human beings. The elimination or mitigation of the allergenic proteins is highly desired for the enhancement and potential utilization of buckwheat as a food crop. The prevalent allergen of common buckwheat, Fag e 1, is β polypeptide of the most abundant storage protein, 13S globulin. Because the 13S globulin is composed of multiple diversified subunits, α polypeptide, the counterpart of β polypeptide in 13S globulin subunits, was characterized in depth in this study to better understand this allergenic protein. The 13S globulin α polypeptides were categorized into three types and were further grouped into methionine-poor and methionine-rich subunits as major and minor types, respectively. Besides the three known methionine-poor subunits, four new methionine-poor subunits with 0, 2, 4, and 6 tandem repeat inserts were identified. Highly polymorphic band pattern and its correlation between SDS-PAGE and PCR analyses at a single seed level suggested that the large variation among the α polypeptides was explained by the different lengths of tandem repeat inserts. In agreement with the fact that the tandem repeat region is hydrophilic with many arginine residues, digestibility against trypsin, that is one of critical characteristics for food allergen, was different between the subunits with and without tandem repeat inserts.

*Keywords: 13S globulin, seed storage protein, common buckwheat (*Fagopyrum esculentum *Moench)*

Introduction

The allergens of buckwheat seeds have been identified and characterized by several research groups. Even though the allergens varied among patient sera. Park *et al.* (2000) revealed that the 9-, 16-, 19-, and 24-kDa proteins were the most prevalent allergens and that the 30-, 43-, and 67-kDa proteins were the least prevalent allergens. The 24-kDa protein (named Fag e 1), which is recognized as one of the most significant allergens by researchers, is the β polypeptide of the 13S globulin. The Fag e 1-null mutant has been sought, but it has not been discovered. However, the content of a Fag e 1 has been demonstrated to change among cultivars (Maruyama-Funatsuki *et al.*, 2004).

Buckwheat seeds contain 8.5% to 18.9% protein. The most abundant protein is the 13S globulin, which is salt-soluble and accounts for approximately 43% of the total seed protein. The 13S globulin is a storage protein and resembles the legumin-like seed storage protein of other species, such as rice glutelin and soybean glycinin. It is considered a member of the 11S globulin family based on its sedimentation constant, amino acid homology and similarities in biosynthetic and accumulation processes. Like other legumin-like, seed storage proteins (i.e., rice glutelin and soybean glycinin), the buckwheat 13S globulin is composed of multiple subunits, each of which contains acidic (α) and basic (β) polypeptides covalently linked by a disulfide bond.

The subunits of seed storage proteins show different characteristics from each other. However, the subunit composition of buckwheat has seldom been of concern, except in some studies by Cepkova & Dvoracek (2006), Rogl & Javornik (1996), and Bonafaccia *et al.* (1994). In fact, even the differences in the subunit structures and the maximum number of subunit types are not well known. To date, only four types of 13S globulin subunits have been identified.

In our current paper, we identified and characterized the α polypeptides of the buckwheat 13S globulin by SDS-PAGE and 2D-PAGE, coupled with immunodetection using two unique antibodies against the 13S globulin-related rice glutelin and soybean glycinin. Four new subunits with various lengths of tandem repeats were confirmed by PCR analysis at a highly polymorphic single seed level. By showing different digestibilities among the subunits, we proposed the possibility to develop novel buckwheat plants with lowered allergenicity.

Materials and Methods

Plant materials and preparation of the 13S globulin

Flour and seeds of common buckwheat cultivar 'Shinano-ichigo' were obtained from a local milling company in the Nagano prefecture, Japan and a local nursery company in the Shiga prefecture, Japan, respectively. Note that the commercial flour was made of uncounted number of ground seeds and was expected to show averaged and practical characteristics in terms of protein composition. Tartary buckwheat seeds of cultivar 'FT Rotundatiem' were a gift from the National Agricultural Research Center for Kyushu Okinawa Region. The protein fraction of the 13S globulin was extracted from either a single seed flour or commercial flour with the extraction buffer (0.035 M potassium phosphate, pH 7.6, and 0.4 M sodium chloride). The 13S globulin was either dissolved in the sample buffer containing 50 mM Tris, pH 6.8, 2% (w/v) SDS, 0.1% (w/v) bromophenol blue, 10% (w/v) glycerol, and 5% (v/v) 2-mercaptoethanol for SDS-PAGE analysis or dissolved in the lysis buffer containing 9.5 M urea, 2% Triton X-100, 5% 2-mercaptoethanol, and 5% Bio-Lyte 3-10 (Bio-Rad) for 2D-PAGE analysis.

SDS-PAGE, 2D-PAGE, and Western blot Analyses

SDS-PAGE was performed using 14%T acrylamide gel at a constant voltage of 200 V. Isoelectric Focusing (IEF) was performed with IPG strips pH range 4-7 (BioRad) with a 7.5-cm length according to the manufacturer's instruction. Electrophoresis in the first dimension (IEF) was performed at 200 V for 10 min, 400 V for 10 min, 1000 V for 10 min, and 1500 V for 12 hrs. The immunoblotting was performed according to the method mentioned in our previous report (Khan *et al.*, 2008). Anti-rice glutelin and anti-soybean glycinin antibodies (Katsube-Tanaka *et al.*, 2004; Katsube *et al.*, 1999) were used to detect the 13S globulin α polypeptides of common buckwheat.

Genomic DNA extraction and PCR Analysis

Genomic DNA extraction from the 'Shinano-ichigo' single seeds and PCR analysis were performed according to a kit (Ampdirect plus, Shimadzu, Japan). The primers used over the tandem repeat region around 433-663 bp from the 5'-end of the GenBank accession gene D87980 were as follows: forward (left1), AGGATG(C/T)CCGGAGAC(A/G)T(A/T)CCA and reverse (right1), CTAACGTTC(C/T)CATCGAGCTG for the Met-poor subunits.

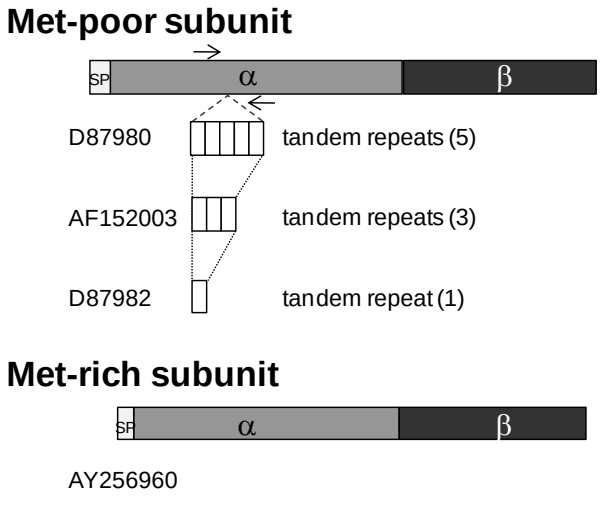
Trypsin digestion

Globulin fractions extracted from the 'Shinano-ichigo' buckwheat flour were incubated with 1/100 (w/w) trypsin (Promega, sequence grade) for two hours at 37°C according to the manufacturer's instruction. Aliquots taken at a given times were electrophoresed and compared by CBB staining and western blotting with the anti-glutelin antibody.

Results and Discussion

The 13S globulin fractions from commercial flour of common buckwheat and tartary buckwheat seeds were resolved by SDS-PAGE, and detected by immunodetection using anti-glycinin and anti-glutelin antibodies. The α polypeptides of common buckwheat cv. 'Shinano-ichigo' were separated into at least 9 major bands (a2-a10) and those of tartary buckwheat were into 7 major bands (ax, a1, a6-a10) (data not shown). The α polypeptides of common buckwheat were ranged from 30 to 47 kDa according to the mobility in SDS-PAGE.

For a detailed understanding in the variations of the 13S globulin α polypeptides, 13S globulin α polypeptides from 'Shinano-ichigo' commercial flour were analyzed by 2D-PAGE using anti-glycinin and anti-glutelin antibodies. The major 8 bands recorded on SDS-PAGE gels were resolved into several distinct spots with different pI values and with slightly different molecular weights on 2D-PAGE gels, forming a horizontal streak (data not shown). Each horizontal streak of spots was collectively named, instead of naming each spot individually in this study. When the spots were examined with the anti-glycinin and anti-glutelin antibodies, most spots reacted against either of two antibodies with different degrees except the spot a9.



	Mol%		kDa		pI	
	Met	Cys	α	β	α	β
D87980	0.35	1.06	41.3	21.1	5.2	9.1
AF152003	0.37	1.12	37.5	21.5	5.4	9.2
D87982	0.40	1.19	33.5	21.4	5.2	9.7
AY256960	3.53	1.55	28.5	20.2	5.0	6.7

Figure 1. Schematic representation of the 13S globulin subunits.

Primary structures of the known 13S globulin subunits (pro-form, α and β polypeptide with signal peptide) of common buckwheat were compared (GenBank accession No. D87980, AF152003, D87982, AY256960). The subunits are classified as Met-poor and Met-rich according to the methionine content of each subunit. The Met-poor subunits have tandem repeats with different lengths (shown as a number in brackets with the α polypeptide). Horizontal arrows denote the position for PCR primers.

A database search and the organization of all retrieved data showed the existence of three types of methionine scarce subunits (Met-poor subunits) and one methionine abounding subunit (Met-rich subunit) (Fig. 1). The three Met-poor subunits contain variable tandem repeat sequences. For example, GenBank accessions D87982, AF152003, and D87980 have one, three, and five tandem repeats respectively, whereas the Met-rich subunit of GenBank accession AY256960 has no tandem repeat sequence. Because the number of types observed in the SDS-PAGE of the α polypeptides were greater than the number of known subunits, PCR primers were designed at conserved positions (horizontal arrows, Fig. 1) over the region containing the tandem repeats of the Met-poor subunit to explore novel genes. The PCR amplification with the crude genomic DNA produced seven types of bands, which were named mp2-mp8 (data not shown). The DNA

sequences of the representative amplified bands (mp2-mp8) were determined and aligned with the Met-poor subunits of the GenBank accessions: D87980, AF152003, and D87982. The sequence analysis revealed that the clones for bands mp3, mp5, and mp7 correspond to D87980, AF152003, and D87982, respectively. Additionally, the clones for bands mp2, mp4, mp6, and mp8 were from novel genes with 6, 4, 2, and 0 tandem repeats, respectively.

When the PCR band patterns were compared with SDS-PAGE patterns examined using the same seeds with those for PCR analysis, the occurrence pattern of the band mp3 showed good correlation with that of the SDS-PAGE band a3 (data not shown). In addition, the mp2-mp8 bands and a2-a8 bands were observed at equally spaced intervals (data not shown). The above results led us to hypothesize that the a2-a8 bands are correlated with mp2-mp8, respectively. In other words, the bands a2, a3, a4, a5, a6, a7, and a8 have 6, 5, 4, 3, 2, 1, and no tandem repeats, respectively.

The amino acid sequences deduced from PCR amplified bands mp2-mp8 were compared with those of D87980, AF152003, and D87982 (Fig. 2). The alignment clearly showed the tandem repeat regions had less diversity, except the lengths of the bands. However, it is notable that the tandem repeat regions and their vicinities had many arginine residues, and the regions were hydrophilic according to a method used by Kyte & Doolittle (1982), indicating the regions are likely to be exposed at the molecular surface. This feature of the tandem repeat regions strongly suggests the regions are susceptible to trypsin.

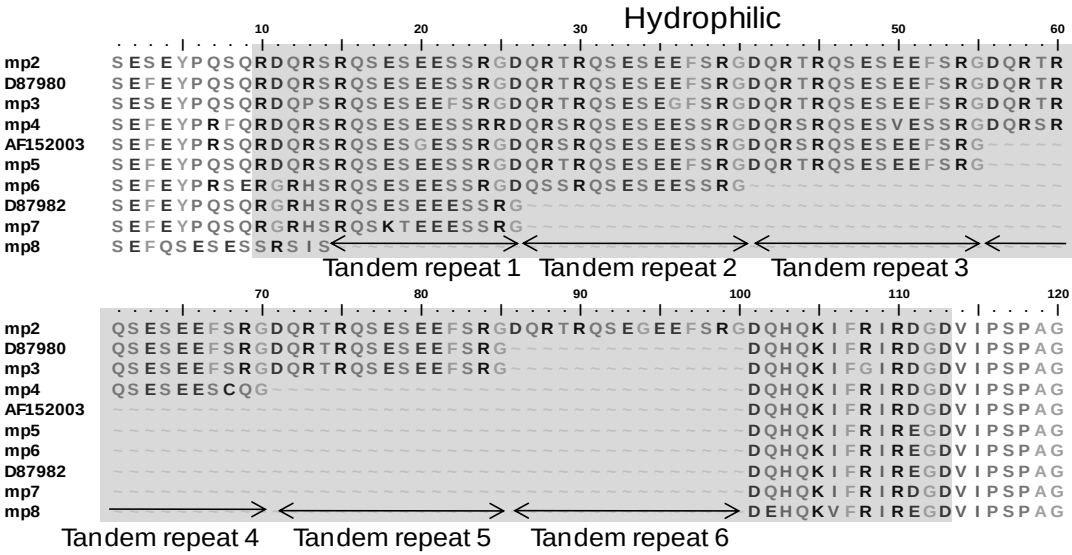


Figure 2. Deduced amino acid sequences and hydrophobicities over the tandem repeat inserts of the Met-poor subunits.

The amino acid sequences deduced from the PCR bands amplified for the Met-poor subunits (mp2-mp8) were compared with that of D87980, AF152003, and D87982. The amino acids spanning 108-212 residues from the N-terminus of the mature α polypeptides (D87980) were aligned. The position of tandem repeats 1-6 and approximate hydrophilic area were shown by double-headed arrows and gray color shades, respectively.

Trypsin digestions demonstrated that most of the 13S globulin α polypeptides degraded within two hours except for the a8 band, which is likely to have no tandem repeat (data not shown). The intensity of the a8 band detected by anti-glutelin stayed nearly constant during the reaction, and no new, α polypeptides were detected, suggesting the degradation occurred not only at a tandem repeat region but also with the entire α polypeptide sequence. Notably, the a9 band, which

seems to be derived from a Met-rich subunit having relatively higher cysteine contents, was rather resistant to digestion according to the CBB staining (data not shown).

It has been described that resistance to digestion and processing is one of the more important characteristics for food allergenic proteins (Bannon, 2004). Sen *et al.* (2002) showed peanut allergenic protein stability upon proteinase digestion affected IgE-binding epitopes intactness. Even though the difference in the digestibility of the β polypeptides was empirically not revealed in this study, the tandem repeat insertion into the α polypeptides might have influenced the β polypeptide conformation as well by a conserved inter-polypeptide disulfide bond with one of the involved Cys residues located 18-residues upstream from the tandem repeats. If the hypothesis is correct, the Met-poor subunits of the 13S globulin may have different digestibilities and distinct potential allergenicities. Although our preliminary analysis on pepsin digestibility demonstrated little difference among the subunits (data not shown), reducing the content of the trypsin-resistant subunit, the a8 α polypeptide with no tandem repeats, might be useful for the development of novel buckwheat with lowered allergenicity. Further investigation on IgE binding of buckwheat hypersensitive patients' sera should be required.

Acknowledgements

The authors thank Mr. Satoru Yamaguchi for his technical assistance. Germplasms of tartary buckwheat seeds were kindly provided by the National Agricultural Research Center for Kyushu Okinawa Region. This work was supported in part by grants to T.K.-T. from the Ministry of Education, Culture, Sports, Science, and Technology, Japan for Scientific Research (C) (20580013, 2008-2010; 23580020, 2011-2013) and by a fellowship to N. K. from the Japan Society for the Promotion of Science.

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Isolation of *soc-tuf* Gene Encoding Chloroplast Elongation Factor Tu (EF - Tu) Protein from Sugarcane (*Saccharum officinarum*)

Dhika Amanda[a], Sony Suhandono[b *]

School of Science and Technology, Institut Teknologi Bandung,
LABTEK XI-Genetic Laboratory, Ganeca 10, Bandung 40132, Indonesia.
Tel.: +62 22 251-1575; +62 22 250-0258; fax: +62 22 253-4107. amanda.dhika@alumni.itb.ac.id ;
**Corresponding author: sony@sith.itb.ac.id*

Abstract

Molecular chaperone is a molecule preventing the aggregation, denaturation, and inactivation of various proteins when the cells were exposed to extreme environmental conditions, such as high temperature or drought. One of such proteins is an Elongation factor Tu (EF - Tu). We have isolated an elongation factor Tu gene from sugarcane from cDNA which was heat-treated at 45°C for 16 h using reverse transcriptase PCR. The isolation was made in two steps. The first steps using internal primer developed using *tuf* gene sequence from maize available in the GenBank. Since partial cDNA sequence needs to be filled in, the sequence from sugarcane which is available in KEGG genome was added into a new primer designed to make overlapped PCR. This technique was used to clone the gene into bacterial expression vector pET-32b in order to test the *soc-tuf* gene function as molecular chaperone in bacterial system. The results showed that the gene had homology of 94% with maize *tuf* gene and 97% with sorghum amino acid sequence. The deduced amino acid sequence had homology of 92% with maize and 95% with sorghum, respectively. Gene analysis was conducted by generate phylogenetic molecular tree. Based on the tree, Sugarcane *tuf* sequence was actually clustered with *Sorghum bicolor* and *Zea mays* sequence. Thus, it had high possibility that *soc-tuf* gene from sugarcane having similar motif, protein structure, and can function as molecular chaperone as *tuf* gene from maize.

*Keywords: chloroplast protein elongation, environmental stress, heat tolerance, sugarcane (*Saccharum officinarum*)*

Introduction

Elongation factor Tu (EF - Tu) is a protein that involved in translational process. By binding to GTP and aminoacyl– tRNA, EF - Tu also translocate them into ribosome A-site (Brot, 1977; Riis, *et al.,* 1990). EF - Tu has three domains GTP-binding protein which also has similar characteristic with Ras proteins superfamily (Warren, *et al.,* 2001) belongs to the family of G proteins and is highly conserved in all organisms (Bhadula *et al.,* 2001).

EF – Tu in organelles (chloroplast and mitochondria) has the same function as EF - 1α in eukaryotic cytosol (Miller & Wissbach, 1977). Interestingly, the organellar EF – Tu shows more similarities to prokaryotic EF – Tu than to eukaryotic EF - 1α (Lee, *et al.,* 1999). Previous studies show that chloroplast EF – Tu has 80% similarity to prokaryotic EF - Tu and has 80 – 90% similarity to other EF – Tu from higher plants (Bhadula *et al.,* 2001; Riis, *et al.,* 1990). This is reflects their origin via ancient endosymbiosis (Gray, 1992). In most of lower photosynthetic eukaryotes, chloroplast elongation factor Tu (EF - Tu) is encoded by *tuf* gene in chloroplast genome (Baldauf & Palmer, 1990). While in higher plants, the genes encoding EF – Tu are located in the nucleus (Moriarty, *et al.,* 2002; Baldauf & Palmer, 1990) and after translation, the protein is transported into chloroplast.

In prokaryotes, EF - Tu is also involved in heat tolerance mechanism as molecular chaperones, that prevent the degradation of cellular proteins during heat – stress, increase the refolding of unfolded proteins, and increase the stability of protein (Moriarty, *et al.,* 2002; Momcilovic & Ristic, 2004; Rao, *et al.,* 2004 Ristic, *et al.,* 2008). During heat shock treatments, EF–Tu

molecules are accumulated in cytoplasm and near cytoplasmic membrane of *E. coli* (Caldas, *et al.,* 1998). Moreover, *E. coli* EF–Tu protein has ability to remain soluble and maintain its activity to 45°C (Caldas, *et al.,* 1998). It also binds other proteins and extends their stability up to 50°C (Caldas, *et al.,* 1998). Previous studies showed that plants also expressed chloroplast EF–Tu protein when it was exposed to high temperatures (Momcilovic & Ristic, 2004; 2007; Rao, *et al.,* 2004; Ristic, *et al.,* 2004; 2008). Exposure to a long term heat stress increased the level of chloroplast EF–Tu protein in mature wheat plants (Ristic *et al.,* 2008). The level of heat-induced EF–Tu protein in heat-tolerance wheat cultivars is greater than EF–Tu level in heat-sensitive cultivars (Ristic *et al.,* 2008). The same pattern was also observed in maize that heat sensitive maize line ZPL 389 did not accumulate EF-Tu under heat stress, while heat tolerant line ZPBL 1304 accumulate EF–Tu (Momcilovic & Ristic, 2004; 2007). Therefore, only plants, which show better tolerance to heat stress, express high amount of heat-induced EF–Tu protein. However, the evidence that chloroplast EF-Tu protein involved in heat tolerance, so far, reported only in maize (Bhadula *et al.,* 2001; Momcilovic & Ristic, 2004; Rao *et al.,* 2004; Ristic *et al.,* 2004).

In this study, EF-Tu from sugarcane (*Saccharum officinarum*) was used. Sugarcane is C4 plant commonly found in Southeast Asia region. Sugarcane has 3-4 times longer harvesting period than maize. It is assumed that sugarcane has been exposed to heat stress longer than maize and have higher temperature tolerance. The purposes of this research were to isolate *soc-tuf* gene encoding chloroplast elongation factor Tu (EF-Tu) from sugarcane and to investigate the gene similarities using phylogenetic tree. Therefore, this study was conducted based on the hypotheses, if *soc-tuf* gene is significantly homologous with *tuf* from maize or highly conserved, thus there is possibility that the protein may function to increase heat tolerance.

Materials and Methods

Samples preparation and treatment with high temperature

In this study, sugarcane plantlets with approximately of 4-6 cm of length were used. Before isolation, the plantlets were treated with different duration of incubation. Sugarcane plantlets were incubated at 45°C (Ristic, *et al.,* 2002) in incubator for 2, 4, 6, 14, and 16 h. The same treatment was also made to control plantlets exposed at 37ºC. Sugarcane from each treatment was taken and cleaned from the attached medium. Then, 600 mg of samples were used for RNA isolation total RNA from sugarcane was isolated by using Trizol® Solution Kit (Sigma, USA), followed by cDNA synthesis.

Primer design and tuf gene isolation from cDNA samples

There was no publication on sugarcane *tuf* gene so far, thus forward primer EF-TU-S for *tuf* gene amplification was designed by using maize tuf sequence. Meanwhile, maize reverse primer DO35690 (5'-TTACCACCCTCACGGATAGCAAACCTC-3') (Ristic, *et al*., 2004) was used to amplify sugarcane *tuf* gene. It was assumed that these primers will amplify about 1370 bp of sugarcane *tuf* gene. Amplification was generated using KAPA 2G™ Robust HotStart PCR system (KAPA Biosystems, USA) and sugarcane cDNA was used as a template.

Construction of full length tuf gene using overlap-extension PCR

Based on multiple sequence alignment, the amplified sugarcane *tuf* gene was not completed. To obtain full length sequence, sugarcane sequence was aligned with sugarcane EST from KEGG genome (http://www.genome.jp/kegg). Reverse primer was designed containing both sugarcane consensus sequence and expression vector sequence. While forward primer was designed containing both of *soc-tuf* partial sequence and expression vector sequence. Primers used to generate overlap-extension PCR were, forward primer EFTU-DA.F1 and reverse primer EFTU-DA.R1, respectively. Amplification was generated by using Phusion® Polymerase PCR

system (Finnzymes, USA) with touchdown PCR. Samples were visualized using DNA electrophoresis. Relevant DNA fragment was extracted from agarose gel and purified for further study.

Construction of tuf gene into expression vector pET-32b using PCR-cloning

In order to investigate the ability of sugarcane *tuf* gene, the gene was inserted into an expression vector pET-32b using PCR cloning (Bryksin & Matsumura, 2010). Instead of using two type of primer (forward and reverse primer), PCR cloning only used one DNA fragment derived from overlap–extension PCR product containing the gene of interest and two vector-homologous sequence at 3' and 5'- end of the sequence. This vector-homologous sequence acts as a primer and anneals to the plasmid vector to initiate the amplification.

Sequence analysis and construction of phylogenetic tree

Multiple sequence alignment was generated using CLUSTAL W in Bioedit and Geneious alignment in Geneious 4.8.5. Nucleotide and amino acid homology were analyzed using BLASTn and BLASTx from NCBI (http:// www.ncbi.nlm.nih.gov). Phylogenetic tree was constructed using MEGA 5.0 software.

Results and Discussion

Nucleotide sequence homology and similarity analysis

Based on BLASTn result (Table 1), sugarcane *tuf* sequence had 97% homology to *Sorghum bicolor* hypothetical protein sequence with 100% query coverage. *Sorghum bicolor* hypothetical protein has not been annotated yet. However, this protein has similar characteristics with EF-Tu proteins. In order to make the result more reliable, sugarcane *tuf* sequence was aligned with maize sequence and the result showed that sugarcane sequence had 94% similarity with maize *tuf* sequence encoding elongation factor Tu (EF–Tu) with 100% query coverage. E-value for these alignments are 0, thus the result was reliable.

Table 1. BLASTn result from sugarcane EF - Tu sequence using eucaryotes database

Accession Number	Species	Query Coverage	E-value	Homology
XM_002452345.1	*Sorghum bicolor* hypothetical protein, mRNA	100%	0	97%
NM_001156938.1	*Zea mays* elongation factor Tu (LOC100284040), mRNA >gb|EU966875.1| *Zea mays* clone 297889 elongation factor Tu mRNA, complete cds	100%	0	94%

In order to predict whether the nucleotide differences within sugarcane sequence has frame shift effect at amino acid sequence or not, sugarcane sequence must be analyzed by using BLASTx. Based on BLASTn result, sugarcane *tuf* sequence had 97% homology to *Sorghum bicolor* hypothetical protein sequence with 100% query coverage. The result (Table 2) showed that the differences within sugarcane nucleotide residues do not change amino acid sequence significantly. Sugarcane sequence still had 95% homology with *Sorghum bicolor* hypothetical protein and also 92% homology with *Zea mays* elongation factor Tu.

In order to investigate the homology between sugarcane and procaryotes *tuf* sequence, another BLASTx and BLASTn were conducted using procaryotes protein database. Based on BLASTn result, sugarcane *tuf* sequence had 67% homology to *Escherichia coli* (acc.

ZP_07125171.1) elongation factor Tu sequence with 90% query coverage. Moreover, The BLASTx result (Table 3) showed that sugarcane sequence still had 70% homology with *Thermus scotoductus* SA-01 and *Thermus Aquaticus* elongation factor Tu, also had 69% homology with *Thermus thermophilus* HB27 elongation factor Tu.

Table 2. BLASTx result from sugarcane EF - Tu sequence using eucaryotes database

Accession Number	Species	Query Coverage	E-value	Homology
XP_002452390.1	*Sorghum bicolor* hypothetical protein SORBIDRAFT_04g024850 >gb|EES05366.1|	99%	0	95%
NP_001149568.1	*Zea mays* LOC100283194 >gb|ACG35888.1| *Zea mays* elongation factor Tu	99%	0	92%

Table 3. BLASTx result from sugarcane EF - Tu sequence using procaryotes database

Accession Number	Species	Query Coverage	E-value	Homology
YP_004201365.1	Elongation factor Tu [*Thermus scotoductus* SA-01]	90%	3e-149	70%
1EFT_A	The crystal structure of elongation factor EF-Tu from *Thermus Aquaticus*	90%	3e-149	70%
YP_005299.1	Elongation factor Tu [*Thermus thermophilus* HB27]	90%	1e-148	69%

Based on BLAST results, it can be concluded that the sequence isolated from sugarcane was the *tuf* gene coding sequence. Characterization of sugarcane *tuf* gene was conducted by using various bioinformatics tools. FGENESH 2.6 from Softberry (www.softberry.com showed that *soc-tuf* cDNA sequence consisted of 1404 nucleotide residue which encodes 466 amino acid.

Construction of phylogenetic tree

Phylogenetic tree (Figure 1) of *tuf* sequences constructed based on sample sequence aligned with the various *tuf* sequences which were retrieved from GenBank and analyzed using Neighbor-Joining method with Kimura 2 parameter. The phylogenetic tree will confirm the BLASTn result. Tentative result of the BLASTn was used to collect other sequence related to the sample sequence, and construct the tree.

Sugarcane sequence is clustered with *Sorghum bicolor* with value 0.021 and *Zea mays* sequence with value 0.055 and also clustered at the same group as *Oryza sativa* with value 0.115 and *Triticum aestivum* with value 0.117 which belong to Poaceae family (the scale or branch length refers to the evolutionary distances used to infer the phylogenetic tree).

Based on sequence homology, several groups were formed with *Thermus thermophilus* as an outgroup. The homology of *tuf* sequence within plants (intra-clade) were higher than the inter-clade homology of *tuf* sequence. Thus, plant species formed a single cluster. While, bacterial species also formed a single cluster. Sugarcane sequence is actually clustered with *Sorghum bicolor* and *Zea mays* sequence at almost no nucleotide difference. It is also clustered at the same group as *Oryza sativa* and *Triticum aestivum* which are belong to Poaceae family. Due to *soc-tuf* gene has significant homology with maize *tuf*, thus there is high possibility that it also has similar motif, protein structure, and can function as molecular chaperone as *tuf* gene from maize in order to improve heat tolerance abillity in organisms.

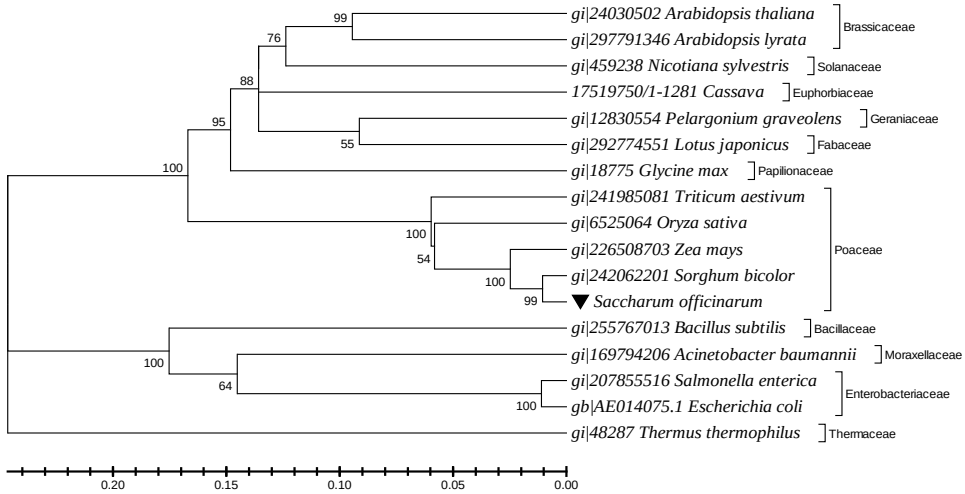

Figure 1. Neighbor-joining tree of *tuf* gene sequence using Kimura 2 parameter model with Gamma distribution.

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Endophyte Microbes from Oil Palm (*Elaeis guineensis*) Tissues and Its Potential as a Biocontrol for *Ganoderma boninense In Vitro*

Wisnu Adi Wicaksono, Rika Fithri Buana, Elizabeth Caroline Situmorang

Plant Production and Biotechnology Division,PT SMART Tbk, Bogor, Indonesia
**) Corresponding author: Plant Production and Biotechnology Division, PT SMART Tbk*
Jl. Raya Padjajaran No 78 FG, Bogor, Indonesia
Tel.: +6251 8310854; fax: + 6251 8310854
wisnu_adi@hotmail.com

Abstract

Endophyte microbes are microbes that colonize internal plant tissues without causing visible damage to their host plant and they can act as promising biological control agents in oil palm. The objective of this research is to obtain endophyte microbes which produce high chitinase activity and can inhibit *G. boninense* growth. Endophyte microbes are isolated from roots of oil palm planted in Padang Halaban estate, North Sumatera. Dual culture are performed in PDA medium using *G. boninense* from field. Chitinase assay was measured by spectrophotometry method. Potential isolates have been identified based on ITS rDNA using ITS1 and ITS4 primers for fungi and based on 16S rDNA using 9F and 1510R for bacteria. These endophyte fungi can inhibit *G. boninense* growth with inhibition ratio < 64%. T1 isolate has the highest activity of chitinase with 2.07 U/mL. There are four potential isolates of endophyte fungi: *Aspergillus, Fusarium, Hypocrea, and Trichoderma.* B2.1.2 isolate of bacteria has the highest inhibition ratio (67%) but B13.10.4 isolate has the highest chitinase activity with 3.43 U/mL. This result can be assumed that inhibition activity do not have any corelation with chitinase activity. The identification of potential isolates showed that they are *Serratia, Burkholderia, Acinetobacter*, and *Bacillus.*

Keywords: root-endophytes microbe, Ganoderma boninense, Elaeis guineensis, *chitinase activity, ITS rDNA, 16S rDNA*

Introduction

Basal stem rot (BSR) disease caused by *Ganoderma boninense* is the most destructive disease in oil palm industries (Flood *et al.* 2000). Mostly, visible disease symptoms of BSR would appear at a late stage. Development of BSR infection could happen at 6 until 12 months (Darmono, 1996). The infected oil palm would decay at basal tissue with the result that infected plant would fall down before the economical time. At the endemic location, plant could infected less than 2 years of planting period. Many strategies have been conducted to control BSR included cultural, chemical and clean clearing. Unfortunately, those strategies have not been successful, for example, the use of chemical fungicide in the field has not been proven as an effective control method (Sapak *et al*., 2008).

Biological control can be promising strategy to control BSR. One of microbes group which could use for biocontrol agent is endophyte microbes. Endophyte microbes is microbes that colonize internal plant tissues without causing visible damage to their host plant. Recent surveys of various host plants have demonstrated that endophyte microbes are ubiquitous in plant species (Shiomi *et al.*, 2006). As internal colonisers, therefore they are more able to compete within the vascular systems with capacity to prevent infection of *G. boninense*.

The objective of this study was to isolate the endophyte microbes for *G. boninense* biocontrol based on antagonistic characteristic and chitinase activity and also to identify endophyte microbes based on ribosomal DNA.

Materials and Methods

Root sampling and isolation of endophyte microbe

Oil palm roots were obtained from Padang Halaban Estate PT SMART, Tbk in North Sumatera, Indonesia. The age of palm was 28 years with symptomless BSR (Basal Stem Rot) at endemic areas. Random palms were sampled with the roots diameter 0.5 cm, taken about 1.0 m away from their bases at 25 – 30 cm depth.

Root samples were surface sterilized by dipping in 5.25 % sodium hypochlorite, and subsequently in 50, 70, and 90 % of ethanol then rinsed twice with sterilized water. Root section was transferred to Nutrient Agar (NA) for bacteria culture and Potato Dextrose Agar (PDA) for fungi. For bacteria were incubated at 37^{o}C and for fungi at 28^{o}C.

Microscopy observation of endophyte microbes

a. Bacteria Gram staining. One loop of bacteria was dropped onto a slide glass and air dried. The slide was stained with crystal violet for 1 min and then washed with alcohol, iodine for 1 min, and distilled water respectively. After that, the slide was added with safranin for one min and washed again with distilled water to remove any staining solution. The slide was examined under a bright-field microscope (Model Nikon Eclipse-50i, Japan).

b. Morphological and histological identification of fungal endophytes. Morphological identification of fungal isolates was according to colony or hyphal morphology of the fungal culture, surface and reverse colony color, and colony texture. Histological identification of fungal endophytes was carried out by observing the characteristics of the spores or conidia, and reproductive structures (sexual and asexual) under a bright-field microscope (Model Nikon Eclipse-50i, Japan).

In vitro screening of endophyte microbe against *G.boninense*

The endophytes microbe isolates were screened for characteristic to inhibit *G. boninese in vitro* by dual culture test and chitinolytic activity assay.

Dual culture test (Jinantana & Syariah 1997). A 5 mm diameter agar disc was taken from a week-old PDA culture of *G. boninense*. For bacteria, agar disc with *G. boninense* was placed in central PDA and 6 cm diameter filter paper with 24 hours old of endophyte bacteria was placed around of *G. boninense* isolate. For fungi, agar disc with *G. boninense* was placed 6 cm away from agar disc with fungi isolate. For control, agar disc *Ganoderma* were inoculated without endophyte microbes. All of the antagonistic were conducted triplicate and incubated in 28 ^{o}C. The ability of the endophyte microbes to inhibit the growth of *G. boninense* was determined after 7 days incubation by measuring the diameter of the *G. boninense* colony in control plate (R1) compare with the diameter of the *G. boninense* colony in plate with endophyte microbe (R2).

Chitinolytic activity assay. Bacteria and fungi isolates were inoculated to liquid chitin medium with 0.3 % chitin (w/v) and incubated at 37 ^{o}C with orbital shaking 150 rpm. The chitinolytic assay was measured after 1, 2, 3, 4, 5, 6, and 7 days after incubation using Spektrophotometer UV-Vis at 420 nm. One unit of chitinase was represented as nmol N-acetylglucosamine min^{-1} mL^{-1} protein. N-acetylglucosamine was used as a standard with variation concentration 0-200 ppm.

Identification of endophyte microbes

Isolation 16s rRNA gene for bacteria and ITS rRNA gene for fungi. Single colony of 24 hours bacteria from NA was inoculated to 5 mL Luria Broth (LB) and incubated for 18 hours at 37 ^{o}C with orbital shaking 150 rpm. The culture was centrifuged at 6000 rpm for 5 min. The pellet was used for the isolation of DNA using Wizard Genomic (Promega) by manufacture procedure. One of 5-day-old fungi colony from PDA was inoculated into 25 mL Potato Dextrose Broth (PDB) and incubated for 5 days at room temperature. The mycelium was used for the isolation DNA using Plant Genomic DNA (Sigma) following the manufacture's procedure. The 1500 bp of *16S rRNA*

gene for bacteria was amplified using 9F (GAG TTT GAT CCT GGC TCA G) and 1510R (GGT TAC CTT GTT ACG ACT T) primers and 600 bp of *ITS rRNA* gene for fungi was amplified using ITS 1 (TCC GTA GGT GAA CCT GCG G) and ITS 4 (TCC TCC GCT TAT TGA TAT GC) primers. Polymerase chain reaction was carried out in a thermal cycler (Applied Bio system Verity) in a total volume of 50 uL containing 2.5 mM MgCl2, 5uL of 10X Taq Buffer, 200 mM dNTPs, 50 pmoles of each forward and reverse primer, Dream *Taq* Polymerase (Fermentas), and 10 ng DNA template. PCR was performed by initial denaturation at 95 ^{o}C for 4 min, followed by 35 cycles of denaturation at 95 ^{o}C for 1 min, annealing at 55 ^{o}C for 1 min, extension at 72 ^{o}C for 1 min, with final extension at 72 ^{o}C for 10 min.

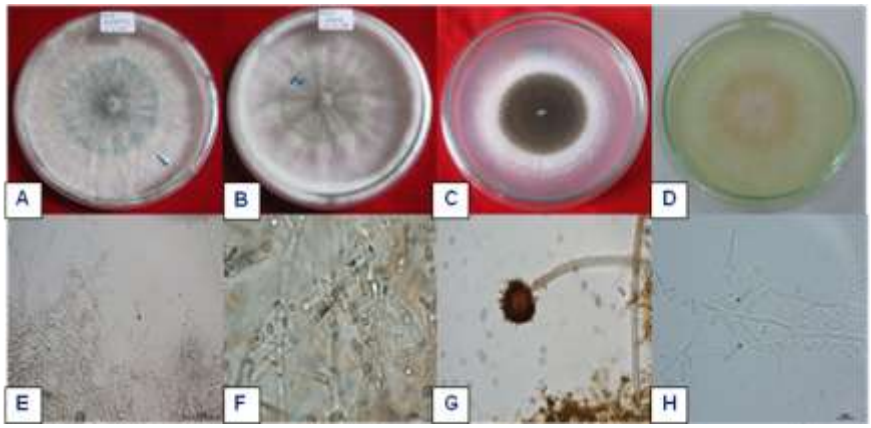
Cloning and transformation. *Escherichia coli* DH5α cells were transformed with PCR-amplified *16S rRNA* genes and *ITS rDNA* genes ligated in pGEM easy vector plasmid (Promega) using Fermentas DNA ligation kit (Fermentas). The plasmid was transformed to *E. coli* DH5α using heat shock treatment. The transformed cells (100 uL) were spread on LA plates containing X-Gal (50 ug/ml), IPTG (100 ug/ml) and ampicillin (50 ug/ml). The plates were incubated at 37^{o}C for 16 hours to screen blue and white colonies. Positive result was confirmed by PCR using M13F and M13R primers of white colonies.

Gene sequencing. The *16s rRNA* and *ITS rRNA* gene was sequenced to determine the homology with the known sequences in the NCBI database. DNA sequencing was done on FirstBase using ***M13*** forward ***sequencing primer*** (GTAAAACGACGGCCAGT).

Results and Discussion

Seventeen of endophyte bacterias were isolated from oil palm root with chitinolytic index ranged from 1.00 to 3.43 (data not showed). The ability of bacteria to form clear zones was measured qualitatively through chitinase activity of bacteria. The chitinase enzymes produced by bacteria are able to diffuse to the media. It can be seen from the presence of clear zones on the media. Most of the bacteria are Gram-negative (66%). According to Bell *et al*.,(1995), the abundance of Gram-negative endophytic bacteria in the oil palm roots was supported by population endophytes in other plant

Endophyte fungi of T1 and T13 isolates sp. are white color colony and scattered greenish patches become visible as the conidia are formed (Figure 1A). Microscopic of T1 and T13 isolates showed that the hyphae are septate and hyaline. Phialides are branched, flask-shaped, and attached to the conidiophores. Conidia have round or ellipsoidal form and have green color with 3 µm average diameter (Figure 1E). V2 and V3 isolates have white colony form in PDA initially, but typically become green (Figure 1B). Phialides are branched and tapered at tips. Spherical conidia gathered at tip of phialides in a tight, and ball-shaped (Figure 1F). A1 and A2 isolates have varies colony color from black to pale (Figure 1C). A1 and A2 isolate showed that hyphae are septate and hyaline. Conidiophores arise from the basal foot cell found at the supporting hyphae and terminate in a vesicle at the tip (Figure 1G). Mycelium morphology of F1 and F2 isolates are mostly white, but sometimes can be purple (Figure 1D). Some species also produce distinctly different conidia in the aerial mycelium (referred to as microconidia). Aerial mycelium is the growth of hyphae above the agar surface and often form a convex shape (Figure 1H).



(A,E: T1 and T3 isolates; B,F: V2 and V3 isolates; C,G: A1 and A2 isolates; D, H: F1 and F2 isolates)

Figure 1. Morphological and histological of fungal endophytes.

Antagonistic test was used to measure the ability of bacteria in inhibiting the growth of *Ganoderma in vitro*. Inhibition ratio of bacteria and fungi against *G. boninese* has different ranges. For bacteria, B2.1.2 isolate has the highest inhibition ratio (67%), while B24.1.7 and B28.5.3 isolate has the lowest inhibition ratio (26 %) and V3 isolate has the highest inhibition ratio (64 %) while the F2 has the lowest inhibition ratio (33 %) (Table 1). There are two possible mechanisms to inhibit growth of *G. boninense*, both are antifungal compound and chitinase activity. The chitinase could lysis cell wall of *G. boninense*. In addition, bacteria and fungi also capable to produce secondary metabolites, these can inhibit the growth of pathogenic fungi. According to Sidduqque *et al*., (2009), *Trichoderma* can produce volatile compounds with marked gas formation and capable to inhibit the growth of *G. boninense* up to 70% by dual culture method . A compound produced by *Trichoderma* is known as 6 - pentyl-alpha-pyrone (6PAP) which is a secondary metabolites compound (Coney *et al*. 1997).

Table 1 Antagonistic potential of endophyte microbe in dual culture test

Microbe isolate	Inhibition ratio (%)
a. Bacteria	
B2.1.2	67 a
B93.22.83	37 b
B28.5.3	26 d
B50.1.3	34 c
B24.1.7	26 d
B13.10.4	34 c
b. Fungi	
T1	58 b
T13	61 ab
V2	63 a
V3	64 a
A1	45 c
A1	37 d
F1	38 cd
F2	33 d

Means in the same column with different alphabet(s) are significantly different ($p < 0.05$) according to Duncan test.

All microbe isolates could produce chitinase enzyme activity although with different pattern (Table 2). B13.10.4 isolate of bacteria has the highest chitinase activity (3.43 U/mL at 5 days of incubation).Whilst, the highest enzyme activity of chitinase in fungi was T1 isolate (2.07 U /mL at 7 days of incubation). Although B2.1.2 and V3 isolates have the highest inhibition ratio, they did not produce enzyme with highest activity. According to Aktuganov (2003), chitinase activity was not related to antifungal activity. The bacteria with high antagonist activity is not always have a high chitinase activity.

Synergism between the antifungal compounds and enzymes of bacteria can enhance the role of bacteria as bio-control pathogen (Sheri *et al*., 2002). Some studies suggest that the enzyme chitinase production of the genus *Trichoderma* spp. is more effective than chitinase enzyme produced by other organisms, to inhibit a various plant pathogenic fungi (Lorito *et al*., 1994). Several studies are also reported that *Trichoderma* was capable in producing chitinase enzymes that play a role in several fungal pathogens including *Sclerotium rolfsii* and *Rhizoctonia solani*, (Haran *et al*., 1996; Harman *et al*., 1993).

Table 2 Chitinase activity enzyme from chitinolytic microbe

Microbe isolate	Enzyme activity (U/mL)	Time incubation (days)
a. Bacteria		
B2.1.2	1.54 c	5
B93.22.83	1.51 c	6
B28.5.3	1.44 cd	7
B50.1.3	1.28 d	5
B24.1.7	2.08 b	2
B13.10.4	3.43 a	5
b. Fungi		
T1	2.07 a	7
T13	1.75 b	6
V2	1.51 c	2
V3	1.16 de	2
A1	0.99 e	6
A2	0.93 e	6
F1	1.56 c	6
F2	1.30 d	6

Means in the same column with different alphabet(s) are significantly different ($p < 0.05$) according to Duncan test.

Table 3 Identification of selected microbe based on ribosomal DNA

Isolate	Identity	Homology (%)
B2.1.2	*Burkholderia sp*	99
B93.22.83	*Serratia sp*	99
B24.1.7	*Acinetobacter sp*	99
B13.10.4	*Bacillus cereus*	99
T1	*Trichoderma asperellum*	99
V3	*Hyphocrea virens*	99
A1	*Aspergillus sp*	99
F1	*Fusarium oxysporum*	99

The fungi and bacteria were identified base on their ribosomal DNA. Identification of fungi showed that there are *Trichoderma, Hypocrea, Aspergillus*, and *Fusarium* (Table 3). These four fungi are known to be a natural bio-control of fungal pathogens (Krupke *et a*l. 2003; Rubini *et al*., 2005; Adriana & Sergio 2001). Identification of potential isolates showed that they are *Serratia,*

Burkholderia, Acinetobacter, and *Bacillus* (Table 3). These results proved that several bacteria and fungi are potential as bio-fungicide because of their high inhibition ratio and activity of chitinase.

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Chitinase Activities of Oil Palm Root at Early Infection of Arbuscular Mycorrhizal Fungi

Happy Widiastuti

Indonesian Biotechnology Research Institute for Estate Crops
Jl Taman Kencana No. 1 Bogor, Indonesia 16151
Corresponding author: happywidiastuti@yahoo.com

Abstract

The activity of chitinase of oil palm root at the early of arbuscular mycorrhizal fungi (AMF) colonisation was studied. The results showed that early in colonisation (0-5 weeks), chitinase activity in roots increased nevertheless eventually suppressing occurs 5-8 weeks after inoculation. Inhibition of chitinase activity is influenced by AMF species and fertilization. Suppression of endochitinase activity of effective species is higher than the species that are less effective, especially in the fertilization treatment. The activity of exochitinase was depressed in the presence of AMF colonisation. In the treatment without fertilizer, suppression of exochitinase activity of inoculated roots with *G. margarita* is higher than those inoculated with *A. tuberculata*. Inhibition of chitinase activities on secondary roots that occurs at the inoculation of *G. margarita* is higher than those inoculated with *A. tuberculata* and vice versa in the primary root.

Keywords: chitinase activities, palm oil, early colonisation, arbuscular mycorrhizal fungi

Introduction

Arbuscular mycorrhizal fungi (AMF) is a fungus symbioses with the oil palm. Biochemical processes involved in plant symbiosis with fungi has been studied. Differences in proteins both quantitatively and qualitatively has been shown between AMF inoculated and uninoculated plants. AMF colonization seems to be in place for increasing the size of low protein expression as shown in tobacco (Dumas *et al*, 1996). The emphasis is more consistent in the chitinase activity which is also have a role in defense plants in the early stages of AMF colonisation. Penetration of AMF in root involves a series of changes in morphology and physiology in plants and fungi. However, as occurs in plant-pathogen interaction mechanisms of induction and suppression associated with plant defense is the key to the colonization of AMF and its compatibility with the host plant (Garcia-Garrido & Ocampo, 2002).

The effectiveness of plant defense responses associated with the speed of the process of introduction of specific signaling molecules called elisitor. Elisitor can be secreted by the microbes that infect or as a result of breakdown of plant cell walls. Regulatory mechanisms of defense responses through degradation of the molecule can be issued elisitor AMF for example by hydrolysis enzymes such as chitinase, glucanase B 1.3, kitosanase. Lambais and Mehdy (1996) observed a suppression of activity endochitinase up to nearly twice in the AMF inoculation treatment and the level of infection-suppressing this enzyme was higher in the effective strain AMF compared to strains that are less effective. This research aim was to study plant defense responses during early colonisation, especially AMF chitinase activity.

Materials and Methods

Planting materials used were germinated oil palm from IOPRI Medan. Germinated oil palm was grown in sterile sand until the age of 3 months. AMF inoculation on the palm was made by the

method of pre-nursery i.e by developing AMF colonization in *P phaseoloides* prior to colonisation to oil palm seedling. *P. phaseoloides* grown in polybags 50 x 50 cm in sized without holes containing 10 kg of sterilized Cikopomayak acid soils. Inoculum dose and optimum dose of fertilizer was based on the optimization experiment (Widiastuti *et al*, 2002). Plants were maintained in a greenhouse by watering with cooling boiled water. Harvesting was done at weeks 0, 3, 5, 8, and 11 after inoculation by washing the roots with water taps. Observations were conducted on fresh and dry weight of seedlings, chitinase activitieas (Lambais & Mehdy, 1996), and the percentage of AMF colonisation. Chitinase activity was determined by counting the hydrolyzed N acetyl glucosamine using BSA as protein standard. The two factors tested were AMF species (without AMF, *A. tuberculata*, and *G. margarita*) and fertilizing (without and with fertilizer). The inorganic fertilizer dose of control was based on Lubis (1992). Experimental design used was completely randomized group design with factorial pattern.

Results and Discussion

AM fungi colonisation in oil palm root

Chemical analysis showed that the soil was very acid (pH H_2O 3.8), the content of C, N, P_2O_5, K_2O, CaO, MgO were very low at 1.59; 0.097; 0.013; 0.01; 0.066; 0.054% and 16.23 Aldd me 100 g^{-1} respectively. Colonisation of AMF began weeks after inoculation in both the primary and secondary roots. However, colonisation in secondary roots was higher than those in primary roots. Moreover, colonisation of AM fungi in no fertilization treatment was higher than those both *A. tuberculata* and *G. margarita* (Fig 1).

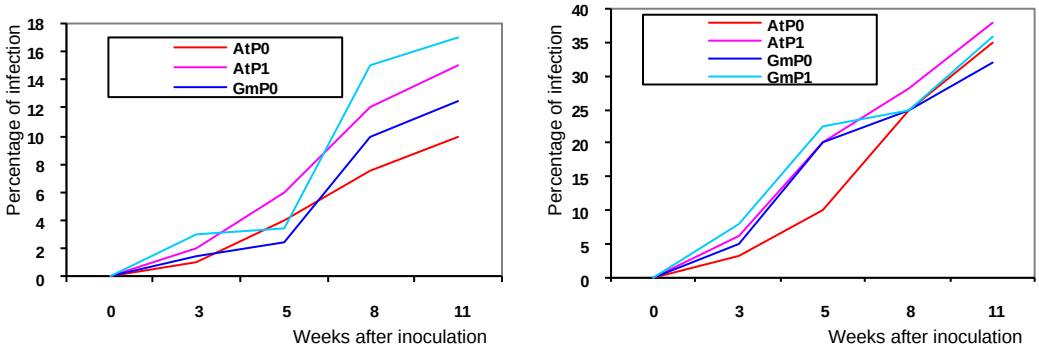

Figure 1. Percentage of AMF colonisation in primary (left) and secondary (right) oil palm roots.

Chitinase activity in the primary root

The analysis showed that in the primary roots which were not inoculated with AMF, the activity of exochitinase was higher compared to those of the inoculated one. Exochitinase activity increased until the fifth week after inoculation and subsequent declined at week eight and very low at week 11. These results indicated that the exochitinase activity was temporary and there is emphasis of exochitinase activities particularly began 8 weeks after AMF inoculation. The effect of fertilization showed a trend that in palm roots without AMF inoculation and inoculated with *G. margarita*, fertilization causes the higher activity of exochitinase compared to those not fertilized. However, these results differed with exochitinase activity of palm roots inoculated with *A. tuberculata.* The exochitinase activities of the root that are not fertilized, is higher compare to those fertilized. Emphasis of the exochitinase activity in the inoculated roots of palm inoculated with *A. tuberculata* both at without fertilization and fertilized was higher than the activity exochitinase palm roots inoculated with *G. margarita*.These results indicate that in the primary root, the activity of exochitinase influenced by AMF species and the presence of fertilization.

Endochitinase activity was lower in primary roots compared to exochitinase activity. Endochitinase activity in primary roots that not inoculated with AMF was higher compared to those inoculated. Unlike to the exochitinase activity, the peak of endochitinase acitivity occurred at week 5 and declined until week 8. Activity of endochitinase of oil palm root unfertilized inoculated with *A. tuberculata* is higher than those of inoculated with *G. margarita*. This result was in line with exochitinase activity (Fig. 2). With the application of fertilizer, the activity endochitinase of primary palm root inoculated with *G. margarita* was higher than those of the inoculated with *A. tuberculata*. These results indicated that the dose of fertilizer and type of AMF affect endochitinase activity on secondary roots.

The data of chitinase activity in general, showed that in the primary root chitinase activity of oil palm uninoculated with AMF was higher than those of inoculated with AMF. This trend happened in the oil palm root inoculated with *G. margarita,* but the opposite occurred in oil palm roots inoculated with *A. tuberculata*.

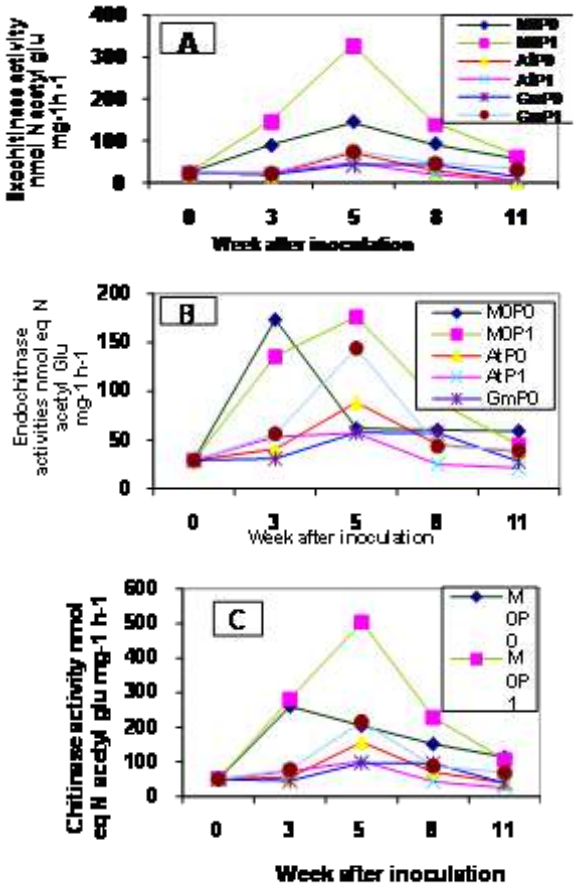

Figure 2. Exochitinase activity (A), endochitinase (B), and total chitinase (C) of primary oil palm roots in response to treatment.

Chitinase activity in the secondary roots

Exochitinase activity on the secondary roots of palm oil was much higher compared with exochitinase activity in the primary root. This difference reached 10 times. Endochitinase activity in oil palm roots without AMF inoculation was higher than those of inoculated with AMF. In the secondary roots inoculated with *A. tuberculata*, endochitinase activity without fertilization was higher than those fertilized root. These results are similar to those in primary roots. But for oil palm roots

inoculated with *G. margarita*, there was no difference of exochitinase activity between a fertilized and unfertilized. In addition, it was shown that in fertilization oil palm root, the emphasis of chitinase activity was slower compared to those of without fertilized.

Activity of endochitinase of secondary roots of palm was lower than exochitinase activity on the same root. In addition, endochitinase activities of palm root that were not inoculated was higher compared to those inoculated with the AMF. In secondary oil palm roots inoculated with *A. tuberculata* but unfertilized, endochitinase had a higher activity compared with those of fertilized, but in contrary to the roots of oil palm inoculated with *G. margarita*. These results were similar to those observed in primary roots. At the root of the fertilized palm, the activity of the endochitinase the activity of root inoculated with *G. margarita* was higher compared to those inoculated with *A. tuberculata,* whereas the opposite occured in the treatment without fertilization. These results were in line with that occured in the primary root. The high of endochitinase activity in roots inoculated with *A. tuberculata* followed by a higher emphasis anyway.

General description for chitinase activity in the secondary roots of palm oil showed that the activity of chitinase in the palm oil root that were not inoculated with AMF was higher than that inoculated with AMF (Fig 3.). In oil palm inoculated with *A. tuberculata*, fertilization actually decreased the activity of chitinase in secondary roots. While palm oil inoculated with *G. margarita*, there was no difference between a fertilized and unfertilized.

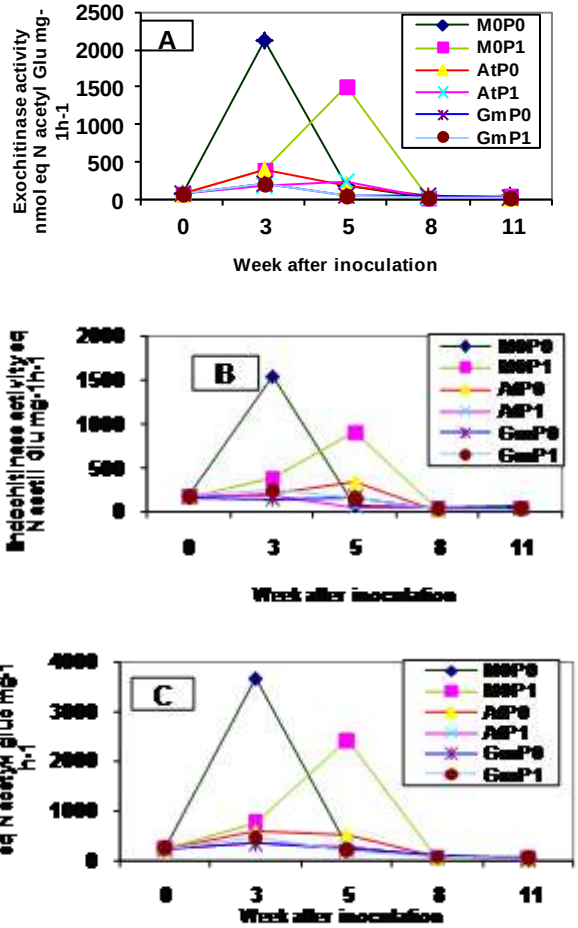

Figure 3. Activity of exochitinase (A), endochitinase (B), and total chitinase (C) of secondary oil palm roots in response to treatment.

Possible differences in plant response to AMF colonisation in particular is evident from the activity of chitinase can be a bookmark for the selection of the effectiveness of AMF. In the present study demonstrated that inoculation suppressed the activity of chitinase. Suppression level was influenced by the AMF species and doseage of fertilizer. Emphasis on treatment without fertilization was highest in week 3 after inoculation, while the emphasis of chitinase activity on fertilization treatment was observed 5 weeks after inoculation. Fertilization appears to slow the emphasis of chitinase activity.

The same is true of the exochitinase activity are fertilized palm that inoculated with *G. margarita*. Despite this emphasis on endochitinase activity of *G. margarita* inoculation lower compared to those inoculated with *A. tuberculata*. Total chitinase activity in the secondary roots either by fertilization or without fertilization showed that the inoculation of *G. margarita* more suppresse than thoses the inoculation with *A. tuberculata*.

Changes in isoenzyme patterns and biochemical properties of several enzymes associated with plant defense as chitinase has been shown for tomato root colonization by AMF (Pozo *et al*., 1996) with the induction of new isoforms. These hydrolytic enzymes act as a defense against pathogen attack because of its potential hydrolyze fungal cell wall polysaccharides (Pozo *et al*., 1996). Induction of this enzyme activity in AMF symbiosis may also be involved in the protection of plants against pathogenic fungi (Dumas-Gaudot *et al*., 1996).

The present study indicated that there was an emphasis on the chitinase activity which was influenced by inoculation with AMF, AMF species, and fertilization. Fertilization slow emphasis chitinase activity. In addition, the data showed generally lower total chitinase activity in the presence of fertilization compared with those not fertilized, especially in the inoculation with *A. tuberculata*. While at the inoculation with *G. margarita* fertilization increased the chitinase activity. The same thing also happened in controls. The results in this study seemed in line with Blee & Anderson (1996) suggested that P can regulate plant defense responses. The results suggested that fertilization decreased 1.3 glucanase (occurs with β mRNA). The delay of suppression of chitinase activity supposed to be caused by the slow of AMF colonisation in the presence of fertilization.

Emphasis on fertilization treatment to endochitinase activity in roots inoculated with *A. tuberculata* is higher than those on inoculated roots of *G. margarita* and vice versa in the treatment without fertilizer. In a previous study demonstrated that *A. tuberculata* is more effective in improving growth and P uptake of oil palm seedlings in comparison with *G. margarita* (Widiastuti *et al*, 2002). In addition Widiastuti *et al* (2005) showed that the optimum dose of inorganic fertilizer of oil palm inoculated with *G. margarita* was higer compared to those inoculatred with *A. tuberculata*. These results suggest that suppression of effective species to endochitinase activity is higher than those to species that are less effective, especially in the fertilization treatment. Similar to Lambais & Mehdy (1996) the effective strain is more pressing AMF endochitinase activity compared to those that are less effective. However this only occurs at an optimal symbiosis is the application of fertilizer, while the treatment without fertilization occurs the opposite of the less effective strain is more pressing endochitinase activity compared to effective strain.

In this study exochitinase depressed activity in the presence of AMF colonisation, however, there were differences in emphasis between the root level of the inoculated *A. tuberculata* and *G. margarita*. In the treatment without fertilizer, suppression activity of inoculated roots exochitinase of *G. margarita* was higher than *A. tuberculata*. While the result of fertilizer there was a difference in primary and secondary roots. The emphasis on secondary roots that occurs at the inoculation *G. margarita* was higher than *A. tuberculata* and vice versa in the primary root. These results indicate that in the less effective strain, the emphasis on exochitinase activity higher compared to the effective strains, especially on secondary roots. These results were different from those expressed Lambais and Mehdy (1996) who reported increased activity exochitinase up to 3 times the effective strain AMF. In this study exochitinase suppression activities occured on the effective strain is lower than those that are less effective. The results of this study indicated that

plants respond differently to AMF colonisation compared to pathogen infection. The mycorrhizal plant has a defense mechanism that temporally and very weak in contrast to pathogen.

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In vitro Test of Rhizosphere Chitinolytic Bacteria as a Biocontrol for *Ganoderma boninense*

Rika Fithri Buana[*], Wisnu Adi Wicaksono, Elizabeth C. Situmorang

Plant Production and Biotechnology Division, PT SMART Tbk, Bogor, Indonesia
** Corresponding author: Plant Production and Biotechnology Division, PT SMART tbk*
Jl. Padjajaran Raya No. 78F-G, Bogor, Indonesia
Tel.: +62 251 831 0854; fax: +62 251 831 0854.
rika.fithri@gmail.com

Abstract

G. boninense is a fungal pathogen that causes basal stem rot in oil palm (*Elaeis guineensis*). Chitinolytic bacteria are abundant in soil and well known as a natural biocontrol of fungal pathogen. The objective of this research is to obtain rhizosphere chitinolytic bacteria as a candidate of biocontrol agent for *G. boninense*. Nineteen indigenous bacteria from Padang Halaban Estate, North Sumatera, were tested for their antagonistic properties against *G. boninense* by *in vitro* dual culture on PDA plates. Their chitinolytic activities were tested using colloidal chitin as the substrate. Chitinase activity was determined colorimetrically by detecting the amount of N-acetylglucosamine released from colloidal chitin substrate. Crude enzyme secreted by the bacteria were characterized by SDS-PAGE with chitinase from *Trichoderma* as a control. Six of the isolates have an inhibition activity against *G. boninense* growth with B3.4 showed the highest percentage inhibition ratio growth or PIRG (58.75%). Inhibition of *G. boninense* growth *in vitro* might due to a competition for nutrients or other antifungal compound from certain bacteria which diffuses in the agar. B3.2 showed the highest activity of chitinase (10.44 U/mL), but B3.4 has lower activity of chitinase (7.08 U/mL). It can be assumed from the result that PIRG does not have correlation with the activity of chitinase. The results of enzyme characterization showed that B3.3, B3.2 and B3.4 has a molecular mass of 37kDA, which is a similar molecular mass with chitinase from *Trichoderma viridae*

Keywords: rhizosphere bacteria, Ganoderma boninense*,* Elaeis guineensis*, chitinase*

Introduction

Basal stem rot disease (BSR) caused by *Ganoderma boninense* is currently a major disease in oil palm plantations (Darmono, 1998). The disease can destroy up to 80% of the stand palms by the time when the palms are halfway through their normal economic life span (Idris, 2003). *Ganoderma boninense* is a saprophytic fungus activated by favorable conditions to behave parasitically (Statmets, 2004). Like all fungi, it has no chlorophyll and thus, lack photosynthetic capability. Instead of manufacturing their own food, fungi absorb nutrients from either living or dead host tissue (Haniff, 2005). the fungus typically attack already weakened oil palm as *Ganoderma* seldom seriously infects undamaged trees (Paterson, 2007). The effects of *Ganoderma* infection on productivity decline in palm crops have been concerned since replanting of oil palm land was began in South-East Asia, especially in Malaysia and Indonesia (Turner, 2003).

BSR has been an endemic disease found in PT. SMART Tbk. oil palm plantation especially in Padang Halaban, North Sumatera, Indonesia. Biological control of pathogenic fungi provides an attractive and alternative management for fungal diseases without the negative impact of synthetic antifungal agents that can cause environmental pollution and may induce pathogen resistance (Haas & Défago, 2005). Plant disease control by chitinolytic bacteria has long been reported (Sneh, 1981). Several strains of bacteria, such as *Aeromonas caviae*, *Bacillus sp*., *Serratia plymuthica*, and *Enterobacter agglomerans* are well-known chitinolytic bacteria (Das *et al*., 2010). Some *Trichoderma* sp. have been described as biological control agents against fungal pathogens and widely used as biocontrol because of its mycoparasitic activity (De La Cruz, 1992). Chitinolytic

enzymes have been considered as an important factor to control soilborne pathogens because of their ability to degrade fungal cell walls, of which a major component is chitin (Chet, 1987).

Chitin is widely available in the soil derived from the decay and degradation of dead cells, so that some bacteria that live on the ground are good in degrading chitin (Suryanto *et al*., 2005). The aim of this study was to isolate chitinolytic bacteria from rhizosphere soils in Padang Halaban estate and to screen their antagonistic activity against *G. boninense*.

Materials and Methods

Isolation of bacteria

Rhizospheric soil were collected near the root of the uninfected plant in endemic site of *G. boninense*. Suspension were made by adding 10 g of soil to 100 mL sterile basic salt solution (0.85% NaCl). Ten fold serial dilutions of these suspensions were made and plated on Nutrient Agar (NA). The colony were selected and purified with 4-way streak method on NA.

Dual-culture technique

Dual culture were made to obtain the percentage inhibition of radial growth (PIRG) of *G. boninense* (Bivi, 2010). Potato Dextrose Agar (PDA) was poured onto 9 cm diameter Petri dishes. Isolated bacteria was streaked into the PDA plate 2.5 cm from the edge of Petri dish. A 5 mm agar disc cut from the side of an actively growing pure culture of 5-day old *G. boninense* placed 2.5 cm from the edge at the opposite side of the same Petri dish. For the control plate, only *G. boninense* was placed in a similar manner without bacteria on a fresh Petri dish. The experiment was made in three replication each bacteria. The plates were incubated at 28 ^{o}C for five days. Results revealed as mean colony growth of the causal pathogen in the presence of the bacteria and its growth on the control plate (without the bacteria). The outcome of two readings was calculated into the formula for the PIRG as below :

$$\% \text{ PIRG} = \frac{R1 - R2}{R1} \times 100$$

Where, PIRG = percentage inhibition of radial growth; R1 = radial growth of *G. boninense* in the absence of bacteria (control); R2 = radial growth of *G. boninense* in the presence of bacteria.

Chitinase assay

Chitinase activity was determined colorimetrically by detecting the amount of *N*-acetylglucosamine (GlcNAc) released from a colloidal chitin substrate (Reissig, 1955). Selected isolates were inoculated into 100 ml of 10% chitin medium and incubated at 37 ^{o}C on rotary shaker 120 rpm for 5 days. Flask were removed every 24 hours and pipetted 10 mL from each mixture were filtered through filter paper. The filtrate as a crude enzyme were pipetted 1.5 ml into 0.75 ml phosphate buffer and 1.5 ml of 0.3% coloidal chitin in 15 ml Corning centrifuge tube. The reaction mixture were incubated in 37 ^{o}C for 30 min, and centrifuge the mixture to separate the product from the remaining substrate. Pipetted 2.5 ml supernatant into 5 ml Schales reagent and 2.5 ml distilled water. The mixture was boiled for 10 min to stop the reaction of the remain enzyme. The reaction was measured with spectrophotometer at 420 nm. *N*-acetylglucosamine was used as a standard. One unit of chitinase acivity was defined as the amount of enzyme that released 1 µmol GlcNAc or its equivalent from colloidal chitin in 1 min (Zilda, 2006).

Protein characterization

Crude enzyme secreted by the bacteria were characterized by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) with 12 % separator gel and 4% stacking gel. Crude supernatants protein were mixed with 2x sample buffer (1:1), boiled for 4 min, then load. The

gel were stained with 0,1% Coomassie brilliant blue R-250, and destained with 10% acetic acid and 20% methanol.

Results and Discussion

There are 19 indigenous bacteria isolated from rhizosphere soil in Padang Halaban. Screening of biocontrol bacteria was carried out by dual culture. It showed that six of them have an inhibitory activity against *G. boninense* growth (Table.1). B3.4 showed the highest PIRG of *G. boninense* amongs other isolates, it showed 58.75% and the B3.3 is the lowest (12.20%). The inhibition of *G. boninense* growth might due to the presence of chitinase or other anti-fungal properties produced by the certain bacteria. Many publications have reported that soil bacterias are capable in producing anti-fungal metabolites properties that inhibited mycelia growth of phytopathogen fungi (Fernando & Linderman,1994; Sisler, 1969; Yiu-Kwok *et al*., 2003). Many of the anti-fungal properties affect the physiological activities of pathogenic fungi (Vesperman, 2007).

Table 1. Antagonistic potential of soil bacteria in dual culture test

Bacterial isolates	PIRG (%)
B3.3	12.20 d
B14.1	35.56 b
B3.1.1	22.22 c
B3.4	58.75 a
B11.2	31.11 b
B3.2	16.67 d

Mean in the same coloumn with different alphabet(s) are significantly different (P<0.05) according to DMRT

Bacteria B3.2 showed the highest activity of chitinase (10.44 U/mL) and the lowest was B14.1 (7.08 U/mL) on day four of incubation. The result revealed that PIRG has no correlation with chitinase activity. Bacteria with the highest PIRG (B3.4) showed less chitinase activity than bacteria with lowest PIRG (B3.3). This result is in accordance to Aktuganov (2003) statement that there is no correlation between PIRG and the ability of bacteria in producing chitinase. It can be assumed that other anti-fungal property was responsible for the inhibitory effect in dual-culture of B14.1.

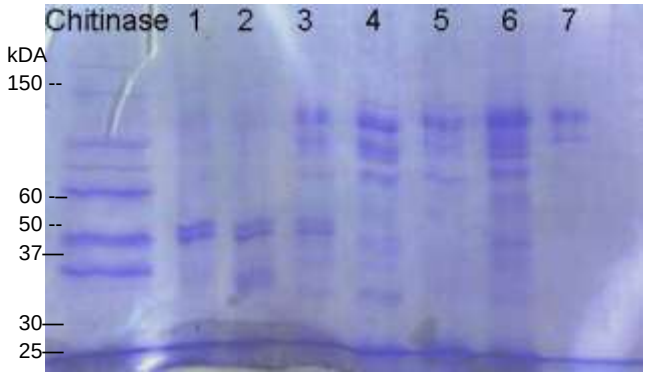
Table 2. Chitinase activity enzyme from isolated bacteria

Bacterial isolates	Enzyme activity (U/mL)	Incubation time (Days)
B3.2	10.44 a	4
B3.3	8.43 b	4
B3.4	9.94 a	4
B14.1	7.08 c	4
B3.1.1	8.37 b	4
B3.2.2	8.31 b	4

Mean in the same coloumn with different alphabet(s) are significantly different (P<0.05) according to DMRT.

Crude supernatan protein secreted by six bacterial isolates were characterized. Chitinase from *Trichoderma viridae* were used as a control. The results revealed that the six isolates have different banding pattern as shown in Figure 1. Chitinase from *Trichoderma* has a molecular mass of 37 kDA (De Marco, 200), and most of chitinases have molecular mass of between 28 kDA - 43 kDA (Nielsen, 1997). Result showed that B3.2, B3.3, and 3.4 have a similar band between 30 kDA – 40 kDA, in a range with most of chitinases molecular mass. The three other isolates showed a

similar band of 50 kDA. Singh *et al,* (1998) reported that chitinase from *Paenibacillus* sp. has molecular mass around 50 kDA.



First lane represent the control of chitinase from *Trichoderma viridae*; Lane 1, B3.2; Lane 2, B3.3; Lane 3, B3.4; Lane 4, B14.1; Lane 5, B3.1.1; Lane 6, B3.2.2; Lane 7, negative control.

Figure 1. SDS-PAGE analysis of total supernatan protein of isolates, stained with comassie brilliant blue.

The result showed that B3.2 was the best rhizosphere chitinolytic bacteria. The most promising bacteria is B3.4. It is because bacteria with more 50% PIRG can be used as bio control (Bivi, 2010). Synergism between chitinolytic and antagonistic bacteria could enhance the role of bacteria as biocontrol pathogen. Combination of these two bacteria can be use as a biocontrol candidate.

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The Effect of Mulch, *Trichoderma,* and Arbuscular Mycorrhizal Fungi (AMF) Biofertilizer on the Growth of Oil Palm Seedlings Inoculated with *Ganoderma*

Happy Widiastuti

Indonesian Biotechnology Research Institute for Estate Crops
Jl Taman Kencana No 1 Bogor, 16151, Indonesia
Corresponding author: happywidiastuti@yahoo.com

Abstract

A Greenhouse experiment was carried out to determine the effect of mulching, *Trichoderma* sp., *Metharizium anisopilae*,. arbuscular mycorrhizal fungi (AMF) biofertilizer on the growth of oil palm seedlings. The experiment was conducted in 70x80 cm sized of polybags filled with 50 kg of Latosol Ciomas soil. Mycorrhizal inoculum is in the form of spores, hyphae and infected roots. Germinated oil palm was planted in polybag, then covered with empty fruit bunches of oil palm (EFBOP) as mulch. The dose of inorganic fertilizer was 50% for the application of AMF biofertilizer while on the other treatment the dose of fertilizer was 100%. The experimental design was complete randomized design to test nine treatments: 1) without any fertilizer, 2) 100% inorganic fertilizer, 3) EFBOP, 4) EFBOP+*Trichoderma pseudokoningii*+*Trichoderma* DT38, 5) EFBOP+*T. pseudokoningii*+ *Trichoderma* DT41, 6) EFBOP+*T. pseudokoningii* + *Trichoderma* DT38 + *Metharizium anisopilae*, 7) EFBOP+*T. pseudokoningii*+*Trichoderma* DT41+*Metharizium anisopilae,* 8) EFBOP+*T. pseudokoningii*+*Trichoderma* DT38+ *Metharizium anisopilae+AMF biofertilizer*, 9) EFBOP+*T. pseudokoningii*+*Trichoderma* DT41+*M. anisopilae+AMF biofertilizer*. The results showed that application of EFBOP as mulch enhanced the gowth of oil palm seedling and combination with DT 41 and both *T. pseudokoningii* and *Metharizium* produce the similar growth with those 100% inorganic fertilizer treatment. While the addition of EFBOP+*Trichoderma* DT41+*T. pesudokoningii* produced the highest seedling dry weight. The application of AMF biofertilizaer reduced the dose of inorganic fertilizer yielded a similar seedling dry weight with those fertlized with 100% of inorganic fertilizer.

Key words: oil palm, mulch, empty fruit bunches oil palm, arbuscular mycorrhizal fungi, acid soil

Introduction

One method of organic matter application is to use it as mulch. Mulch is organic or inorganic materials either natural or artificial which is placed above the soil and have a role to protect and cover it. Some of the benefits of mulching is to minimize the occurrence of surface runoff, increase water infiltration, protect the soil against rain, modify soil temperature, suppress weeds, reduce the rate of dry surface soil, increase soil biological activity and modifies the level of nutrient availability as well as to maintain or increase the availability of organic material soil (Baon, 1998). Basically the main function of mulching is to maintain the soil moisture. Water is a very important factor in the cultivation of plants including crops. The application of mulch is not accompanied with tillage practice. Muruganandam *et al* (2009) showed that the activity of enzymes that play a role in N mineralization in no-tillage soil is significantly higher compared to conventionally cultivated soil. While White & Rice (2009) suggested that the biological activity in no-tillage soil was detected higher compared to conventional cultivated soil.

In the microbiological aspects, organic materials addition means the addition of carbon and energy sources to improve microbe activities. The same thing seems to occur in peat soil. Komariah *et al* (1993), Nurani *et al.* (2007) reported that the use of microbial consortia and EFBOP increase soil pH and base saturation and lower CEC of peat soil as well as the C/N ratio. Microbes, in addition to functioning release nutrients or nutrient mineralization in the process of decomposition is also able to degrade toxic compounds (Sparling, 1998). However, microbe use root exudate as

source of carbon, energy and other nutrients to support their live. Brockling *et al*. (2008) suggests that plants can regulate microbial communities through root exudates.

The application of inorganic fertilizer as well as pesticide in higher dose will disturb the activities of soil microbes caused declining biological soil properties. These imbalances soil properties affect the soil microbial communities to control soil borne pathogens and microbes that play a role in the health of the land and crops. This is supported by research conducted by Vallad *et. al.* (2003) which proves that the application of organic fertilizer (compost) reduce plant disease attack. Rhizosfer manipulation seems necessary to maintain land productivity. Rousk *et al* (2009) suggested that the decrease in pH will increase five times the population of fungi, and *vice versa* for the bacteria. The aim of the research was to determine the effect of mulching*, Trichoderma* sp. , *M. anisopilae*, and AMF biofertilizers on the growth of oil palm seedlings.

Materials and Methods

The experiment was conducted in a greenhouse using a 70x80 cm sized polybags filled with 50 kg of Ciomas soil. Germinated oil palm was used as plant material and planted in polybag and add with empty fruit bunches oil palm (EFBOP) as mulch. DT38 and DT41 isolates were cultured in the media then transferred to PDA medium and inoculated into polybags. Mycorrhizal inoculum is in the form of spores, hyphae and infected roots. Germinated oil palm was planted on the polybag and the EFBOP was added as mulch. In the treatment of mycorrhizal fertilizer, the dosage of inorganic fertilizer was 50% of the recommendation dose while others were 100% of inorganic fertilizer. The experiment was arranged in completely randomized design to test nine treatments i.e. 1) without fertilizer, 2) 100% dose of inorganic fertilizer, 3) EFBOP, 4) EFBOP+*T. pseudokoningii*+DT38, 5) EFBOP+*T. pseudokoningii*+DT41, 6) EFBOP+*T. pseudokoningii*+DT38+ *Metharizium anisopilae*, 7) EFBOP+*T. pseudokoningii*+DT41+*Metharizium anisopilae*, 8) EFBOP+ *T. pseudokoningii*+DT38+*Metharizium anisopilae*+AM fungi, 9) EFBOP+*T. pseudokoningii* DT41+ *M. anisopilae*+AM fungi. Each treatment was repeated 3 times. Observations made on the growth of oil palm.

Results and Discussion

Observations of plant height showed that fertilization increased height of plants while EFBOP solely suppressed the height growth of plant (Table 1). The application of *Trichoderma* sp. and *M. anisopilae* and arbuscullar mycorrhizal fungi (AMF) enhanced the height of palm seedling.

Table 1. The height and leaf number of oil palm seedlings (9 months) in each treatment tested

Treatment	Height (cm)	Leave number
Withou fertilizer	106 ab	19 a
100% inorganic fertilizer	119 ab	19 a
EFBOP	81 a	20 a
EFBOP+Trichoderma DT38+T pseudokoningii	130 b	20 a
EFBOP+ Trichoderma DT41+T pseudokoningii	130 b	20 a
EFBOP+ Trichoderma DT38+T pseudokoningii +Meth	129 b	19 a
EFBOP+ Trichoderma DT41+T p+Meth	133 b	20 a
EFBOP+ Trichoderma DT38+T p+Meth+AMF	126 b	20 a
EFBOP+ Trichoderma DT41+T p+Meth+AMF	123 ab	18 a

Note: Figure (s) in each column followed by the same letter is not different according to Duncan ($P < 0.05$).

However, the application of EFBOP as mulch in term of plant height was not significantly different compared with those were not treated (without fertilizer) and 100% inorganic fertilizer. These results indicated that the plant height variable is not affected by EFBOP and inorganic fertilizer application. The absence of the influence of fertilization and the addition of EFBOP as mulch plants may caused the soil already contains enough nutrients. Inoculation treatments DT 41 + *T. pseudokoningii* or DT 38 + *T. pseudokoningii* without or in combination with *M. anisopilae*, and EFBOP as mulch significantly resulted in higher plant height compared to the application of EFBOP. These results indicated that the application of *Trichoderma* sp. DT 38, DT 41, *M. anisopilae*, *T. pseudokoningii* in combination with EFBOP yield positive effect on plant height. Positive influence is probably due to the decomposition process carried out by DT 38 and DT 41, while *M. anisopilae* seems to have an effect on the prevention of *Oryctes*.as a plant pest. The same was observed in the treatment of DT 38 + *T. pseudokoningii* + plus *M. anisopilae* and AM fungi. AM fungi can be symbiotic with the roots of oil palm and the symbiosis can enhance nutrient uptakes, especially P which contributes to increase plant height. In addition, the treatment was shown that application of AMF (fertilizer 50%) showed a similar plant height with plants that 100% fertilized .These results demonstrated that the application of AMF biofertilizer increased the efficiency of inorganic fertilizer by 50%. The increasing inorganic fertilizer efficiency by using AMF symbiosis has also been reported by other researchers. Some mechanisms that occur are by increasing the reach of roots, and mineralization of organic P through the extraction phosphatase. The application of DT 41 + *T. pseudokoningii* + *M. anisopilae* + and AMF biofertilizer was not significantly different with those of DT 38 + *T. pseudokoningii* + *M. anisopilae* + and AM fungi in term of plant height.

Treatment of EFBOP increased the number of leaves compared to 100% inorganic fertilizer and the unfertilizer treatment (Table 1). The application of EFBOP provides the same number of leaves with the inoculation of DT41 or DT38 and the *T pseudokoningii* accompanied by the application of *M. anisopilae* and AMF biofertilizer. However there is no significantly differences between the number of leaves of all treatments tested in the blank, 100% inorganic fertilizer, or mulching EFBOP. Interaction between AM fungi with DT 38 is better compared to the interaction of those fungi with DT41 especially demonstrated by the number of leaves.

The fresh weight of leaves increased significantly with the addition of inorganic fertilizers as well as giving EFBOP as mulch. Similar results were also observed in the treatment of EFBOP addition which is accompanied by DT 38, DT 41, *T. pseudokoningii, M. anisopilae* and AMF biofertilizer. It was shown that the efficiency of inorganic fertilizer can be achieved by the application of AMF biofertilizer combined with either DT 41 or DT 38. Similar results are also shown on the variables fresh weight of stem. Fresh weights of oil palm seedlings stem with inorganic fertilizer is higher than thethe blank (Table 2). Nevertheless, the fresh weight of stem between 100% inorganic fertilizer and application of EFBOP as mulch and blank werenot significantly different. The addition of DT 41 and DT 38, which is accompanied by *T. pseudokoningii* and *M. anisopilae* results a fresh weight of stem that were not significantly different compared to those with the addition of EFBOP as mulch or 100% inorganic fertilizer. The application of EFBOP as mulch, AMF biofertilizer and 50% inorganic fertilizer yield fresh weight of stem that is not significantly different to 100% inorganic fertilizer treatment. These results indicated that the application of AMF biofertilizer improves inorganic fertilizer efficiency as much as 50%.

Compared to the fresh weight of the stem appears that the effect of the treatments being tested was more visible on the fresh weight of leaf variables. Application of EFBOP accompanied by DT 41 and *T. pseudokoningii* addition produced the highest fresh weight of leaves. Similar results were also observed in the treatment of EFBOP as mulch with DT 38, DT 41 or accompanied by *M. anisopilae*. As for DT 38 leaf fresh weight significantly higher compared with the blank when combined with *M. anisopilae* and AMF biofertilizer.

Table 2. The fresh weight of oil palm seedlings (9 months) in each treatment tested

Treatment	Fresh weight (g)				
	Leaf	Stem	Shoot	Root	Total
Without fertilizer	450 a	833,33 a	1283,33 a	783 a	2066 a
100% inorganic fertilizer	850 b	1433,33 ab	2250 ab	1017 ab	3267 b
EFBOP	863,33 b	1583,33 ab	2466,67 ab	967 ab	3433 b
EFBOP+DT38+T p.	733,33 ab	1683,33 ab	2416,67 ab	933 ab	3349 b
EFBOP+DT41+T p.	1066,67 b	2000 b	2733,33 b	1183 b	3916 b
EFBOP+DT38+T p+Meth	833 b	1566,67 ab	2400 ab	933 ab	3333 b
EFBOP+DT41+T p+Meth	883,33 b	1750 ab	2633,33 ab	1000 ab	3633 b
EFBOP+DT38+T p+Meth+AMF	900 b	1533,33 ab	3433,33 ab	783 a	4216 b
EFBOP+DT41+T p+Meth+AMF	716,67 ab	1416,67 ab	2133,33 ab	1017 ab	3150 b

Note: Figure (s) in each column followed by the same letter is not different according to Duncan (P < 0.05).

Fertilization treatments increased fresh weight of shoots as well as the addition of EFBOP as mulch. The application of DT 41+*T. pseudokoningii* and DT 38+*T. pseudokoningii* either accompanied by *M. anisopilae* or not resulted in fresh weight of shoots that are not significantly different compared to those with 100% inorganic fertilizer and EFBOP as mulch. This result shows that the effect of DT 41, DT 38 and *M. anisopilae* is not significantly different on the fresh weight of shoots. However the application of DT 41+*T. pseudokoningii* and DT 38+*T. pseudokoningii* accompanied by *M. anisopilae* and AMF biofertilizer yield shoots fresh weight equal to 100% fertilization. These results indicated that AMF biofertilizer application increased the efficiency of inorganic fertilizer, although this treatment may not significantly increased the fresh weight of shoots of oil palm seedlings.

Root fresh weights were not significantly different in the treatment of 100% inorganic fertilizer, EFBOP as mulch, and EFBOP+DT 38 + *T. pseudokoningii* and DT 41+*T. pseudokoningii* accompanied by *M. anisopilae* or without *M. anisopilae*. The same result observed in the treatment of DT 41 or DT 38+*T. pseudokoningii*+*M. anisopilae* accompanied wth AMF biofertilizer. Other study demonstrated that the application of DT 41 and DT 38 or AMF biofertilizer increase the root fresh weight. The same result was observed in the total oil palm seedlings fresh weight.

Table 3. Dry weight of oil palm seedlings (9 months) in each treatment tested

Treatment	Dry weight (g)				
	Leaf	Stem	Shoot	Root	Total
Without fertilizer	123,33 a	231,67 a	355 a	138 a	493 a
100% inorganic fertilizer	220 a	361,67 a	582 ab	215 ab	797 b
EFBOP	205 a	453,33 a	658 ab	182 ab	840 b
EFBOP+DT38+T p.	201,67 a	386,67 a	588 ab	278 b	866 b
EFBOP+DT41+T p.	225 a	463,33 a	688 b	212 ab	900 b
EFBOP+DT38+T p+Meth	196,67 a	460 a	657 b	140 ab	797 b
EFBOP+DT41+T p+Meth	188,33 a	438,33 a	627 ab	165 ab	792 b
EFBOP+DT38+T p+Meth+AMF	168,33 a	410 a	578 ab	128 a	726 b
EFBOP+DT41+T p+Meth+AMF	126,67 a	425 a	551 ab	145 ab	698 b

Note: Figure (s) in each column followed by the same letter is not different according to Duncan (P < 0.05).

The addition of 100% inorganic fertilizer increased dry weight of oil palm stems as well as the treatment of EFBOP as mulch (Table 3). Similar result was observed in mulching treatments EFBOP with by DT 41, DT 38 which with *T. pseudokoningii* and *M. anisopilae* and AMF biofertilizer. Nevertheless, the results of statistical tests indicated that there is no significanly differences between dry weight of plants fertilized with 100% treatment and other treatments. Similar results

were also found in variable weight of dry leaves, shoot, roots, and oil palm seedling. It seems that the application of EFBOP as mulch can be combined with the inoculation of *Trichoderma* sp, *M. anisopilae* and AMF biofertilizer. Hence the application of AMF biofertilizer reduced the doseage of inorganic fertilizer as much as 50%. The effect of treatment to *Ganoderma* infection is likely could be suppressed in this experiment.

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The Use of RAPD Marker on Gambier's Breeding Program

Hamda Fauza[a*], Jamsari [a], Istino Ferita [a], Ade Noverta [a], Azmi Dhalimi [b], Ahmad Denian [c], Murdaningsih H. Karmana[d]

[a]*Faculty of Agriculture of Andalas University, Padang, Indonesia*
[b]*Center for Agricultural Technology Assessment and Development, Bogor, Indonesia*
[c]*Medicinal and Aromatic Crops Research Institute, Bogor, Indonesia*
[d]*Faculty of Agriculture of Padjadjaran University, Bandung, Indonesia*
**Corresponding author: Faculty of Agriculture Andalas University, Kampus Limau Manih 25163*
Tel: +62 751 72776; Fax.: +62 751 72702; mobile : +62 8156119287 ;
hamdafauza@yahoo.com

Abstract

Low productivity is one of the problems faced by farmers in gambier (**Uncaria gambir*)* plants cultivation. This problem is mainly caused by the use of low yield gambir cultivars. Although breeding effort has been initiated since several years ago, no significant result has been achieved until now. This is due to inadequacy of molecular investigations in this species. Molecular aspects are commonly related with protocol for DNA preparation and molecular markers which are important so that it will speed up the breeding program. The objectives of the research were to optimize the DNA isolation procedures using different protocol and type of materials, to optimize DNA amplification procedures, and (3) to find out RAPD primers indicating high level of polymorphisms. The research was done from May to November 2009 in the Laboratory of Biotechnology and Plant Breeding, Faculty of Agriculture, Andalas University. DNA isolation procedure and type of materials were assessed. DNA isolation was performed using four different protocols. Type of materials tested consisted of seven treatments. Optimizing PCR condition and RAPD primer selection delivered basic information of optimum PCR condition. A number of 50 RAPD primers were randomly selected. The results recommended the application of CTAB-based method protocol, using young meristem leaves for DNA isolation.

Keywords: gambier, breeding, RAPD

Introduction

Low productivity is a major problem in gambier (*Uncaria gambir)* plant development. Crop productivity ranges between 400 and 600 kg per ha (Dinas Perkebunan Sumatera Barat, 1998; Roswita, 1990). Meanwhile the potential of this plant can reach 2100 kg per ha of dry resin (Sastrahidayat & Soemarsono, 1991). The low productivity is due to the use of low yield varieties. Meanwhile, the use of improved varieties is one determining factor in increasing crop productivity. In the cultivation of gambier, there is no information of using high yielding varieties.

Compared with other commodities, research and development of gambier plant breeding is still far behind. With the use of biotechnology is expected the lag, especially in the field of plant breeding can be pursued. Studies of molecular aspects of the basic information that will be useful as an initial step in gambier plant breeding programs

During the previous decade, strategy for the evaluation of genetic variability was carried out through the approach of anatomy, morphology, embryology, and physiology. This approach now has been fasilitated with molecular techniques. The development of biotechnology in particular science called molecular markers based on polymorphisms found in proteins or DNA, has been widely facilitate research in disciplines such as taxonomy, ecology, genetics, and plant breeding (Weising, *et al*, 1995).

In the field of plant breeding, using fingerprinting techniques have been applied to various aspects and types of plants. Various examples can be mentioned, among others, is its application to rice. Wu and Tanksley (1993) reported the identification of allele-specific microsatellite found in

indica and japonica rice types. The results are then followed by the use of PCR techniques to identify microsatelite polymorphism possessed by the upland rice and other rice cultivars. Virk *et al* (1995) using the RAPD technique for the distinction between the types of rice cultivation and to identify possible duplication of cultivars. Besides the rice crop, the application of molecular markers has also been applied to other fields. Meanwhile, for gambier plants, Fauza, *et al.* (2007) in early research on diversity studies of gambier concluded that RAPD markers have a broad genetic variability. But in this study there are several obstacles in the method of DNA isolation and RAPD-PCR method technique is not stable yet, and limited use of polymorphic primers for gambier plants.

This study aimed to obtain the DNA isolation method for the gambier plant, to find information on material characteristics for DNA isolation, to find a method (program) appropriate in RAPD-PCR technique for gambier plant, and to obtain primers polymorphic in plants gambier characterization techniques based on RAPD-PCR.

Materials and Methods

The research was done from May to November 2009 in the Laboratory of Biotechnology and Plant Breeding, Faculty of Agriculture Andalas University. Two informations namely: DNA isolation procedure and type of materials were assessed. Experiment of DNA isolation procedure performed four protocols as treatments, i.e.: (1) Saghai-Maroof, *et al.* (1984), (2) Doyle and Doyle (1990), (3) Krizman, *et al.* (2006), and (4) An Michiels et al. (2003). Experiment for type of materials applied seven treatments, namely: (1) fresh young leaf, (2) fresh old lead, (3) fresh young leaf stored at -20ºC for one week, (4) fresh young leaf stored at 4ºC, (5) young leaf stored at room temperature (25ºC) for one week, (6) young leaf stored with silica gel for one week (7) etiolated fresh young leaf. Optimizing PCR condition and RAPD primer selection; these experiments delivered basic information of optimum PCR condition that could be used in variability and pedigree analysis based on RAPD molecular technique. Optimization had been done by applying some levels of annealing temperature i.e.: 35°C; 37°C; 40°C; 42°C and 45°C. These annealing temperatures will be combined with one or two cycle groups. Besides some level of DNA concentration (5; 10; 15; 20; 25; 50; and 100 ng/reaction) and concentration of primer (5; 10; 20; 50; and 100 pmol/reaction) applied. A number of 50 RAPD primers were randomly selected.

Results and Discussion

Optimization of DNA Isolation

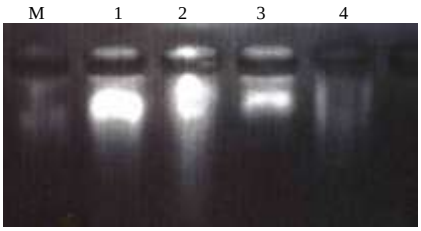
Of the four isolation procedures attempted, it seems that the protocol based on Doyle and Doyle (1990) produced the highest total DNA concentration. The ratio of the intensity of DNA fragments isolated by Doyle and Doyle (1990) showed 2-5 times higher than the standard λ DNA used. With the DNA isolation protocol based on Saghai-Maroof *et al.* (1984), there was no alleged DNA pellets obtained. While isolation by using a protocol based on Krizman *et.al* (2006) and based on An Michiels *et. al*, (2003) for the isolation of DNA-containing plant samples sap, the results obtained was also not optimum .

The success of protocols based on Doyle and Doyle was likely caused by the presence of phenol compounds was mixed with Chloroform-Isoamylalcohol in one stage of isolation. Phenol is known as a compound that is effective enough to separate the protein from cell organelles. It is known that plant cells are very rich in polyphenols. These substances are contaminants for the DNA isolation from gambier plant tissue. However for other species, the use of phenol during DNA isolation process is needed (Jamsari, 2003)

Of the several types and characteristics of materials used for DNA isolation was found that young leaves of gambier plants in fresh condition and young leaves stored in silica gel, were good

materials for the isolation of gambier plant DNA. It is characterized by the discovery of DNA pellet after the extraction process, which in turn is also evidenced by the results of electrophoresis analysis. While other materials did not show DNA pellet after the precipitation process.

At this stage the attempt to isolate DNA from various plant tissues by several methods was carried out. Isolation of DNA from fresh young leaves by the CTAB method (Doyle and Doyle, 1990) and DNA with DNA Kit produced better quality. While the isolation of DNA from young leaves that were stored with silica gel with CTAB method produced DNA with poorer quality than those from fresh leaves, but by using a DNA kit produced DNA with a fairly good quality. In principle, the resulting DNA can be used to the process of PCR-RAPD amplification. Optimization results of DNA with CTAB method are shown in Figure 1.



M = Lambda 50. 1,2,3 = fresh young leaves, 4 = young leaves stored in silica gel

Figure 1. The examples of optimization results of gambier plant DNA isolation.

Optimization of RAPD-PCR technique

Optimization of RAPD amplification was done by modifying the temperature and time of the stages of the amplification process of some methods and programs from various sources, including: method of Williams, *et al*. (1990) and Ready to Go PCR Kit (RTG-PCR Kit). Based on the results of the optimization program that will be applied in the amplification program was that recommended by Williams, *et al*. (1990), as showed in Table 1.

Table 1. RAPD PCR amplification program of gambier plant

Process	Temperature (°C)	Time	Total cycles
Initiation of denaturation	96	2 min	1
Denaturationi	94	30 sec	45
Anneling	36	1 min	
Extention	72	2 min	
Final extention	72	5 min	1
Pause	4	~	

Primer Selection

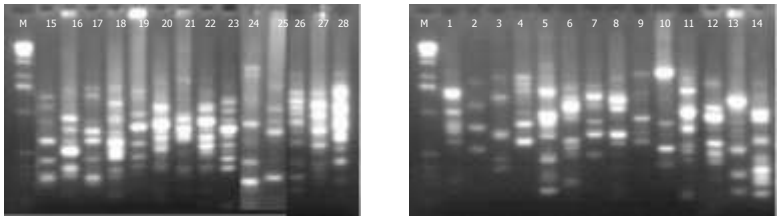
To obtain a high degree of polymorphism, primers were selected by using random operon dekamer primer technology (Almaeda, USA). As the DNA template used in the selection of this primer DNA pool consisting of five DNA genotype gambier based phenotypic appearance is thought to have a distance kinship. Selections of primer for subsequent experiments based on the number of bands (fragments) and the sharpness of the resulting ribbons. Selection was carried out on 50 types of primer RAPD method and program optimization. The primer selection was conducted at two-phase. In the first phase used 25 primers.Three of the 25 primers, were used were for the analysis of individual selection, the OPN-06, OPE-18 and OPY-08. Each band primer produced between 0-5 bands.

Table 2. List of RAPD primer and PCR product characteristics obtained

No	Primer Name	Secuences	Number of fragments	Fragment Characteristics
1.	OPA-11	CAA TCG CCG T	8	Fragment is quite clear, but it's rather difficult to be scoring
2.	OPA-12	TCG GCG ATA G	4	Fragments are clear and easy to be scoring
3.	OPA-15	TTC CGA ACC C	6	Fragments are clear and easy diskoring
4.	OPA-19	CAA ACG TCG G	8	Fragment is quite clear, but it's rather difficult to be scoring
5.	OPB-01	GTT TCG CTC C	14	Fragment is quite clear, but it's rather difficult to be scoring
6.	OPB-06	TGC TCT GCC C	10	Fragment is quite clear, but it's rather difficult to be scoring
7.	OPB-09	TGG GGG ACT C	7	Fragment is quite clear, but it's rather difficult to be scoring
8.	OPB-11	GTA GAC CCG T	9	Fragment is quite clear, but it's rather difficult to be scoring
9.	OPF-08	GGG ATA TCG G	5	Fragments are clear and easy to be scoring
10.	OPK-06	CAC CTT TCC C	8	Fragment is quite clear, but it's rather difficult to be scoring
11.	OPL-13	ACC GCC TGC T	11	Fragment is quite clear, but it's rather difficult to be scoring
12.	OPN-19	GTC CGT ACT G	7	Fragments are clear and easy to be scoring
13.	OPR-11	GTA GCC GTC T	8	Fragmen ts are clear and easy to be scoring
14.	OPT-16	GGT GAA CGC T	11	Fragments are clear and easy to be scoring
15.	OPW-11	CTG ATG CGT G	6	Fragments are clear and easy to be scoring
16.	OPX-01	CTG GGC ACG A	7	Fragments are clear and easy to be scoring
17.	OPX-04	CCG CTA CCG A	8	Fragments are clear and easy to be scoring
18.	OPX-07	GAG CGA GGC T	11	Fragment is quite clear, it's rather difficult to be scoring
19.	OPX-09	GGT CTG GTT G	7	Fragments a re clear and easy to be scoring
20.	OPX-15	CAG ACA AGC C	7	Fragments are clear and easy to be scoring
21.	OPX-19	TGG CAA GGC A	8	Fragments are clear and easy to be scoring
22.	OPY-04	GGC TGC AAT G	11	Fragments are clear and easy to be scoring
23.	OPY-20	AGC CGT GGA A	10	Fragments are clear and easy to be scoring
24.	OPB-17	AGG GAA CGA G	8	Fragments are clear and easy to be scoring
25.	OPF-05	CCG AAT TCC C	6	Fragments are clear and easy to be scoring
26.	OPE-18*	GGA CTG CAG A	12	Fragments are clear and easy to be scoring
27.	OPN-06*	GAG ACG CAC A	10	Fragments are clear and easy to be scoring
28.	OPY-08*	AGG CAG AGC A	13	Fragments are clear and easy to be scoring

* The best primer on the first stage selection.

The second stage was the repeatation of the selection of 25 primers and the best three in the first phase. DNA template used consisted of five different genotypes of first stage. Based on the results of 28 RAPD primer amplification with DNA pool gambier, all tested primer products were found in the DNA mixture. It means thar all the selected RAPD primers has the potential to be used in the selection of individual gambier plant DNA. Products produced ranged from 4-14 fragments of DNA. Fragments generated from the primer selection of this second stage were better than the first stage. This is due to the different DNA pool used in this experiment. Therefore RAPD primer that can be recommended for the analysis of kinship is the OPN-06, OPE-18 and OPY-08. More detail can be seen in Table 2 and the results of amplification of DNA pools with 28 primers RAPD gambier shown in Figure 2.



Description: sample mentioned in each of them according to the primary number in Table 2.

Figure 2. Amplification of gambier DNA pools with 28 primer RAPD.

Conclusions

The isolation of DNA for gambier generated good quality and quantity of DNA using the CTAB method by protocol of Doyle and Doyle (1990) using young leaf tissue. Meanwhile, a program that should be applied for RAPD-PCR amplification was a program recommended by Williams, *et al*. (1990). About 50 selected primers could be used in gambier DNA amplification, but three primers showed a high number of bands and fragments namely: OPE-18, OPN-06 and OPY-08.

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Shorea leprosula: the Most Commercial Trees to Improve "Production-Natural Forest" Productivity

Wahyudi [1] Indrawan A.[2] Irdika Mansur [3] Prijanto Pamoengkas [3]

[1] *Department of Forestry, Palangka Raya University*
isanautama@yahoo.com
[2] *PhD, Faculty of Forestry Bogor Agricultural University*
[3] *Faculty of Forestry, Bogor Agricultural University*

Abstract

Deforestation rate in Indonesia is 1,8 million ha per year. This is due to low productivity of natural forest which is just 0,25 m^3 ha^{-1} $year^{-1}$ (compared with teak plantation forest: 8-10 m^3 ha^{-1} $year^{-1}$). Decrease in natural regeneration and forest plantation were caused by stand competition and limited of light in the forest floor. Efforts to improve production-natural forest productivity was by applying the Selective Cutting and Strip Planting (SCSP) system. This system made optimum open area (strip) which is suitable to maximize the growth of Dipterocarp species, especially *Shorea* spp as the most commercial trees in the natural tropical forest. This research aimed to analyze and created modeling of growth and yield of *Shorea leprosula* plantation in the SCSP system. The research was conducted on research plots of SCS in logged over – production natural forest of PT Gunung Meranti forest concession, Central Kalimantan Province. Analysis of data used growth modeling for even-aged forest. The result showed that mean annual increment of *Shorea leprosula* plantation at 2, 11 and 16 year olds were 1,06 cm $year^{-1}$; 1,22 cm $year^{-1}$ and 1,31 cm $year^{-1}$ in diameters, respectively. Based on even-aged forest modeling, the first cycles of *Shorea leprosula* plantations was 32 year in the 125,14 m^3 ha^{-1} of logs (40 cm up of diameters), therefore *Shorea leprosula* plantations in the SCSP system could improve the natural forest productivity until 262,72 times. The SCSP system with *Shorea leprosula* plants is very applicable in the logged over-production natural forest to improve forest productivity.

Keyword: *Selective cutting and strip planting system,* Shorea leprosula*, growth and yield, productivity*

Introduction

Indonesia is third in the world after Brazil and Zaire in wide of tropical forest regions and has the highest biodiversity too (Whitmore, 1975; Mac Kinnon, et al., 2000). However, the condition of natural forest resources in Indonesia tends to face degradation in the quality and quantity along with environmental changes nationally and also globally (Ministry of Forestry, 2008). Deforestation and degraded forest in Indonesia are caused by increasing of resident and wood requirement (Singh, *et al.* 1995) illegal logging, shifting cultivation, illegal mining, illegal occupation of land, forest fire (Indrawan, 2008) conversion of forest (Saharjo, 2008), and poor forest management (Wahjono and Anwar, 2008).

As comparison, in the year 1990's, logs production in Indonesia were 28 million m^3 coming from 59,6 million ha of production forest. But in the year 2007, logs production decreased to become 9,1 million m^3 from 27,8 million ha of production forest only. Deforestation and degraded forest will continue to happen if there isn't repair of production forest management system in Indonesia. Some researches of silvicultural system in Indonesia have been conducted since 1993 and applying of Selective Cutting and Strips Planting (SCSP) silvicultural system with intensive silvicultural technique has been done limited to 25 forest concessions since 2005, using species of Dipterocarp specially *Shorea* spp. *Shorea leprosula* is one of Dipterocarp species recommended to be developed in strips area in SCSP system. Afterwards, research on influencing of gap size and slope to increase growth and yield of *Shorea* spp plantation in the SCSP system is very needed to support this system.

Based on the forest function, forests in Indonesia are divided into three regions i.e. conservation forest, protection forest and production forest. Production forest can be divided into some forest regions, in the form of virgin forest, logged over forest, low potential forest, bushes-scrub, grassland and critical land. Logged over forest and low potential forest can be managed by Selected Cutting and Strips Planting silvicultural system using *Shorea* spp, especially *Shorea leprosula*.

This Research aimed to compile growth and yield modeling of *Shorea leprosula* that is developed in the strips area. Research was expected to be used by stakeholders, specially for user of SCSP system.

Methods

Research was conducted in the Permanent Sample Plots of Selective Cutting and Strips Planting in the District of Mandau Talawang, Central Kalimantan Province that was planted in 2008 (age of 2 years), 1999 (age of 11 years), and 1994 (age of 16 years). Data were collected in 2010.

Measured parameters were diameters and high of *Shorea leprosula* at the aged 2 years, 11 years, and 16 years. Growth and yield of *Shorea leprosula* pattern was formed according to to the increment and time (years) functions through polynomial equation (Brown, 1997; Burkhart, 2003) that was:

$$y = c_1 + c_2x + c_3x^2$$

Where: y : diameter (average)
x : time (years)
c_1, c_2, c_3 : coefficient

Results and Discussion

The research results of *Shorea leprosula* plantation in the Permanent Sample Plots of Selective Cutting and Strips Planting that is planted in 2008 (age of 2 years), 1999 (age of 11 years), and 1994 (age of 16 years) which were collected in 2010 were shown in Table 1.

Table 1. Mean annual increment of *Shorea leprosula* in the PSP of SCSP at 2, 11, and 16 years old

Age (year)	Live (%)	MAI	
		Diameter (cm)	High (m)
0	100	0	0
2	84.22	1.06	1.40
11	61.87	1.22	0.94
16	61.21	1.31	0.82

Table 1 showed that diameter mean annual increment (MAI) of *Shorea leprosula* since the first time until 16 years old was always inreasing. MAI of *Shorea leprosula* was 1,36 cm/year at 16 years old with 61,21% of live. At the 16 years old, diameter and high of *Shorea leprosula* plants were 21,22 cm and 13,1 m respectively. Growth and yield of *Shorea leprosula* was always increasing until it achieved 30 to 40 cm in diameters (Wahyudi *et.al*. 2011).

Growth and yield of trees in the e*ven-aged stand forest* were different with those of *uneven-aged stand forests*. Modeling must consider variation coefficient and deviation standard of data. There were phenomena in the field that some plants in the monocultural plantation indicated differences in growth level for each species. There are some species which grow very fast, fast, slow and very slow. This phenomena is overcome by tending and thinning periodically.

Growth of plants can be assumed from time function. Diameter of plants will be getting bigger progressively, but periodically growth must be mentioned in the model, so it needs the time series of data to create modeling of growth and yield. Therefore, sigmoid curve to draw the growth of yield of *Shorea leprosula* also needed the time series data. Modeling must accommodate all information about species, environment, tending, increment and so on, which was made available and trustworthy of growht and yield to create the justified modeling (Grant et al. 1997; Porte & Bartelink 2001; Vanclay 2001). Modeling can simplify the complicated calculation composed of several equations at the same time with some simulation expected.

A **B** **C**

Figure 1. *Shorea leprosula* plantation in the Selective Cutting and Strips Planting system at 2 years old (A), 11 years old (B) and 16 years old (C).

Modeling using polynomial equation based on increment of *Shorea leprosula* and time functions (Brown 1997; Burkhart 2003) is as the following:

$Y = 0{,}0297x^2 + 0{,}8208x + 0{,}3728$ ..... $R^2 = 86{,}89\%$

y : final diameter ; x : time (year) .

This model predicted that to achieve 50 cm up of diameter average, the time needed was 32 years as shown in Figure 2. Correlation coefficient of this equations was 86,89%, accordingly, it indicated that this equation can be used to predict growth and yield of *Shorea leprosula* plantation on the Selective Cutting and Strips Planting system.

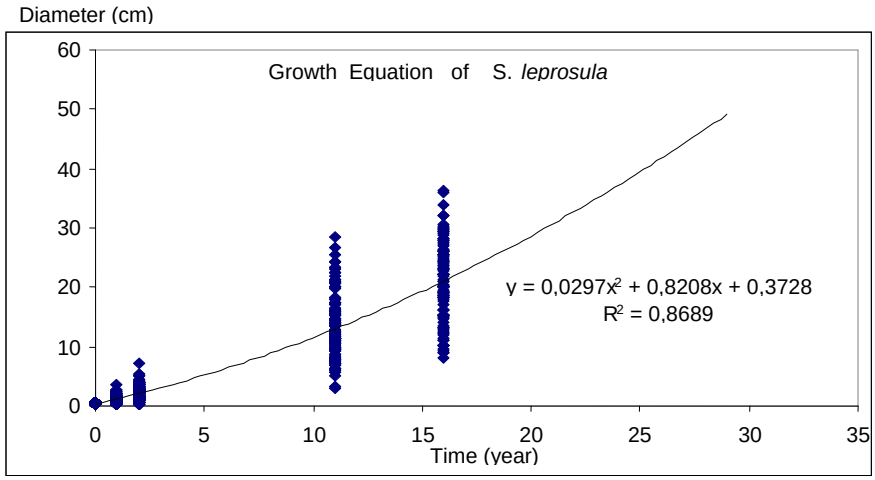

Figure 2. Growth and yield of *Shorea leprosula* plantation using modeling of polynomial equation.

Mean production of logs in PT Gunung Meranti (forest concession) for 4 years (2007 to 2010) was 22,3 m^3 ha^{-1} of logs. Whereas, based on even-aged forest modeling, the first cycles of *Shorea leprosula* plantations for 32 years, the production was 125,14 m^3 ha^{-1} of logs (40 cm up of diameters), therefore *Shorea leprosula* plantations in the SCSP system could improve the natural forest productivity until 262,72 times. The SCSP system of *Shorea leprosula* is very applicable in the logged over-production natural forest to improve forest productivity. So, *Shorea leprosula* is most commercial trees to improve "Production Natural Forest" productivity in the tropical forest.

Conclusion

Shorea leprosula plantation in the Selective Cutting and Strips Planting silvicultural system can improve Production Natural Forest productivity in the tropical forest with applying the cutting cycles as long as 32 years and predicted to produce 125,14 m^3 ha^{-1} of logs.

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Patogenity Test of Two Isolates of *Ganoderma* on Sengon Seedlings

Elis N. Herliyana [a,*], Irfan K. Putra [a], Darmono Taniwiryono [b], Hayati Minarsih [b]

[a] *Department of Silviculture, Faculty of Forestry, Bogor Agricultural University, Bogor, Indonesia*
[b] *Indonesian Biotechnology Research Institute for Estate Crops (IBRIEC), Bogor, Indonesia*
** Corresponding author: Department of Silviculture, Faculty of Forestry, Bogor Agricultural University, Kampus IPB Darmaga Bogor (16680), Indonesia*
Fax 0251626806,
elisherliana@yahoo.com; elishe@ipb.ac.id

Abstract

Sengon tree (*Paraserianthes falcataria* (L.) Nielsen) currently becomes a major forest tree species widely planted by smallholders in Indonesia. Sengon often used by farmers as component planted in agroforestry system as well as shade trees planted in between other crops such as coffee and cacao. *Ganoderma* infection causebasal stem rot disease which is becoming more prevalent and causing significant loss in sengon tree and other estate crops. This research is based on the attack of *Ganoderma* tothe shade tree, sengon. Therefore, it is important to do a specific research on the process of the inoculation of *Ganoderma* to prevent such attack. The aim of this research is to understand the effect of the inoculation to the sengon seedlings. There are two majors in the research i.e. non inoculation and inoculation treatments. Each treatment consisted of three observation blocks that are considered equal and each block consisted of four plants (the sengon seedlings age were one and a half months) as replicates. The non inoculation treatments were all combinations of root and foodbase treatments. Foodbase treatment itself is divided into two i.e. the sengon wood piece with varying size (3, 4, and 5 cm diameters) and PDA (Potato Dextrose Agar) without inoculation of *Ganoderma* spp. from Ciamis area/region (isolates of *Ganoderma* from lamtoro) and isolates of *Ganoderma* from sengon plants) derived from the collection of Forest Pathology Laboratory and will be called SP1 and SP2. The result showed that the control has a better average growth compared to the inoculation treatment. The f pathogenity test shows that *Ganoderma* SP2 has more pathogenic than *Ganoderma* SP1. the heights of seedlings that were inoculated with *Ganoderma* SP2 are l lower than the heights of seedlingss that were inoculated with *Ganoderma* SP1. Root treatments application showed that both controls and treatments blocks have better growth from sengon seedlings using root cutting treatment. The effect on the growth of sengon seedlings showed that inoculated seedlings in the wood with 3 centimeters diameter as a foodbase inhibit both their height and leaves growth compared to the seedlings with 4 centimeters or 5 centimeters diameter of wood of the same foodbase.

*Keywords: foodbase, Ganoderma, sengon (*P. falcataria*), Basal stem rot.*

Introduction

Sengon tree (*Paraserianthes falcataria* (L.) Nielsen) currently becomes a major forest tree species widely planted by smallholders in Indonesia. In Java, there is a total area of 400.000 ha of community forest dominated by sengon from which 895.000 m3 wood are produced annually. The wood of this quick growing tree is processed further by paper industries to become pulp or sawed by lumber industries as a raw material for soft wood board. Sengon often used by farmers as component planted in agroforestry system as well as shade trees planted in between other crops such as coffee, cacao and banana. *Ganoderma* infection is becoming more prevalent and causing significant loss in sengon tree in Indonesia. Once the tree is infected by this cosmopolitan fungal pathogen, sooner or later its base will become totally rotten leading to the tree dead. The disease incidence ranges from 3% to 26%. One hundred percent disease incidence has been reported to occur in the second generation of replanting.

This basal stem rot disease spread amongst trees primarily through roots contact. The fungus has rigid fruiting bodies plate (conk), with or without stem, the top surface often looks shiny waxy brown to dark brown in color, the bottom surface looks porous creamy white in color.

Theyoften found attached to the base of the rotten tissues near soil surface. Basidiospores produced from pores at bottom surface plays in important roles in the disease spread from the infected tree to the fresh cut of the stumps. The disease symptoms look similar with those caused by heavy water stress displaying weak pale to yellowish in color of the tree canopy which is unrecoverable and leading to leaf dryness. The disease is difficult to be controlled, because when the symptom is observed, the infected tree is usually cannot be saved anymore by any control means. Turner (1981) in Zakaria *et al*. (2004) reported that at least there are 15 species of *Ganoderma* in various places in the world, which caused basal stem rot disease.

Ganoderma is a fungus causing basal stem rot disease that usually attacks the roots of the host range. This fungus attacks is commonly found in various types Leguminoceae (Henessy and Daly 2007), Palmae (Turner 1981 in Zakaria *et al*. 2005), Rubiaceae (Hindayana *et al*. 2002). The authors also found *Ganoderma* that attack the ebony tree (Ebenaceae). This research is based on the attack of *Ganoderma* to ashade tree, sengon. Therefore, it is important to do a specific research on the process of the inoculation of *Ganoderma* to prevent such attack. The aim of this research is to understand how the effect of the inoculation to the sengon seedlings to find the control technique.

Methodology

This research was conducted from October 2009 - March 2010. This research was held in a greenhouse of Department of Silviculture and Forest Disease Laboratory of the Department of Silviculture, Bogor Agricultural University.

Preparation of Equipment and Materials Research

The study was started by the preparation of sterile soil and foodbase that include a piece of wood with diameter 3, 4 and 5cm and foodbase a PDA (Potato Dextrose Agar). The supply of sterile soil was done by autoclaving of soil mixed media and charcoal husk compost with the ratio of 2:1:1. Autoclaving was done by the time adjacent to weaning so that the soil conditions results in steaming plastic bags are not contaminated. The PDA foodbase was prepared in two ways that are not inoculated or inoculated.

PDAs which were not inoculated by *Ganoderma,* was prepared simply by making sterile PDA medium which is then placed in polybags at weaning. The preparation of PDA inoculated media by *Ganoderma* was done in conjunction with the preparation of pieces of wood that also were infected with fungus. Isolates of *Ganoderma* have been cultured in a sterile culture into sterile jars containing both types of foodbase. Maturity level of culture can be seen quite well from the morphology of the fungus and spread on a PDA or timber in the jar. The uninoculated pieces, supplies quite done with skinning sengon logs that had previously been cut with a length of 5cm and various diameters i.e. 3, 4 and 5 cm. The pieces of wood were then boiled in a pan for a few hours before sterilized in an autoclave.

Weaning and Maintenance

Weaning were performed in the afternoon to prevent the death of seedlings due to the stress. Weaning also performed each treatment to be easy in the preparation plant. After that, sengon seeds will be slightly softened, about one or two days prior to adapt from the stress.

Providing treatment

There are two majors in the research i.e. non inoculation and inoculation treatments. Each treatment consisted of three observation blocks that are considered equal and each block consisted of four plants (the sengon seeds age were one and a half months) as replicates. The non inoculation treatments were all combinations of root and foodbase treatments. Foodbase treatment itself is divided into two i.e. the sengon wood pieces with varying size (3, 4,

and 5 cm diameters) and PDA without inoculation of *Ganoderma* from Ciamis area/region (isolates of *Ganoderma* from lamtoro) and isolates of *Ganoderma* from sengon plants) derived from the collection of Forest Pathology Laboratory and will be called SP1 and SP2. The total of treatments are 10. Inoculation treatments were a combination of various treatments of root and foodbase which has inoculated with *Ganoderma*. Types of *Ganoderma* were also included in a combination. The total number of inoculation is 15 treats. Types of *Ganoderma* were also included in a combination. Total for this inoculation tratement is 30 treatments.

Observation of the Treatment and Data Collection

The observation of the treatment was carried out daily with the parameters of the number of young leaves and seeds of high accretion sengon. Data were collected on a tall sheet and analyzed using SPSS. At the end of the study, the root shoot ratio data was taken to see the most balanced growth among the treatments.

Data was taken on the inoculated sengon with *Ganoderma*, Determination of the presence or absence of fruiting body of *Ganoderma* was documented. The seeds which were harvested and cleaned were then grouped by the type of treatment and then the roots were separated from the shoots (stems). Part of the seed that is classified as part of the plant roots are located just below the former location of the branch or branches from the first base.

After the separation of shoots and roots, the weight of each seedling roots and stems were measured to determine the fresh weight of roots and shoots of seedlings. After the fresh weight measurements, parts of seeds were wrapped in a paper and dried in the oven for 24 hours at 110^0 C. The second measurement was done for both roots and shoots of each seedling to calculate the dry weight of roots and shoots.

Data Analysis

The experimental design was used complete randomized design based on the assumption that the research conducted on a homogeneous condition. Homogeneity test is based on experiments which only require element differentiation of treatment given. The calculation is performed with use of SPSS, while Duncan's test was used for further testing.

Results and Discussion

In general, the result of control treatments measurement has a better growth compared to the inoculation treatment. This result can be seen on the accretion parameter of heights and number of leaves. It is possible to do the observation on the treatment of the inoculation with the negative growth tendencies. The height measurement seed, according to the regulation of The Minister of Forestry no.3 in 2004, is the height measurement from the base of the plant seed to the top growing spot using centimeter unit. From that definition, if the growing point is dead, then the growing spot underneath can replace it, so the height will be reduced.

The pathogenicity test shows that *Ganoderma* SP2 has more pathogenic than *Ganoderma* SP1. This result can be seen on the heights of seeds that were inoculated with *Ganoderma* SP2 are lesser than the heights of seeds that were inoculated with *Ganoderma* SP1.

The root treatment application shows that both controls and treatments blocks have a better growth than sengon seedlings using root cutting treatment. This result corresponds with Deselina works (1999), where root cutting will produce more vigor seeds and stronger roots. This positive result of root cutting application in inoculation treatment, reveals that infection rate of *Ganoderma* is lower than the healing process as the effect of the cutting.

The effect on the size of sengon seedlings showed that inoculated seedlings in the wood with 3 centimeters diameter as a foodbase hampered both their height and leaves growth compared to the seedlings with 4 centimeters or 5 centimeters diameter in the same foodbase. This result

indicates that wood pieces with 3 centimeters diameter as a foodbase, is more optimal in spreading *Ganoderma* than the 4 or 5 centimeters.

Application of foodbase type differentiation in sengon seedlings showed that wet foodbases such as PDA is easier than dry foodbases such as wood pieces, in transmitting *Ganoderma* into plant's roots. This can be seen from the calculation where the inoculated sengon seedlings with the wet foodbase such as PDA, has a higher disruption than the dry one. These results occurred in height parameter. Root sprout ratio measurement in this research shows an effect of the *Ganoderma* infection on the plant. General calculation of root sprout ratio will generally support the result using SPSS. Root cutting treatments on seedling growth sengon resulted in high seedling leaves and seedling growth better than the group without cutting the roots of sengon seedlings (Figure 1 and 2).

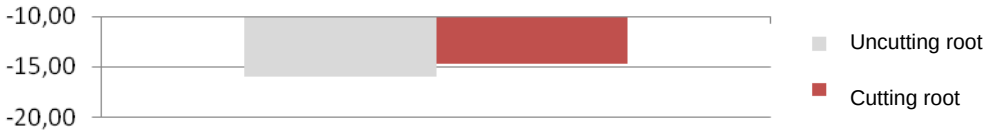

Figure 1 Comparison of the rate of increase in the child leaves the root treatment. Grey block shows the uncutting roots and red block shows the cutting roots.

Figure 2 Comparison of the high rough at the rate of root treatment. Grey block shows the uncutting roots and red block shows the cutting roots.

Conclusion

The control treatments shows better growth compared to the inoculation treatments. Inoculation treatments at high pathogenicity on seedlings derived from *Ganoderma* sengon sp2. These were shown by the magnitude inhibition. These obtained from the average growth of the inoculated seedlings sengon with parameters of the number of leaves and seedlings height. Observations showed that sengon seedling growth better by cutting the roots treatments, both in the controls and inoculation treatments.

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A PCR-based Technique for Detection *Cylindrocarpon destructans*, the Causal Agent of Grapevine Black Foot Disease

Raed A. Haleem[a,] *, Samir K. Abdullah[b], Jaladet M. S. Jubrael[c]

[a] *Plant Protection Department, Faculty of Agriculture and Forestry, University of Duhok, Iraq*
[b] *Department of Biology, Faculty of Science, University of Zakho, Iraq*
[c] *Scientific Research Center, University of Duhok, Iraq*
** Corresponding author: Plant Protection Department, Faculty of Agriculture and Forestry, University of Duhok, Iraq. Tel.: +4947504015992*
raed@uod.ac

Abstract

The symptoms of Grapevine black foot disease caused by *Cylindrocarpon destructans* including reduction of the plant vigour with small-sized trunks, reduction in root biomass, black discolouration and brown to dark streaks in wood mainly at the base of the rootstock. According to morphological and cultural characteristics of *Cylindrocarpon*, all isolates from infected roots of declined plants were identified as *C. destructans.* PCR technique was performed for accurate identification of *C. destructans* isolates. Ten isolates of the fungi were selected from different locations. Those isolates were subjected to species-specific PCR assay. Total genomic DNA was isolated from pure cultures of the isolates. The average DNA yields ranged between 1.5-6.7 µg/ml with a purity 1.6-1.8. The specific primers for *C. destructans* were used to amplify the ITS region of nuclear ribosomal DNA (rDNA) containing ITS1, ITS2 and the intervening 5.8 rRNA genes of *Cylindrocaropon.* TheITS sequence could successfully and appropriately confirmed that all isolates were correctly identified as *C. destructans*. This fungus obtained from this study was reported for the first time in Iraq.

Keywords: Black foot disease, Cylindrocarpon destructans, Grapevine, PCR

Introduction

Species of *Cylindrocarpon* Wollenw. are common soil inhabitants that often associated with roots of herbaceous woody plants. Grasso and Magnano di San Lio (1975) described black foot symptoms from nursery plants with black discoloration and gum inclusions in xylem vessels of affected rootstocks. Scheck *et al.* (1998) also described black foot symptoms as dark-brown to black streaking in the vascular tissue of young (2–5 year-old) grapevines.

There are two negative impacts of this disease on nursery seedling production. First, these fungi cause seedling mortality in nurseries up to 50% (Anderson *et al.* 1962; Anonymous, 1993). Second, infected seedlings have a lower survival rate after out planting to reforestation sites. An important factor compounding this problem is that in some cases the symptoms are not visible on infected seedlings but disease can develop after transplantation.

Detection of plant pathogens directly from infected tissues has been reported for several agricultural plant pathogens (Le´vesque *et al.* 1994 & O'Gorman *et al.* 1994). In addition, PCR has been used to detect soil pathogens directly from infested soil (Henson *et al*., 1993). This molecular detection approache is ideally suited for the study of root rot organisms because of the difficulty in isolating and identifying some of these fungi. Hamelin *et al*. (1996) designed species-specific primers (Dest1 and Dest4) to detect *C. destructans* from conifer seedlings. Using these primers in direct PCR assays on DNA extracted from *C. destructans* cultures isolated from grapevines in Portugal, obtained a DNA fragment of 400 bp; The universal primer ITS4 and the fungus-specific primer ITS1F were used in a first-stage fungus-specific amplification, followed by a second-stage amplification with the primers Dest1 and Dest4 using the PCR product from stage one. This is a simple and reliable method for detection of *Cylindrocarpon* spp. directly from infected grapevines

(Nascimento *et al*., 2001).The objective of this study was develop an efficient and reliable detection method based on PCR for the identification and detection of *C. destructans* from infected seedlings.

Materials and Methods

Fungal isolation

Fungal isolation was done using two methods, i.e. isolation method from vine root and moist chamber isolation method.

Isolation from vine roots

Small pieces of vine roots collected from five locations in Duhok governorate (Bajelor, Badi, College nursery, Malta nursery and Nizarke) were surface sterilized respectively by placing in 70% ethanol for 30 s, 1% NaOCl for 1 min and again in 70% ethanol for 30 s and then dried by filter papers. Pieces of sterilized tissues were plated onto 2% potato dextrose agar (PDA) (Himedia Laboratories Pvt. Ltd. - India) containing 0.25 mg/ml chloramphenicol. Hyphae growing out from the tissue pieces were cut and subcultured onto fresh PDA plates, and incubated at 25±2 °C (Van Niekerk *et al*, 2004).

Moist chamber method

Cuttings were made from vine roots and placed in 90 mm petridishes containing sterilized moist filter paper. Plates were incubated at room temperature until fungal growth observed. Propagules (spores, mycelia) were transferred to Potato-dextrose-Agar (PDA) plates. Pure cultures of each isolate were obtained by excising a hyphal tip from colony margins and plating it onto fresh PDA.

Phenotypical characterization

All isolates were grown on PDA and MEA at 25°C in darkness or under NUV + fluorescent illumination with a 12-h photoperiod (Philips /36W) for 10 days until cultures sporulated. The colonies were further incubated for 20 days to determine the presence or absence of chlamydospores. The diameter of 20 chlamydospores per isolate was measured. Length and width of 40 conidia (microconidia and one-, two-, and three-septate macroconidia) were measured. Isolated fungi were identified based on the characters in culture and on natural substrates (Domsch *et al,* 1980; Watanabe, 2002; Petit & Gubler, 2005).

DNA extraction and PCR amplification of ITS region

Ten isolates (DC1-DC10) were selected to confirm the identification by a specific primer of the ITS region. Isolates were collected from five locations in Duhok governorate (Bajelor, Badi, College nursery, Malta nursery and Nizarke).

Genomic DNA was extracted according to a method reported by Borges *et al.* (1990). The specific primers of *C. destructans* ITS region (Dest1 5'-TTGTTGCCTCGGCGGTGCCTG-3', Dest4 5'-GGTTTAACGGCGTGGCCGCGCTGTT-3') were used to amplify the ITS region of nuclear ribosomal DNA (rDNA), containing ITS1, ITS2 and the intervening 5.8 rRNA gene (Hamelin *et al.,* 1996). The PCR reactions were carried out in a total volume of 25 µl, in thin-walled, 0.5 µl Eppendorf tubes.Master mix was prepared for 12 samples of each fungus (10 isolates plus 2 control) by mixing 30 µl of 10XPCR, 30 µl of dNTPs, 24 µl forward primer, 24 µl Reverse primer, 12 µl MgCL2, 4.8 µl of Taq polymerase enzyme and de-ionized distilled water was added to a final volume of 252µl. The solution mixed and spun for 10 second in a microcentrifuge. Then, the mixture was distributed in PCR tubes. All these steps were done on ice. Amplification was carried out in an automated thermal cycler (Delphy 1000, Oracle Biosystems, MJ Research Inc., Watertown, MA, USA) according to the following program: An initial denaturation at 94°C for 4 min, after which 30

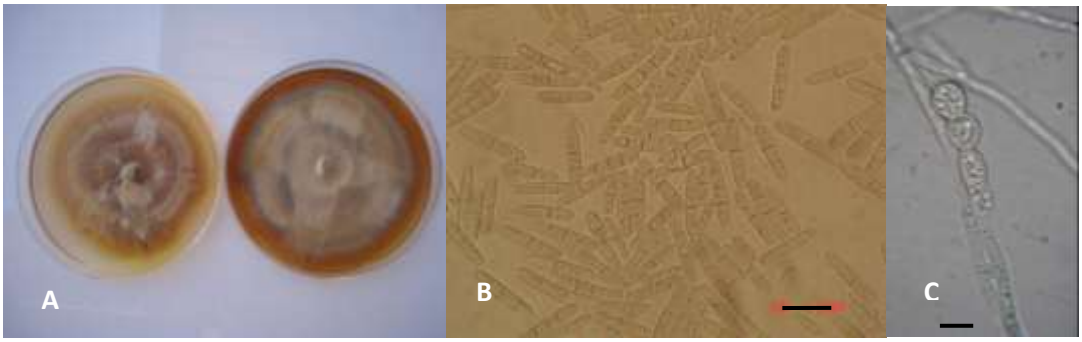
cycles of denaturation (1 min at 94°C), primer annealing (1 min at 58°C) and primer extension (1.5 min at 72°C) were performed (Alaniz *et al.,* 2007). A final extension was performed at 72°C for 10 min Amplification reactions were conducted at least twice, in two separate experiments. For each isolate, 5 µl of PCR products were mixed with 7µl loading buffer and then analyzed by electrophoresis in 2% (w:v) agarose gels with 1xTBE buffer visualized by UV fluorescence.

Results and Discussion

Phenotypical characterization of *C. destructans*

Cylindrocarpon destructans (Zinssm.) Scholten, Neth. JL. PL. Path. 70 (Suppl. 2) 9 (1964). Fig. (1) A – C. Telemorph: *Neonectria radicicola* (Gerlach & L. Nillsson) Mantiri and Samules Canada J. Bot. 79: 339 (2001).

Colonies on MEA reached a diameter of 78 mm on PDA and 80 mm on MEA after 20 days at 25°C. Colony surface slimy to felty; aerial mycelium typically sparse to felty, white to buff or a shade of brown. Colony reverse was orange to dark brown. Conidiogenous cells formed apically on densely, irregularly branching clusters of cells borne laterally on otherwise undifferentiated vegetative hyphae. Conidiophores 65 µm tall. Macroconidia cylindrical, mainly 4-celled, 18 – 45 (-47) × 4 – 8 µm. Microconidia, cylindrical, 1-celled, 8 – 11 (-12) × 3 – 4 µm. Chlamydospores yellowish brown, ovate to ellipsoidal, a few in a chain, 8 – 10 (-15) µm in diameter. Conidial dimensions were in concordance with the previous identification (Petit & Gubler, 2005).



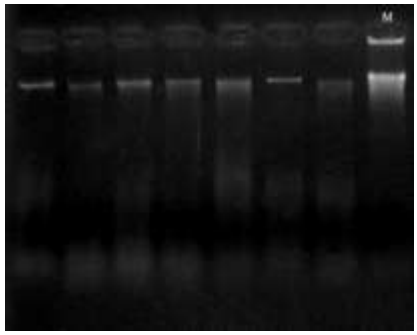
A) Twenty- day old colony on PDA-left, and MEA-right . B) Microconidia and macroconidia. Scale bar: 30 µm, C) Mycelia and Chlamydospores, Scale bar: 15 µm.

Figure 1. *Cylindrocarpon destructans*.

Molecular detection of *C. destructans*

Genomic DNA isolation and purification

Suitable yields of genomic DNA were obtained from repeated experiments with an average yield of 1.5-6.70 µg/ml and a purity of about (1.6-1.8) determined by spectrophotometer ratio A260/A280. The molecular weight of DNA samples was estimated using 1% agarose gel electrophoresis containing ƛ DNA sample as control (Fig. 2). Ratios above 2.0 correspond to RNA contamination, while ratios below 1.6 suggest protein contamination (Sinha *et al.*, 2001).

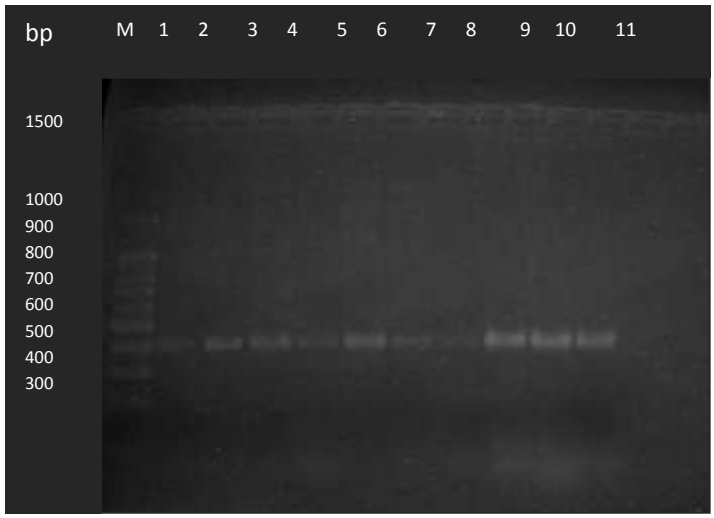


M: represents unrestricted λ DNA as a standard molecular weight marker. Lane1- 7: Whole Genomic DNA of some *C. destructans* isolates isolated from different locations of Duhok Governorate.

Figure 2. Agarose gel electrophoresis 1% at 70 volt for 45 minutes.

Species specific primers

All isolates of *C. destructans* collected from different locations of Duhok Governorate were amplified by two specific primers (Dest1, Dest4) which were designed by Hamelin *et al,* (1996). A PCR fragment of about 400-bp was obtained for all of them. The agarose gel electrophoresis of amplified products with this specific primer is shown in Figure (3). Other investigators have used the same primers to amplify the ITS region of *C. destructans* (Hamelin *et al.,* 1996; Alaniz *et al.,* 2007).



Lanes 1 (DC1)-10(DC10), *C. destructans* isolates. Lane 11, negative control of sterile distilled water; lane M, 1Kb Plus DNA Ladder.

Figure 3. Agarose gel of the PCR products using primer pairs Dest1 – Dest4.

This is the first molecular detection work on *C. destructans* in Iraq. In this study, the differences in the intensity of bands were not taken in consideration; despite they may reflect the differences in copy number of the priming sites in the individual genome. The band intensity may also be attributed to the difference of DNA concentration of individual isolates. This method did not require going through all classical methods and only in a few hours the results could be obtained. This approach is particularly well suited to soil organisms that are difficult to identify or isolate because of the presence of other aggressive species.

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Photosynthetic Light Reactions in C_4 Photosynthesis

Naoya Nakamura[a], Yuri Nakajima Munekage[a], Akiho Yokota[a*]

[a]*Graduate School of Biological Sciences, Nara Institute of Science and Technology*
** Corresponding author: Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, 630-0192 Japan*
Tel.: +81 0743 72 5568; fax: +81 0743 72 5569.
yokota@bs.naist.jp

Abstract

The most productive wild plants and crops use C_4 photosynthesis (Brown, 1999). C_4 photosynthesis requires the coordinated functions of two cell types in leaves, namely mesophyll (M) - and bundle-sheath (BS) - cells (Hatch, 1987). Atmospheric CO_2 is initially fixed by phosphoenolpyruvate carboxylase (PEPC) in M cells. The resulting products, C_4 acids are transported into BS cells where CO_2 is released by decarboxylation of C_4 acids and refixed by riburose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) functioning in the Calvin cycle (C_3 cycle). Since this process increase CO_2 levels at the site of RuBisCO in the BS cells, the oxygenase reaction of RuBisCO is largely reduced and RuBisCO can achieve maximal catalytic activity of CO_2 fixation. Therefore, C_4 plants have higher potential efficiencies in the use of light, water and nitrogen than C_3 plants (Long, 1999). C_4 plants have evolved from ancestral C_3 plants (Sage *et al.*, 2011). In C_4 plants, not only the CO_2 metabolism but a manner of light reactions which is a process to produce ATP and NADPH used for CO_2 metabolism has been changed. However, mechanism of light reactions and its physiological roles on C_4 photosynthesis are not yet fully understood. Here, we introduce latest findings and perspectives of physiological roles of light reactions in C_4 photosynthesis.

Keywords: C_4 photosynthesis, cyclic electron flow, Flaveria, Zea mays

The structure of mesophyll and bundle-sheath chloroplasts: the regulation of grana formation

Light reactions take place on thlakoid membranes in chloroplasts. The thylakoid membranes are arranged in stacked (grana) and unstacked (stroma lamellae) in C_3 plants. Supercomplex of photosystem (PS) II and light-harvesting complex of photosystem II (LHC-II) are localized in grana thykoids while most of PSI and ATP synthase are localized in stroma lamellae (Albertsson, 2001). The grana stacks are more developed in low light intensity than in high light intensity, the changes are assumed to optimize the photosynthetic efficiency in C_3 plants (Ballantine and Forde, 1970). In NADP-malic enzyme (ME) type C_4 plants such as *Zea mays*, *Sorghum bicolor* and *Flaveria trinervia*, grana thylakoids are observed in the M chloroplasts like chloroplasts of C_3 plants, but BS cells possess chloroplasts where grana thylakoids are largely decreased, as shown in Fig. 1 (Laetsch *et al.*, 1965, Laetsch and Price, 1969, Hofer *et al.*, 1992). Interestingly, grana formation was observed in BS chloroplasts of maize leaves at very low light intensity but was completely abolished at normal light intensity (Brangeon, 1973). Decrease in grana stacks of BS chloroplasts is likely induced by high light signal and those systems would have been recruited from C_3 plants during C_4 evolution.

LHC-II is suggested to be involved in grana formation by binding thylakoid membranes in C_3 plants (Allen and Forsberg, 2001, Standfuss *et al.*, 2005). LHC-II is mainly present as trimer on grana thylakoids. It is reported that in *Flaveria trinervia* the amounts of LHC-II polypeptides in BS chloroplasts is only slightly reduced compared with that in M chloroplasts (Hofer *et al.*, 1992) and a significant amount of LHC-II polypeptides are also detected in fractionated stroma lamellae (Munekage *et al.*, 2010). Moreover, in maize, LHC-II trimer is identified in BS thylakoid membranes

using Blue-Native PAGE, although BS chloroplasts of Maize are almost grana-free (Romanowska *et al.*, 2008). These results suggest that amounts of LHC-II polypeptide and the presence of LHC-II trimer are not sufficient for grana formation. Perhaps the additional mechanism is involved in the formation of thylakoid membranes with reduced grana in BS chloroplasts.

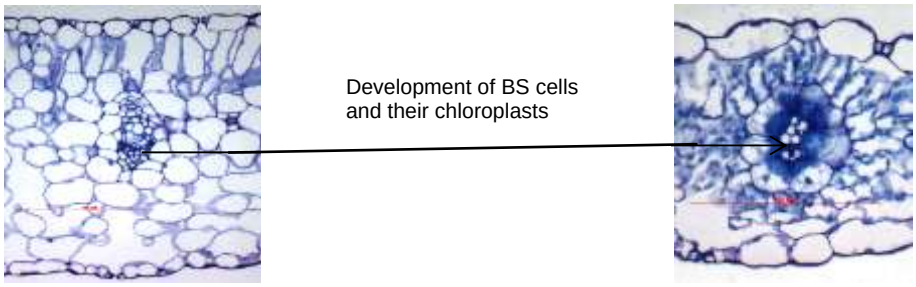

Figure 1. Light micrograph's of leaf transverse section of *Flaveria species.*

Regulation of linear electron flow and cyclic electron flow around PSI in C_4 photosynthesis

Linear electron flow operates to produce the both ATP and NADPH. In linear electron flow, the electrons excised from water molecules at PSII are transferred to PSI through the cytchrome b_6f complex, and finally NADPH is produced. Protons are transported across the thylakoid membranes from stroma side to lumen side during the process of electron transfer. This proton gradient is used for ATP synthesis (Fig. 2). On the other hand, cyclic electron flow around PSI (CEF1) contributes to only ATP production. In CEF1, electrons are returned from accepter side of PSI to plastoquinone which mediate electron transport from PSII to cytchrome b_6f complex (Shikanai, 2007). This electron transport contributes to form proton gradient without NADPH production. Two CEF1 pathways have been identified in C_3 plants.

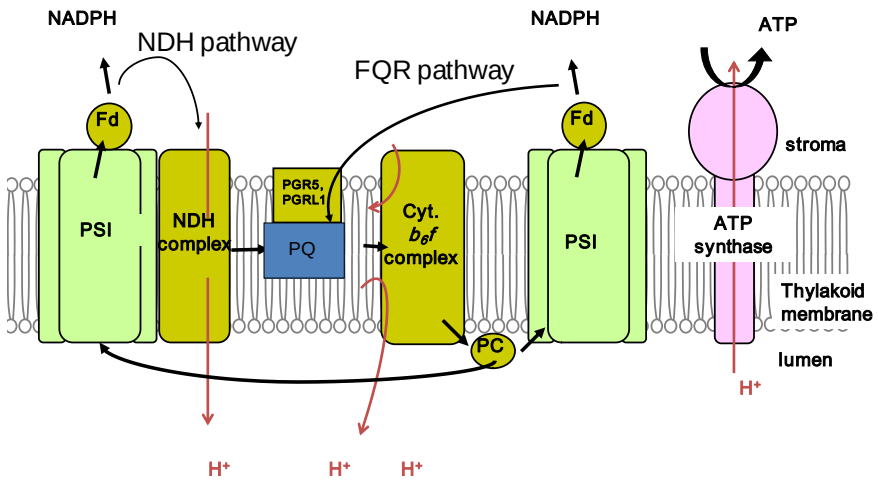

Figure 2. Schematic model of PS1 cyclic electron transport.

The first pathway is called as ferredoxin-plastoquinone reductase (FQR) pathway involving PROTON GRADIENT REGULATION 5 (PGR5) and PGRL1 (Munekage *et al.*, 2002, Dalcorso *et al.*, 2008). The other pathway is NAD(P)H dehydrogenase (NDH) pathway involving a plastidial NDH

complex composed of the eleven plastid-coded subunits (NDH-A to -K) and the over 15 nuclei-encoded subunits (Peng *et al.*, 2011). In C_3 plants, the FQR pathway contributes for maintaining the production ratio of ATP/NADPH and for photoprotection, whereas the NDH pathway functions in stress resistance in C_3 plants (Munekage *et al.*, 2004, Wang *et al.*, 2006, Munekage *et al.*, 2008). Localization of the NDH complex and PGR5 on stroma lamellae and distribution of PSII and PSI on thylakoid membrane suggest that CEF1 takes place in stroma lamellae (Rumeau *et al.*, 2005, Munekage *et al.*, 2010).

In C_4 plants, increased CEF1 activity and decreased linear electron transport is induced. In NADP-ME type C_4 plants, BS chloroplasts of mature leaves show the inhibition of PSII activity (Woo *et al.*, 1970). The composition of PSII complex in BS chloroplasts is investigated by many researchers using *Zea mays*, *Sorghum bicolor* and *Flaveria trinervia*. Several studies are suggested that the subunits of PSII complex, especially those of oxygen evolving complex are largely reduced in BS chloroplasts compared with M chloroplasts (Sheen *et al.*, 1987, Oswald *et al.*, 1990, Hofer *et al.*, 1992). BS chloroplasts produce only limited amounts of ATP and NADPH from liner electron flow activity, because in this chloroplasts, PSII activity is significantly inhibited (Schuster *et al.*, 1985). Since NADPH is supplied by decarboxylation of C_4 acids by NADP-ME, only ATP is required to be produced in BS chloroplasts of NADP-ME type. The ATPs are considered to be produced by CEF1 activity. Significant activity of CEF1 compared with C_3 plants has been reported in *Zea mays*, *Sorghum bicolor* (Herbert *et al.*, 1990, Asada *et al.*, 1993). The higher amounts of NDH complex and PGR5 in BS cell than M cell also supports activation of CEF1 in BS cell of NADP-ME type C_4 plants (Kubicki *et al.*, 1996, Munekage *et al.*, 2010). On the other hand, ATP demands are increased in M chloroplasts in NAD-ME type C_4 metabolism. In *Salsola laricina* and *Halocharis gossypina* carrying NAD-ME type C_4 photosynthesis, lower grana index and higher ratio of PSI/PSII in M chloroplasts than BS chloroplasts are reported (Voznesenskaya *et al.*, 1999). Moreover, subunits of NDH complex are enriched in M chloroplasts of NAD-ME type C_4 plants (Takabayashi *et al.*, 2005). Activated CEF1 likely contributes to the ATP production in C_4 photosynthesis. However, for final conclusion, it is need to investigate phenotype of transgenic plants in which genes involved in CEF1 are knocked down.

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Heavy Metals Concentration in Irrigation Water, Soils and Fruit Vegetables in Coastal Area, Kota Bharu, Kelantan, Malaysia

Aweng, E. R.[a] , Karimah, M.[a] Suhaimi, O.[a]

[a]*Faculty of Agro Industry and Natural Resources, Universiti Malaysia Kelantan, Malaysia.*
** Corresponding author: Faculty of Agro Industry and Natural Resources, Universiti Malaysia Kelantan, Malaysia.*
Tel: +06097717247; fax: +06097717232.
aweng@umk.edu.my

Abstract

This study was conducted to evaluate the levels of selected heavy metals namely Aluminium, Boron, Cadmium, Iron, Lead, Manganese and Zinc in irrigation water, soils and selected fruit vegetables (brinjal, chilli and lady's fingers) which were cultivated in Kampung Badang, Pantai Cahaya Bulan in Kota Bharu, Kelantan, Malaysia. The water used for irrigation in Kampung Badang, Pantai Cahaya Bulan had the highest concentration of Boron (3.5 ppm) followed by Manganese (1.105 ppm), Iron (0.233 ppm), Lead (0.22 ppm), Zinc (0.217 ppm), Aluminium (0.214 ppm) and Cadmium (0.0853 ppm). However, in general, the quality of the irrigation water complied with the Class IV, Interim National Water Quality Standard for Malaysia, INWQS, 1985 (standard for irrigation water) and Food and Agriculture Organization of the United Nations (FAO) Standard (1985) except Cadmium. The irrigation water results were also compared with the Indian Standard (Awashthi, 2000), where in general, all the measured parameters complied with the standards except Cadmium (0.01 ppm) and Plumbum (0.10 ppm). Meanwhile, the concentration of Aluminium, Iron, Boron, Zinc, Manganese, Lead and Cadmium in soils were ranged from 2.858 - 3.5ppm, 3.753 – 3.92 ppm, 3.753 – 3.92 ppm, 0.667 – 1.133 ppm, 0.253 – 0.653 ppm and 0.68 – 1.307 ppm, 0.072 – 0.181 ppm and 0.0283 – 0.0844 ppm respectively. The concentrations of heavy metals in soils were also complied with the Indian Standard (Awashthi, 2000) and the European Union (EU) Standards (2002). In addition, the concentration of Aluminium, Iron, Boron, Zinc, Manganese, Lead and Cadmium in vegetables ranged from 0.071 – 0.22 ppm, 1.28 – 2.76 ppm, 2.367 – 2.467 ppm, 0.273 – 0.32 ppm, 0.064 – 0.098 ppm, 0.0127 – 0.138 ppm and 0.0482 – 0.053 ppm respectively. All heavy metals analyzed in the vegetables also complied with the Indian Standard (Awashthi, 2000), World Health Organization (WHO)/FAO (2007) and EU Standards (2006).

*Keywords***:** fruit vegetables, heavy metal, soil, ferum, manganese

Introduction

Contamination of the environment with toxic heavy metals has become one of the major causes of concern for human kind. Heavy metals in surface water bodies, ground water and soils can be either from natural or anthropogenic sources. The two basic categories of pollution are organic and inorganic (Duke &Williams, 2008). Inorganic pollution is basically from heavy metals. Chemical substances such as heavy metals are one of the factors which contribute to environmental pollution, and it was believed that it can disrupt living ecosystem (Kabata-Pendias & Pendias, 2001). Currently, anthropogenic inputs of metals exceed natural inputs due to increased urbanization and industrialization. Industrial wastes, atmospheric deposition from crowded cities and other domestic wastes are among the major sources of heavy metals in the surface water, ground water and soils (Varalakshmi and Ganeshamurthy, 2010). On the other hand, heavy metals such as Cadmium, Copper, Plumbum, Nickel, Chromium, and Mercury may be present in soil from the parent materials during soil formations (Plaster, 2003). Soil is a supporting layer for all organisms in the world. The most important thing is soil acts as a medium for plant growth which can recycle the nutrient and resources needed by plants. Soil will absorb heavy metals in the polluted river as well as ground water and these will cause side effect for vegetable growth. As root

grows in the soil, it will absorb water and nutrients in solution (Plaster, 2003). Heavy metals that are attached with soil water and soil particles will be absorbed by plant roots and accumulated in vegetables (Alirzaveya *et. al*. 2006). Another patch way for heavy metals to get into vegetables is via irrigation water which is contaminated by heavy metals.

This present study was aimed at finding out the levels of contamination of seven heavy metals viz. Aluminium (Al), Boron (B), Cadmium (Cd), Iron (Fe), Lead (Pb), Manganese (Mn) and Zinc (Zn) in irrigation water, soils and selected fruit vegetables in Kampung Badang, Pantai Cahaya Bulan in Kota Bharu, Kelantan, Malaysia. A number of studies had been conducted by researchers to identify the levels of heavy metals in vegetables from various sources. Anita *et. al*. (2010) stated that, waste water irrigation led to the accumulation of heavy metals in soil and consequently into the vegetables, while, Miclean *et. al*. (2000) found out that the metal accumulation in vegetables grown in the vicinity of industrial sites represents a potential risk for public health. Works by Garcia *et. al*. (1981) had shown that some common vegetables are capable of accumulating high levels of metals from the soils.

Materials and methods

The study sites covered the agriculture area around Kampung Badang, Pantai Cahaya Bulan, Kota Bharu, Kelantan, Malaysia (Figure 1). Kampung Badang, Pantai Cahaya Bulan, a suburban area located in the coastal area in the north west of Kota Bharu.

Figure 1: Study Area.

Samples of the edible fruity vegetables were randomly collected from 5 x 5 m area of two different fields. The fruit vegetables namely brinjal (*Solanum melongena),* chilli *(Capsicum anmum)* and lady's fingers (*Hibiscus esculentus*) were collected at fruiting stage or at least at 60 days after planting. Five fruits were collected for each type of vegetables. The collected samples were stored in polyethylene bags, each sample bag for each vegetable before taking them to the laboratory for the analysis. The soil that was used to grow the vegetables was randomly collected. Each location comprised three samples; one sample was taken from each type of vegetable soils. In addition, in each type of vegetable soils comprised three points, where these three point samples were mixed as one sample with a total weight of about 50 grams. Therefore, a total of three soil samples were collected for this study. A metal spade was used to collect the soil at 5 cm depth from the surface. The samples were then placed in plastic bags, each sample for each bag before taking them to the

laboratory for analysis. The source of irrigation water for this cropland was tap water from Kelantan Water Berhad. Water samples used for irrigation were collected in a 500 ml washed polypropylene bottle and 1 ml of concentrated acid nitric (HNO_3) was added to the samples to avoid microbial activity. For heavy metal extraction, 1g ground wet sample of plant or soil was digested in 3 ml concentrated nitric acid (HNO_3) and 1 ml of concentrated hydrochloric acid (HCl) (3+1, (v/v)). The mixtures were oven heated for 3 hours at 40 ^{0}C. After that, the samples were removed from the oven and let them cool for a few minutes. Then they were diluted with 150 ml de-ionized water. The samples were then adjusted to pH 5 to 6 via acid base adjustment with hydrochloric acid and sodium hydroxide. Then, the samples were analyzed using Spectrophotometer DR5000. Meanwhile, irrigation water analysis followed USEPA Approve for wastewater analyses and adapted from Standard Methods for the Examination of Water and Wastewater (2005) via Spectrophotometer DR5000.

Results and discussion

The results obtained showed that in soil, the concentration of Aluminium ranged between 2.9 ppm and 3.5 ppm, where soil for growing chilli and brinjal recorded 3.5 ppm and soil for growing lady's fingers recorded 2.9 ppm. The concentration of Boron was between 0.67 ppm and 1.13 ppm and the highest recorded in soil for grow lady's fingers. Meanwhile, Cadmium concentration recorded between 0.028 ppm and 0.084 and the highest rercorded in soil for growing chilli. In addition, the concentration of Iron was between 3.75 ppm and 3.92 ppm where the highest concentration recorded in soil for growing brinjal. The concentration of Plumbum was between 0.072 ppm and 0.181 ppm where the highest recorded in soil for growing chilli. Manganese and Zinc were also measured for this study where the results showed that, the concentration of Manganese recorded was between 0.68 ppm and 1.31 ppm and Zinc recorded between 0.25 ppm and 0.65 ppm (Figure 2, 3 and 4). All Pb, Cd and Zn values complied with the Indian Standard (Awashthi 2000) and European Union Standards, EU (2002). Generally, concentration of Iron was the highest followed by Aluminium, Manganese, Boron, Zinc, Lead and Cadmium.

Among the heavy metals measured in three types of fruit vegetables, Boron concentration was the highest (2.37 ppm – 2.47 ppm), where the highest concentration was recorded in brinjal and Iron was the second highest (1.28 ppm – 2.76 ppm), and where the highest concentration was recorded in chilli. Meanwhile, the concentration of Cadmium ranged between 0.048 ppm and 0.053 ppm, where the highest concentration was recorded in brinjal. The concentration of Lead ranged between 0.013 ppm and 0.138 ppm with brinjal that recorded the highest concentration. In addition, the concentration of Zinc was ranged between 0.27 ppm and 0.32 ppm with the highest concentration recorded in brinjal (Figures 2, 3 and 4). All Pb, Cd and Zn values complied with the Indian Standard (Awashthi 2000), WHO/FAO (2007) and Commission Regulation, EU (2006).

The concentrations of Aluminium, Boron, Cadmium, Iron, Lead, Manganese and Zinc in irrigation water that was collected were 0.214 ppm, 3.5 ppm, 0.085 ppm, 0.233 ppm, 0.22 ppm, 1.105 ppm and 0.217 ppm respectively. Pb, and Zn values complied with the Indian Standard (Awashthi 2000), FAO (1985), and Class IV, Interim National Water Quality Standard for Malaysia (INWQS 1985) but not Cd. The concentration of Cd in irrigation water recorded 0.0853 ppm exceeded the safe limits standard (0.01 ppm). The concentration of heavy metals in irrigation water in sequence were B>Mn>Fe> Pb>Zn>Al>Cd.

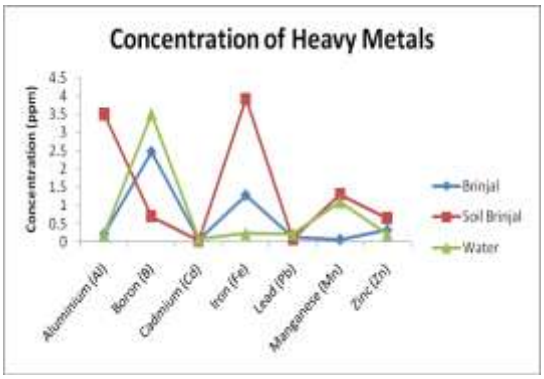

Figure 2: Concentration of heavy metals in brinjal, soil for brinjal and irrigation water.

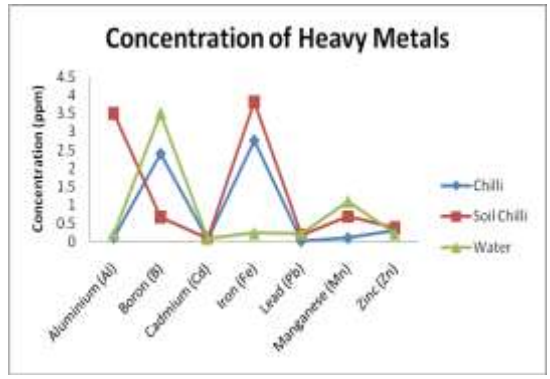

Figure 3: Concentration of heavy metals in chilli, soil for chilli and irrigation water.

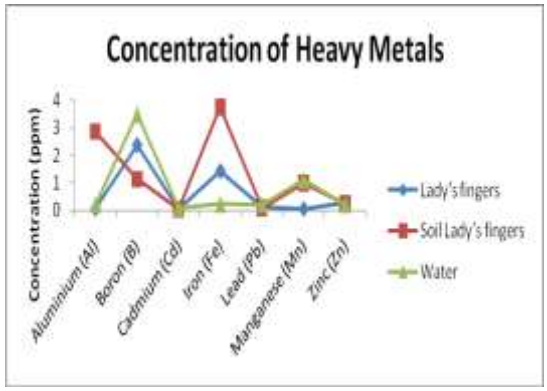

Figure 4: Concentration of heavy metals in lady's fingers, soil for lady's fingers and irrigation water.

The results showed that, the trend of heavy metal concentrations in vegetables was similar (positive correlation) with the trend of heavy metals concentrations in irrigation water. On the other hand, the trend of heavy metal concentrations in soil was also similar with the concentrations in vegetables and irrigation water except for Aluminium (negative correlation). Meanwhile, high concentrations of iron and manganese in soil was believed to be due to salt water intrusion (Figure 2,3 &4).

Conclusions

High concentrations of heavy metals in soil and irrigation water led to the accumulation of heavy metals in vegetables. Heavy metal concentrations varied among the test vegetables, which reflected the differences in their uptake capabilities and their further translocation to edible portion of the plants. The trend revealed that, high concentrations of Cd, Pb and Zn in soil and water led to high concentrations in vegetables, even though the concentrations in all vegetables were below the national and international permissible limits. Irrigation water was a dominant factor to determine the concentrations of heavy metals in vegetables compared to soil because irrigation water normally led to the accumulation of heavy metals in soil and consequently into the vegetables. Based on the

results, it showed that, Boron was found to be the highest in irrigation water as well as in vegetables but not in soil. The lower concentrations of heavy metals in vegetables were believed to be due to the good quality of irrigation water and absence of pollution in soil in the study area. It could be suggested that fruit vegetables in Kampung Badang, Pantai Cahaya Bulan were safe for consumption.

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The Contribution of Agriculture in a Local Greenhouse Gas Turnover

Arief Sabdo Yuwono

Dept. of Civil and Environmental Engineering, Bogor Agricultural University (IPB)
PO Box 220 Bogor, Indonesia. Tel.: +62 251 8627 225
arief_sabdo_yuwono@yahoo.co.id

Abstract

The general objective of this study is to analyze the contribution of agricultural sector in a local scale greenhouse gas turnover. It includes identification and quantification of the entire agricultural activities regarded as greenhouse gas sources as well as those concerned as greenhouse gas sink. The study was carried out in 2010 and based on a series of five years data compiled from a municipality in West Java Province as a case. The greenhouse gas sources were categorized into four sectors covering energy sector, solid waste, agricultural and animal husbandry sectors whereas the greenhouse gas sink was identified merely in agricultural sector. The amount of a single greenhouse gas emission was calculated by multiplication between a source unit and its corresponding emission factor. The total quantity of greenhouse gas emission generated by the municipality was summation of the whole sources from the entire sectors. The amount of the whole greenhouse gas sink was summation of the total sequestered carbon. The study result based on five years compiled data indicated that the average municipality greenhouse emission was in the order of 14.0 MT CO_2-e per year whereas the net sink was approximately 0.05 MT CO_2-e per year. The contribution of agricultural sector was merely 0.08 % of the total municipality greenhouse gas sources while its contribution on the greenhouse gas sequestration (as sink) was 2.39% of the emitted greenhouse gases. It can be concluded that based on the quantitative analysis, the local agricultural sector is a manner to sequester atmospheric carbon.

Keywords: agricultural sector, greenhouse gas, source, sink, turnover.

Introduction

Global warming, or more properly, global climate change (GCC), may be the most serious environmental challenge ever faced by mankind (Mackie and Cooper, 2009). There are many uncertainties surrounding the issue of global warming. However, several things about the phenomenon are certain. It is known that CO_2 and other greenhouse gases, such as CH_4, absorb infrared radiation by which earth loses heat. The levels of these gases have increased markedly since about 1850 as nations have become industrialized and as forest lands and grasslands have been converted to agriculture (Manahan, 2000). Global warming phenomenon which is nowadays concerned as a serious worldwide problem is essentially able to be mitigated locally based on the local governmental development program. The local governments can formulate action plans based on their local greenhouse gas (GHG) mass balance and database. A local GHG turnover is therefore necessary to estimate contribution of each sector involved in emitting the GHG. Greenhouse gases which deal with infrared-absorbing trace gases (other than water vapor) consist of carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O) and chlorofluorocarbons (CFCs). CFC was excluded from this study case due to the lack of CFC distribution and consumption data. The objectives of this study are to quantify the entire agricultural activities regarded as greenhouse gas sources as well as those concerned as greenhouse gas sink and to analyze the contribution of agricultural sector in a local scale greenhouse gas turnover.

Materials and Methods

The study was carried out in 2010 and based on a series of five (5) years data compiled from a municipality in West Java Province, called City of X, as a case. The greenhouse gas sources were categorized into four sectors covering energy sector, solid waste, agriculture and animal husbandry sector whereas the greenhouse gas sink was identified merely in agricultural sector. The amount of a single greenhouse gas emission was calculated by multiplication between a source unit and its corresponding emission factor as indicated in Box 1. The total quantity of greenhouse gas emission generated by the municipality was summation of the whole sources from the entire sectors. The amount of the whole greenhouse gas sink was summation of the total sequestered carbon.

Box 1. General equation and the related emission factor for agricultural sector data source

CO_2, CH_4 and N_2O emission from wetland or dryland paddy field:
- Data source: Centre Agency for Statistics, City of X, 2005-2009
- Emission factor: IPCC Guidelines for National Greenhouse Gas Inventories, 1996
- GHG mass = wetland area x plantation day x emission factor

Carbon sequestration by city re-greening program and estate crops plantation:
- Data source: Centre Agency for Statistics, City of X, 2005-2009 and BPLH, City of X, 2009
- Emission factor: IPCC Guidelines for National GHG Inventories, 1996
- Carbon sequestration = area x emission factor

Greenhouse Gas Source and Sink in Agricultural Sector

Agricultural activities regarded as greenhouse gas sources and sink in The City of X covers wetland paddy cultivation, dryland paddy cultivation, estate crops, and city re-greening program. The amount of the absorbed carbon was estimated simply from the area of the paddy cultivation. The similar case occurs in the estate crops as well. Therefore, the total amount of the absorbed carbon by those plant species was calculated directly based on the area of the cultivation.

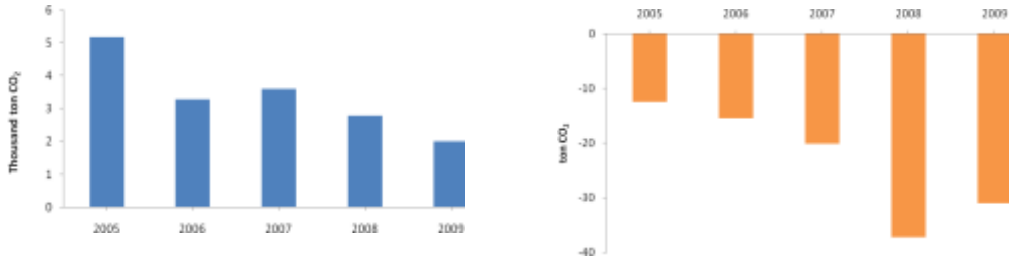
Contribution of Agricultural Sector in the Local Greenhouse Gas Turnover

The identified agricultural activities occurred in The City of X mentioned above are compiled altogether as agricultural sector. The cultivated area by each of these activities was identified and recorded by Centre Agency of Statistics, City of X. It was then compiled for five (5) years (2005-2009), in order to calculate the GHG emission quantity generated by agricultural sector. Other sectors contributing greenhouse gases are energy sector, solid waste generation, and animal husbandry. The contribution of energy sector on the total greenhouse gas production was estimated by multiplying the amount of the energy source quantity by its corresponding emission factor. It covers all fossil fuel consumption for the city electricity, transportation, and industry. The amount of the generated solid waste is assumed in the order of 0.51 kg/capita/day (IPCC, 1966). By using the total population data of the City of X, the total amount of methane emission can be estimated. For the parameter nitrous oxide (N_2O), the estimation method is almost the same as those used in the CO_2 and CH_4 parameters, except for the emission factor. Global warming potential (GWP) of CO_2 is one, N_2O is 21 whereas for CH_4 is 310.

Results and Discussion

Estimation of the emitted CO_2 from the wetland paddy field and dryland paddy field is presented in Figure 1. It shows that in general there is tendency where the amount of emitted CO_2 from the wetland paddy field is decreasing with the year. This is due to the ongoing encroachment of the modern irrigated agricultural area that was converted to be industrial area and other land

uses. In 2009 even, the total area of the wetland paddy field was less than a half of the total area in 2005 meaning that the amount of the emitted greenhouse gas becomes less than a half as well.



(a) (b)

Figure 1. CO_2 emission from wetland (a) and dryland (b) paddy field.

The amount of the sequestered GHG from dryland paddy field however, was not decreasing in the period of 2005-2009. It showed that the CO_2 sequestration from dryland paddy field in 2009 was almost threefold of that in 2005. This was simply due to the growing area of dryland paddy field in the City of X. The amount of methane emission from the wetland and dryland paddy field is presented in Table 1. It revealed that according to the paddy field area, the quantity of the emitted methane varies directly with the total paddy area. The more paddy field area, the higher amount of the methane emission, and vice versa. The generation of the methane in the wetland paddy area is initiated by anaerobic condition of the inundated paddy field as it was commonly practiced in conventional paddy cultivation system in Indonesia. As a result, methanogenic bacteria become more active to generate methane in such kind condition. The relative contribution of agricultural sector on total methane emission of The City X is presented in Figure 2. Agriculture contributes merely 0.01 % of the total methane emission where the largest portion was contributed by domestic solid waste due to the characteristic of the City X as an urban area. Another main issue of an urban area is the scarcity of agricultural land. Therefore, total methane emission generated by agricultural land is consequently relatively small.

Table 1. CH_4 emission from wetland paddy field

Area type	Year	Area (ha)	Plantation day (day/year)	Emission factor[1] (kg/ha/day)	kg CH_4[2]	ton CO_2-e
Wetland	2005	1959	200	0.096	37,613	790
	2006	1242	200	0.096	23,846	501
	2007	1364	200	0.096	26,189	550
	2008	1055	200	0.096	20,256	425
	2009	758	200	0.096	14,544	305
Dryland	2005	40	100	0.063	252.0	5
	2006	50	100	0.063	315.0	7
	2007	65	100	0.063	409.5	9
	2008	120	100	0.063	756.0	16
	2009	100	100	0.063	630.0	13

Data source: Centre Agency of Statistics, City of X (2005-2009)
[1] IPCC Guidelines for National Greenhouse Gas Inventories, 1996
[2] $kgCH_4$ = area x plantation day x emission factor

It is estimated that approximately 5 to 20 percent methane produced and released into the atmosphere is a by-product of the anaerobic decomposition of waste. A significant source of this type of methane production is solid waste disposal in landfills, where methanogenic bacteria break

down organic matter in the waste under anaerobic conditions to produce methane (Anonymous, 2002). The landfilling of municipal solid waste is a significant source of atmospheric methane (CH_4), contributing to10–20% of anthropogenic methane emissions (IPCC, 2001 in Einola et al., 2008). This is in line with the findings of Papageorgiou et al. (2009) stated that disposal of waste in landfills generates methane that has high global warming potential.

The third concerned parameter of GHG in agricultural sector is nitrous oxide (N_2O) where its theoretical emission quantity was determined by areas of the wetland and dryland paddy field that were multiplied by their corresponding emission factors. Relative contribution of agriculture sector on total nitrous oxide emission was very small, i.e. merely 0.1 percent. Agricultural sector was not an important source of GHG from the viewpoint of the generated nitrous oxide (N_2O).

Table 2. CH_4 emission per sector expressed in ton CO_2 equivalent (CO_2-e)

Sector	Average (ton CO_2-e)
Fossil fuel consumption	2,9E+05
Domestic solid waste	8,2E+06
Agriculture	5,2E+02
Animal husbandry	6,6E+02
CH_4 Total emission	8,4E+06

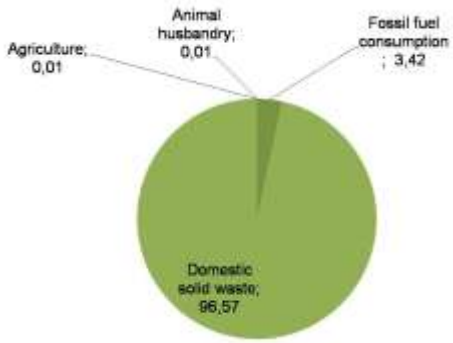
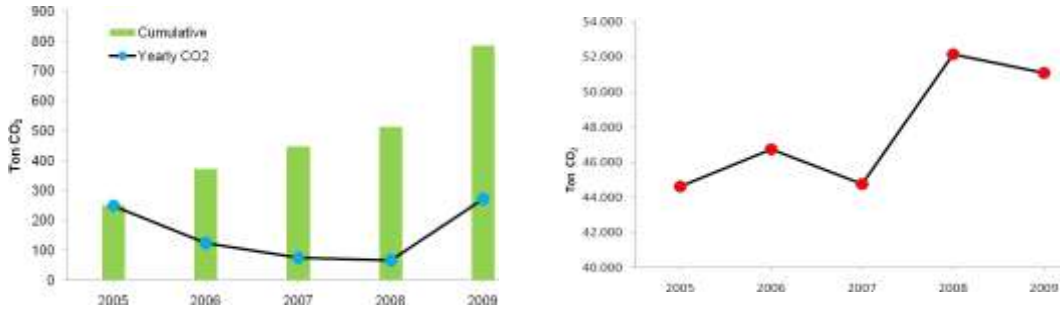


Figure 2. Relative contribution of agricultural sector on total methane (CH_4) emission.

Cumulative and yearly rate of carbon sequestration by means of city re-greening program is presented in Figure 3. The lowest part of the graph occurred in 2008 as a consequence of the re-greening program that achieved 10.0 hectares merely whereas the average area of the re-greening program normally was 23.8 hectares per year. In contrast however, the best success of the city re-greening program was reached in the last year (2009) where The City of X showed their commitment to reduce the greenhouse gas emission as re-greening area covered 41.2 hectares in a year.

Another way of carbon sequestration mode that was contributed by the city was through estate crop plantation (Figure 3). The identified crops in The City of X were papaya, banana, durian, manioc, mango, corn, sweet potato, and others. Total area of the estate crop plantation was in the range of 3049-3849 hectares per year that is spread over the sub-city area. By opening more plantation area, the amount of the captured atmospheric carbon dioxide would become higher. According to de Nevers (1995), the only methods we now know to slow or stop the buildup of CO_2 in the atmosphere are to reduce the use of fossil fuels (gas, oil, coal, peat, lignite) and to stop the deforestation of the tropical rain forests.



(a) (b)

Figure 3. Carbon sequestration by city re-greening program (a) and estate crop (b).

An important illustration of the emission profile of The City of X is an outline of the GHG emission and the related carbon sequestration quantity. Analysis on the compiled data revealed that agricultural sector plays a minor role in emitting greenhouse gas (GHG) as indicated in Table 3. Percentage of the CO_2 emission from agricultural sector was merely 0.17% whereas its contribution on CH_4 and N_2O emission were only 0.01% and 0.06%, respectively. On the other side, however, agriculture contributes about 2.39% (Table 3) sequestration process of the emitted carbon dioxide back to the earth through photosynthesis process. It indicated that agriculture plays a significant role in the greenhouse gas turnover in the local scale.

The quantity of the emitted methane (CH_4) and nitrous oxide (N_2O) were significantly higher than the amount of emitted carbon dioxide (CO_2). Such condition should be noticed carefully since the warming effect of CH_4 and N_2O are much stronger than CO_2. According to Jones (1992), although increasing atmospheric CO_2 is often assumed to be the major contributor to warming, the combined effect of a number of trace gases, principally methane, nitrous oxide and chlorofluorocarbons (CFCs), though present at concentrations that are two to six orders of magnitude lower than CO_2, can rival the effect of CO_2 because, per molecule they absorb infrared radiation much more strongly.

Table 3. Outline of the GHG emission of The City of X and the carbon sequestration

Emission/ sequestration	GHG	Sector	Average quantity (2005-2009) [ton CO_2-e]	%
Emission	CO_2	Paddy field	3.3E+03	0.17
		CO_2 Total emission	2.0E+06	99.83
	CH_4	Agriculture	5.2E+02	0.01
		CH_4 Total emission	8.4E+06	99.99
	N_2O	Agriculture	2.2E+03	0.06
		N_2O Total emission	3.6E+06	99.94
	Grand total emission (source)		14E+06	0.24
	Average contribution of agric. sector		2.0E+3	0.08
Sequestration	CO_2	Sequestration by:		
		• Estate crops	4.8E+04	2.38
		• City re-greening program	1.6E+02	0.01
	Grand total sequestration (sink)		4.8E+04	2.39

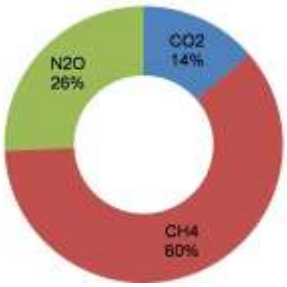


Figure 4. Relative emission of The City of X based on GHG component (2005-2009).

More severe condition occurred in The City of X where GHG parameters of CH_4 and N_2O were absolutely much higher than CO_2 (Figure 4). As a consequence, the warming effect on the atmosphere would be much higher too. The main potential reason for such condition was that the people of The City of X generated solid wastes higher than the other Indonesian urban population.

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The Influence of Global Climate Indices on Rainfall Distribution Pattern and Its Impact on Crop Yield in Gunung Kidul, Yogyakarta, Indonesia

Bayu DA Nugroho [a,*], Hiromitsu Kanno[b], Ryoji Sameshima [b], Hiroshi Fujii [c], Larry CM Lopez[c]

[a] *The United Graduate School of Agricultural Sciences, Iwate University, Japan*
[b] *National Agricultural Research Center for Tohoku Region, NARO, Japan*
[c] *Faculty of Agriculture, Yamagata University, Japan*
** Corresponding author: The United Graduate School of Agricultural Sciences, Ueda 3-18-34, Morioka, Iwate, Japan 020-8550. Tel.: +81 19 643 3408; fax: +81 19 641 9296. u0410004@iwate-u.ac.jp*

Abstract

The correlation between the global climate index (SOI/SST), rainfall distribution and crop yield in rain-fed highland areas was investigated. For this analysis, rainfall data were collected during the rainy season (October-March) for the period 1981 to 2009 in Gunung Kidul district of Yogyakarta Province, which is one of the important rain-fed crop producers in Indonesia. The rainfall distribution pattern found indicates that most of the rainfall occurs in coastal areas, especially in the southern hilly areas, where the occurrence of the Southern Oscillation Index (SOI) and Surface Sea Temperatures (SST) Niño.West are also highly correlated with rainfall. Soybean yield is highly correlated with rainfall while dry land paddies, peanuts and wetland paddies yields show a good correlation with SOI during January-February-March-April (JFMA).

Keywords: crop yield, rainfall, rain-fed area, SOI/SST

Introduction

Research on rainfall variability in Indonesia related to rain-fed agricultural areas is a critical issue due to its relationship with El Niño and La Niña events. The delay of rainfall has a negative effect on agricultural production in Indonesia. El Niño occurrences delayed rice harvesting in Indonesia and creates instability in food security (Harger, 1995). In 1997, rice farming in the sub-districts (Kecamatan) of Java decreased dramatically by 58% in wetland areas and 52% in dry land areas (Irawan, 2002). The abnormal weather caused by El Niño events seriously disturbed crop cultivation and the production of food and other commodities in the affected areas, mainly those located in the rain-fed dependent highland areas.

Rain-fed highland areas are more affected by rainfall variability because of the topography characteristics (Haylock and McBride, 2001). Rainfall variability in Indonesia correlates well with global climate index, such as the Southern Oscillation Index (SOI) and the Sea Surface Temperature (SST) pattern (Saji *et al*., 1999). At the same time, crop production in Indonesia is influenced by rainfall variability and highland topography. Therefore, it is necessary to estimate rainfall variability as influenced by the global climate indices (SOI and SST) in rain-fed highland areas and its correlation with crop yields.

The aims of this study are to analyze the relationship between rainfall, global climate indices SOI/SST and agricultural crops production based on the rainfall variability during the rainy season from 1981-2009 in rain-fed highland areas.

Materials and Methods

Study Area

Gunung Kidul district is one of the rain-fed highland areas of Java Island, located between 7°46'-7°09' S latitude and 110°21'-110°50' E longitude. Based on elevation, Gunung Kidul district as

reported by the Agricultural Service for Food Crops and Horticulture (ASFCH) is divided into three areas of which the northern border ranges between 200-700 meters above the sea level (m); the center area is the lowland area with an elevation of 150-200 m, and the south area with a hilly topography ranges from 0 to 300 m. A total of 92% of the agricultural land area in Gunung Kidul district depends on rainfall, especially on October precipitation, as reported by the ASFCH (2006) and the remaining 8% is irrigated. The mean annual rainfall in Gunung Kidul district is 2041 mm/year based on the record data from 1989-1998.

Secondary Data

Crops yield from 1990 to 2009 and rainfall data from 1981 to 2009 at twelve locations were collected from the rainfall observation stations of ASFCH (2009). The SOI and SST data were collected from the Japan Meteorology Agency (JMA) website (http://www.data.jma.go.jp/gmd/cpd/db/elnino/index/dattab.html) and were averaged in Niño3 (5°N-5°S and 150°W-90°W), Niño.West (15°N-EQ and 130°E-150°E) and IOBW (20°N-20°S and 40°E-100°E) areas, respectively. Spatial data of Gunung Kidul district with a scale of 1:25000 were taken from the National Coordinating Agency for Survey and Mapping (Bakosurtanal) of Indonesia (2007).

Rainfall data were the average of cumulative rainfall during the rainy season (from October to March) from 1981 to 2009 at each station. Steps in the data analyses were as follows: first, mapping of rainfall during the rainy season (6 months, Oct-Mar and then separately Oct-Dec (OND) and Jan-Mar (JFM) from 1981-2009. Second, in order to analyze the distribution of rainfall in Gunung Kidul district, a regression analysis was conducted between rainfall during the rainy season, 6 months and 3 months (OND and JFM) from 1981 to 2009 and the averages of SOI/SST, to identify the global climate indicators that influence rainfall. Third, correlation analysis was conducted between the average of rainfall during the rainy season and crop yields, and finally, correlation analysis between the average of global climate indicators and crop yield during the rainy season was conducted.

Correlation analysis between rainfall in the rainy season and crop yield, global climate SOI/SST and crop yield was calculated only for the period 1990-2009 because of the data availability on crop yield for this period.

Results and Discussion

Distribution of rainfall during the rainy season for the period 1981 to 2009

The analysis of rainfall distribution from 1981 to 2009 was carried out based on a six months (Oct-Mar) and three months (OND and JFM) analysis. The distribution analysis shows that in the southern coastal areas and in the inland western areas rainfall ranged from 1800-2200 mm, while in the lowland central and northern areas rainfall was around 1500-1600 mm (Fig. 1). The southern area is a mountainous area with a maximum elevation of 700 m. It caused moisture from the Indian Ocean carried by the wind and moving horizontally produces dense clouds along the mountain ranges. These clouds cause rain in the upper region known as orographic precipitation (Roe, 2005), which is the reason why the windward side of the mountain range receives much more precipitation than the leeward side.

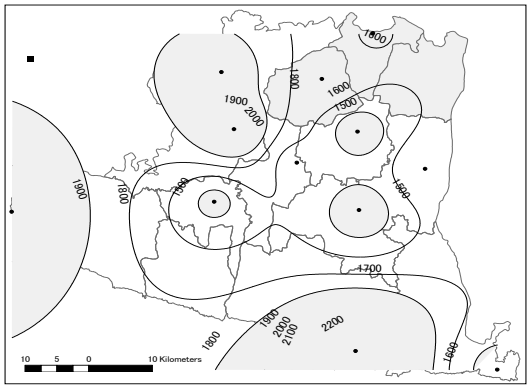

Figure 1. Distribution rainfall map during rainy season in Gunung Kidul district 1981 – 2009.

Relationship between the averages of rainfall and SOI/SST during 1981-2009

In Correlation analysis between the averages of rainfall during the rainy season and the averages of SOI and SST Niño.West in all sub districts, only three sub district, which has significant correlation, Panggang (r = 0.58), Playen (r = 0.44) and Rongkop (r = 0.49) sub-districts in $p<0.01$ and $p<0.05$. Panggang and Rongkop are the southernmost sub districts in the Gunung Kidul district, which directly border with the Indian Ocean, whereas Playen sub district, located in the middle of Gunung Kidul district, is poorly influenced by the Indian Ocean. Therefore, the area along the coast shows a stronger correlation with SOI - SST Niño.West than the center area. The average rainfall for the 9 other sub districts do not have significant correlations with the averages of SOI - SST Niño.West, which are probably influenced by the mountainous terrain indirectly affecting the correlation of rainfall and SOI.

Relationship between average rainfall and crop yield

Correlation analyses between average rainfall and crop yield during JFMA (January-February-March-April) can be seen at Table 1.

Table 1. Correlations between the average of rainfall in rainy season and crops yield in JFMA

Dry land paddy	Maize	Soybean	Peanut	Wetland paddy
-0.15	-0.31	0.60**	-0.04	-0.21

Pearson correlation coefficient
** Significant with $p < 0.01$

Based on Table 1, a strong correlation between average rainfall and crop yield during JFMA is only for soybean yield. Therefore, from PS 2 farmers tend to grow soybean as the main crop rather than dry land paddy in rain-fed highland areas. In the case of cassava yield, statistical analysis cannot be done during JFMA from 1990 until 2009, because it is not harvested during these months. Cassava is an annual plant, which is well adapted to drought and is harvested between July and August.

Relationship between the averages of SOI and crop yield

The relationships between the averages of SOI/SST and crop yield indicated that SOI is correlated with crop yield (dry land paddy, r = 0.50; peanut, r = 0.55 and wetland paddy, r = 0.55), whereas no correlation between SST Niño.West and crop yield was found (Table 2). Based on Table 2, average SOI during JFMA corresponds well with dry land paddy, peanut and wetland

paddy yields, and changes in the value of SOI influencing dry land paddy, peanuts and wetland paddy yields.

Table 2. Correlation between average SOI and crops yield in JFMA

Dry land paddy	Maize	Soybean	Peanut	Wetland paddy
0.50*	0.28	0.25	0.55*	0.55*

Pearson correlation coefficient
* Significant with $p < 0.05$

The usefulness of global climate index and rainfall pattern to determine suitability of crops is needed to help farmers in their decision taking, especially in rain-fed highland areas. Our results indicate that averages of SOI and SST Niño.West are correlated with rainfall, especially in the seaside southern hills areas.

In general, increases in the average SOI/SST Niño.West will influence the amount of rainfall, and directly influence the cropping pattern. Our study found that increasing rainfall during the rainy season (JFMA) from 1990 to 2009 increased soybean yield. Different results show that averages of SOI corresponded well with dry land paddy, peanut and wetland paddy yield during JFMA. Increases of SOI averages in JFMA by +1 will cause the increase of dry land paddy yield during JFMA periods. The same trend can be observed for peanut and wetland paddy yield, in JFMA.

Overall, our results show that most of the crops yield trend during the rainy season does not correspond to global climate indices and rainfall. The main reason may be local cropping patterns that still dominate regardless of the rainfall pattern and global climate index. Further analysis of other rain-fed highland areas, which are different in elevation and in micro-scale (village) by including crop yield data, is necessary.

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An Input-Output Analysis of the Economic Impact for Sustainable Rural Development in Wonogiri District, Indonesia

Shintaro Kobayashi, Shigeki Yokoyama*, Satoshi Uchida, Yoshiko Iizumi

Japan International Research Center for Agricultural Scineces, Tsukuba, Japan
**Corresponding author: Social Science Division, Japan International Research Center for Agricultural Scineces, 1-1 Ohwashi, Tsukuba, 305-8686, Japan.*
Tel.: +81 29 838 6343; fax: +81 29 838 6342.
syokoyam@affrc.go.jp

Abstract

Wonogiri district located in Central Java is the water source of Solo River. There is a large multi-purpose reservoir in the region. However, the reservoir is reported to be losing its function due to rapid sedimentation caused by forest conversion and intensive farming. For sustainable resource management, assessment of development strategies incorporating alternative agro-forest systems is required. This study, as a preparation for the assessment of development strategies, was aimed to grasp regional industrial structure, using the input-output analysis. The results showed that the importance of agriculture in the region was both as an income source especially for unskilled labor and as financial base for purchasing manufactured goods. Therefore, environmentally sound agro-forest systems need to be proposed so that strict environmental restriction on agriculture can be avoided.

Keywords: sustainable rural development, input-output analysis, Wonogiri.

Introduction

Wonogiri district is located in Central Java, Indonesia and is the water source of Solo River (Figure 1). There is a large multi-purpose reservoir in the region, and it has contributed to both flood control and water utilization. However, the reservoir is reported to be losing its functions as a public reservoir due to extremely rapid sedimentation. It is said that main reasons for the rapid sedimentation is forest conversion and intensive farming induced by regional population increase (JICA, 2007). In order to achieve sustainable resource management in the region, assessment of development strategies incorporating alternative agro-forest systems is required. This study, as a preparation for the assessment of development strategies, was aimed to grasp the regional industrial structure and income multiplier effects by sector, using the input-output analysis, an economic analysis method.

Figure 1. Location of Wonogiri District.

Materials and Methods

The input-output analysis was proposed by W. Leontief (e.g. Leontief, 1986) and it was used for projections of impacts of changes in production technology, social environment, consumer preference, or economic policy on an economy. Based on economic statistics or economic survey, simulation models called “input-output model” were formulated. Simplified flow of the analysis was as follows. Firstly, an input-output table which described all transactions among economic sectors was estimated (Figure 2). Secondly, production technologies and traits of transactions were abstracted from the table as the form of parameters called "input coefficients" (Figure 3). Lastly, using the input coefficients, transactions between demand sectors and production sectors and among production sectors were formulated to produce simultaneous equation for simulation (Figure 4). Based on the statistics of Wonogiri district, we estimated an input-output table. Tables 1, 2, and 3 show sector classification of the estimated table.

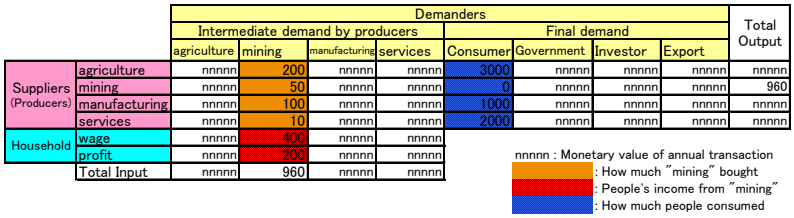

Figure 2. A sample structure of an input-output table. The table is estimated based on economic statistics or economic survey of a target region. Row vectors in the table show the amounts of supply valued at market prices. Column vectors show the purchase of goods and services.

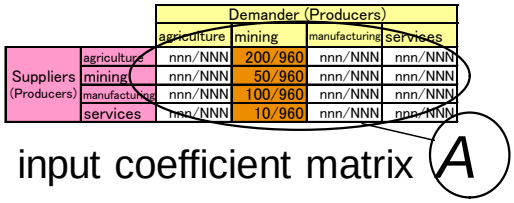

Figure 3. Abstraction of information on production technology as the form of parameters called "input coefficient" A.

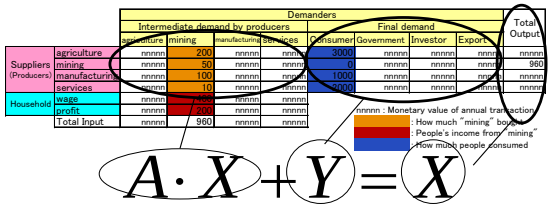

Figure 4. Formulation of technology A, production X, and demand Y in an economy.

Table 1. Production sectors

P1	Farm crops
P2	Livestock and products
P3	Forestry
P4	Fishery
P5	Mining and quarrying
P6	Oil and gas manufacture
P7	Other manufacture
P8	Electricity supply
P9	Gas supply
P10	Water supply
P11	Construction
P12	Trade, hotel, and restaurant
P13	Road transport
P14	Other transport
P15	Communication
P16	Financial services
P17	Real estate and business services
P18	Public administration and social services
P19	Personal and household services

Table 2. Demand sectors

D1	Private consumption
D2	Private investment
D3	Governmental consumption
D4	Public investment

Table 3. Value added sectors to supply primary factors to production

V1	Land
V2	Unskilled labor
V3	Skilled labor
V4	Capital
V5	Natural capital
V6	Indirect tax

In this paper, we drew skyline chart and derive income multipliers of economic activites. Skyline chart revealed trade characteristics of target economy. Drawing method was as follows. An input-output table could be expressed as follows:

$$X = A \cdot X + F + E - M \qquad \text{Eq. 1}$$

where X is a column vector of total output, A is "input coefficient matrix", F is a column vector of domestic final demand, E is an export column vector, and M is an import column vector. Solving Eq. 1, X can be decomposed into 3 inducing factors as follows:

$$X = (I - A)^{-1} \cdot F + (I - A)^{-1} \cdot E - (I - A)^{-1} \cdot M \qquad \text{Eq. 2}$$

where the first term of right-hand side indicates the production induced by domestic final demand F, the second term indicated the production induced by export E, and the last term indicated the potential domestic production to be substituted for import M. Based on Eq. 2, a skyline chart shows each factor's relative contributions to production on the vertical scale, with 100% was representing $(I - A)^{-1} \cdot F$. In addition, the horizontal scale showed the sectoral share in total production.

Income multipliers could be obtained by the following procedures. Assuming that import was in proportion to domestic demand, Eq. 1 could be transformed as follows:

$$X = A \cdot X + F + E - \tilde{M}(A \cdot X + F) \qquad \text{Eq. 3}$$

where $\tilde{M}$ is a diagonal matrix of import coefficients. Solving Eq. 3 for *X*, we obtain the following equation.

$$X = \left\{I - (I - \tilde{M})A\right\}^{-1} \cdot \left\{(I - \tilde{M})F + E\right\} \qquad \text{Eq. 4}$$

where $\left\{I - (I - \tilde{M})A\right\}^{-1}$ is called "Leontief inverse matrix" and includes information on the industrial structure of an economy. Supposing that $\tilde{V}$ is a diagonal matrix of value added ratio to production cost, income multiplier was column sums of a matrix $\tilde{V}\left\{I - (I - \tilde{M})A\right\}^{-1}$.

Results and Discussion

Based on an input-output table of Wonogiri district estimated for year 2005, we analyze current situation of Wonogiri district. Major findings were as follows:

(i) Farm crop sector was the largest income source and accounts for 46% of regional gross domestic products (Figure 5). This implied that environmental restriction on agricultural activities would affect the standard of living in the region. Therefore, environmentally sound agricultural systems including agro-forestry need to be proposed so that strict environmental restriction can be avoided.

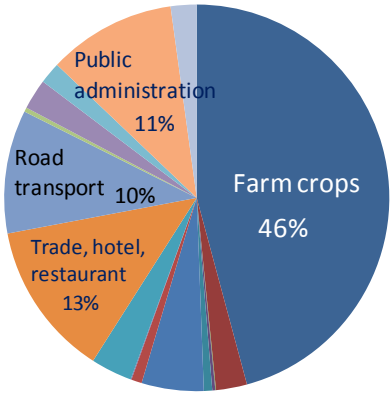

Figure 5. Share of income sources.

(ii) The district exported substantial amount of Farm crops and imports manufactured products (Figure 6). This means that the district participates in a regional division of labor and an interregional trade system. High self-sufficiency of Road transport seems to reflect the active transportation of products including Farm crops.

(iii) Agricultural sectors had tendency to strongly induce income of unskilled labor. Trade, hotel, and restaurant sector had also high value in income multiplier for unskilled labor (Table 4). These facts implied that integrated activities consisting of agriculture and services such as ecotourism was promising for pro-poor development.

This multiplier showed how much income of unskilled labor was induced by one unit of final demand for each sector.

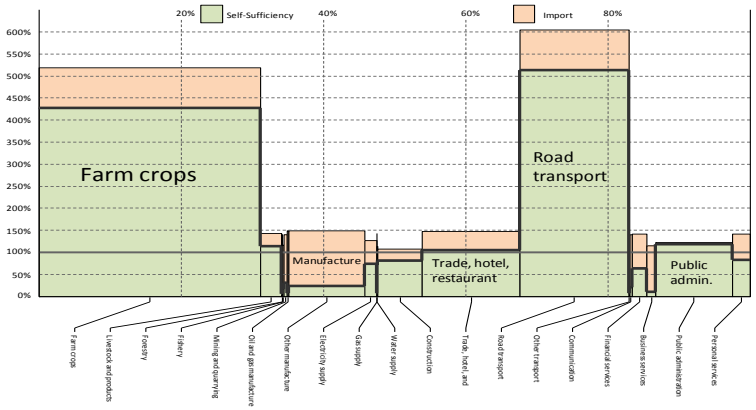

Figure 6. Skyline chart. Horizontal axis shows share of production and vertical axis shows self-sufficiency rate.

Table 4. Income multiplier for unskilled labor

Trade, hotel, and restaurant	0.389
Farm crops	0.388
Forestry	0.324
Personal and household services	0.321
Livestock and products	0.310
Public administration and social services	0.285
Road transport	0.270
Fishery	0.227
Construction	0.218
Other manufacture	0.174
Financial services	0.155
Communication	0.152
Mining and quarrying	0.148
Water supply	0.137
Electricity supply	0.077
Real estate and business services	0.054

Conclusions

Income source share and the skyline chart showed the importance of agriculture both as an income source and financial base for purchasing manufactured goods. Therefore, environmental policies to restrict agricultural activities would affect the standard of living. In order to avoid reducing income level in the region, environmentally sound agro-forest systems need to be proposed. Income multiplier implied that “Trade, hotel, and restaurant”, as well as agricultural sectors, had a strong power to induce income of unskilled labor. Therefore, integrated activities consisting of agriculture and services such as ecotourism were promising for pro-poor development. The remaining issues were crop-wise input-output analysis for decomposing Farm crop sector, backward and forward linkage among sectors, and simulation analysis on income generation effects of proposed community business models.

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Permutation Test in Evaluating the Significance of Plants in PLS-DA Model of Jamu Ingredients

Farit Mochamad Afendi [a, b*], Md. Altaf-Ul-Amin [a], and Shigehiko Kanaya [a]

[a] *Graduate School of Information Science, Nara Institute of Science and Technology, Nara, Japan*
[b] *Department of Statistics, Bogor Agricultural University, Bogor, Indonesia*
** Corresponding author: Graduate School of Information Science, Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan.*
Tel.: +81-743-72-5952; fax: +81-743-72-5953.
farit-a@is.naist.jp

Abstract

PLS-DA (Partial Least Square Discriminant Analysis) model is used to establish relationship between Jamu ingredients, i.e. plants composition in Jamu, and Jamu efficacy to investigate which plants act as main ingredients and which are supporting by checking the plants significance. Permutation testing is used in the investigation by generating the coefficients distribution under null hypothesis, i.e. the plants are not affecting Jamu efficacy. The generation process is performed by permuting the order of the response while maintaining the order of the predictors. The PLS-DA model then is applied to the new dataset after permutation. After repeating this process many times, then the accumulation of the PLS-DA coefficients provides the distribution under the null hypothesis. The proportion of the coefficients larger than or equal to the PLS-DA coefficient using original data then serves as the p-value, which then can be compared to the significance level α. By performing this permutation process 1000 times and α = 5%, we found, over all efficacies, 231 out of 465 plants are significant. Moreover, from literature review, among these 231 plants, the usages of 226 plants on the assigned efficacy are supported by scientific paper.

Keywords: jamu, PLS-DA, permutation test

Introduction

Jamu is an Indonesian herbal medicine made from a mixture of several plants. So, the ingredients, i.e. plants composition used, in Jamu determine the Jamu efficacy. Among the ingredients of Jamu are plants used as main ingredients, which contribute primarily to its efficacy, as well as plants used as supporting ingredients (Pramono, 2007; Redaksi Trubus, 2009). Investigating which plants are main ingredients and which are supporting is important to comprehensively understand the mechanism of plants used in Jamu to achieve specific efficacies. A statistical model can be helpful in this attempt by relating plants usage in Jamu as predictors and Jamu efficacy as response. Plants perform as main ingredients will have significant effect on the model developed.

PLS-DA, a statistical model for classification and discrimination that based on Partial Least Square Regression (PLSR) in which the dependent variable is chosen to represent class membership (Barker and Rayens, 2003), is suitable for this analysis regarding that large number of plants are used in Jamu and; on the other hand, Jamu efficacy are in categorical scale. Hence, the PLS-DA coefficients can be used to explore the relationship between plants and Jamu efficacy. Furthermore, the absence of significance testing on PLS-DA coefficients is replaced by permutation testing, which generated the coefficients distribution under null hypothesis through resampling of the existing data (Good, 2005).

Materials and Methods

Dataset

The data used in the present study are the commercial Jamu registered at The National Agency for Drug and Food Control (NA-DFC) of Indonesia. The data contain 3138 Jamu and in total they use 465 plants. Each Jamu is classified into one of nine efficacy categories, namely: (1) urinary related problems (URI), (2) disorders of appetite (DOA), (3) disorders of mood and behavior (DMB), (4) gastrointestinal disorders (GST), (4) female reproductive organ problems (FML), (6) musculoskeletal and connective tissue disorders (MSC), (7) pain/inflammation (PIN), (8) respiratory disease (RSP), and (9) wounds and skin infections (WND).

In the present study, the plants usage in each Jamu provides the predictors in PLS-DA model while the Jamu efficacy serves as the responses. Let X_{ij} (i = 1, 2, …, n; j = 1, 2, …, m; n = 3138; m = 465) denotes the status of plant j usage in Jamu i, where X_{ij} = 1 if Jamu i use plant j and 0 otherwise, and let Y_{ik} (k = 1, 2, …, 9) denotes the efficacy status of Jamu i, where Y_{ik} = 1 if Jamu i has efficacy in category k and 0 otherwise. Note that $\Sigma_k\ Y_{ik}$ = 1 meaning that each Jamu is classified into one efficacy category only.

PLS-DA Modeling

The details of the PLS-DA modeling are as follows (Barker and Rayens, 2003; Wold et al, 2001). Let **T** (n x c) is a matrix of the underlying factors of **X** and is obtained by maximizing its covariance with the corresponding matrix of the underlying factors of **Y**, that is

$$\mathbf{T} = \mathbf{XW} \quad (1)$$

where **W** (m x c) is a matrix of weight, and c is the number of factors extracted. Matrix **T**, multiplied by matrix of **X**–loadings **P** (m x c), is a good summaries of **X**

$$\mathbf{X} = \mathbf{TP}^t + \mathbf{E} \quad (2)$$

so that the **X**–residuals **E** (n x m) is small. In addition, matrix **T** also a good predictors of **Y**

$$\mathbf{Y} = \mathbf{TQ}^t + \mathbf{F} \quad (3)$$

where **Q** (9 x c) is matrix of **Y**–loadings. The **Y**–residuals **F** (n x 9) express the deviation between the observed and the predicted responses.

Substituting Eq. (1) into Eq. (3) we obtain multiple regression model of PLSDA

$$\mathbf{Y} = \mathbf{XWQ}^t + \mathbf{F} = \mathbf{XB} + \mathbf{F} \quad (4)$$

where the PLS-DA coefficient matrix **B** (m x 9) is calculated as

$$\mathbf{B} = \mathbf{WQ}^t \quad (5)$$

Note that each plant has a set of coefficient containing 9 values, one for each efficacy.

Permutation Test in PLS-DA Model

Permutation testing is a resampling method intended to provide underlying distribution of test statistic under null hypothesis, which then can be used to calculate p value. Unlike the conventional statistical testing, which assuming the null distribution follows some theoretical distribution, the permutation testing provides the null distribution by an empirical distribution which generated through resampling of the data sample at hand. The idea of permutation testing in PLS-DA is illustrated in Figure 3. The details of the steps are as follows.

Step 1. Resampling of Jamu data.
In this step, new Jamu data set under null hypothesis, i.e. plants are not affecting the Jamu efficacy, is generated by resampling the existing Jamu data set. The resampling process is performed by permuting the order of the response randomly while maintaining the order of the predictors. This process ensures the relationship between the predictors and the response, if any, in the original Jamu data set is destroyed. Thus, the result from this resampling is the new Jamu data set under null hypothesis. Let $\tilde{\mathbf{Y}}$ denotes the new response obtained from the permutation process.

Step 2. PLS-DA modeling on the new jamu data set.

PLS-DA model is performed on the new Jamu data set obtained from Step 1. The matrix **X** and $\tilde{\mathbf{Y}}$ provide the predictors and responses, respectively. The coefficient matrix obtained is denoted by $\tilde{\mathbf{C}}$.

Step 3. Accumulation of PLS-DA coefficients

After all permutation rounds R (in the present study we performed R = 1000 times), the PLS-DA coefficient in each round $\tilde{\mathbf{C}}_r$ is accumulated into coefficient distribution **C**, which is the distribution of PLS-DA coefficient under null hypothesis. Let $C_{jk,r}$ denotes the coefficient of plant j on efficacy k at permutation round r.

After the distribution of PLS-DA coefficient under null hypothesis is obtained, the p value in testing the effect of plant j on efficacy k is calculated as the following. Note that the hypothesis to be tested is.

$$H_0: \beta_{jk} \leq 0 \qquad \text{vs} \qquad H_1: \beta_{jk} > 0.$$

Thus, the p value is calculated as

$$p_{jk} = \frac{1}{R+1}\left\{\left(\sum_{r=1}^{R} I(C_{jk,r} \geq B_{jk})\right) + 1\right\} \tag{6}$$

where $I(C_{jk,r} \geq B_{jk})$ is an identity function that equal to 1 if the argument is fulfilled and equal to 0 otherwise. Basically, the p value is the proportion of the coefficients larger than or equal to the PLS-DA coefficient using original data. The null hypothesis is rejected if the p value is smaller than the significance level α.

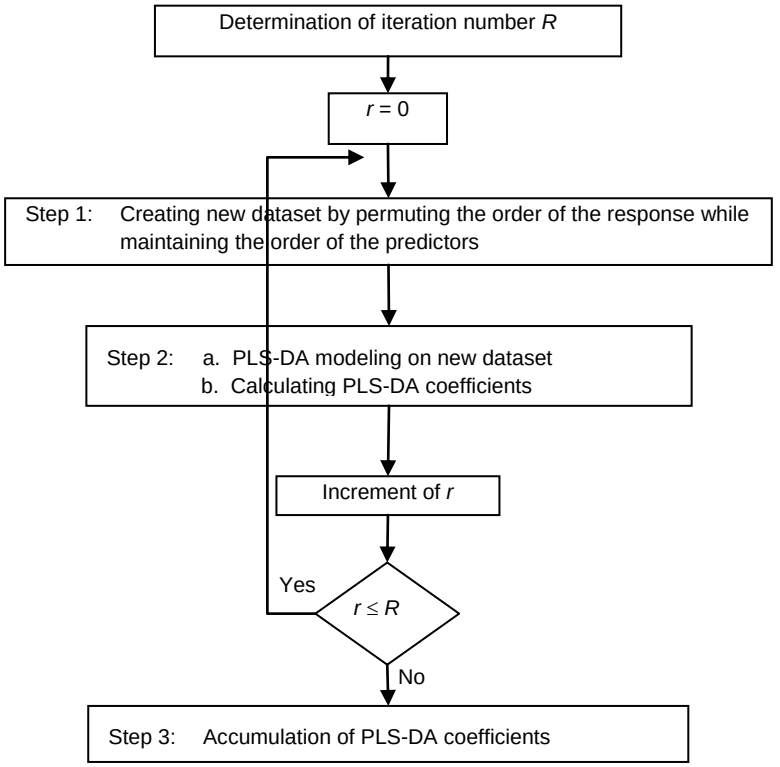

Figure 3. The schematic diagram of the permutation steps used in the present study.

Results and Discussion

In the present study, in selecting the number of components used in PLS-DA, we performed 5-folds cross validation as follows. The Jamu data set are splitted randomly into five sets. In each round, we combined four sets as training set and the other one as testing set. We apply PLS-DA with number of components *q* on training set and use the model to predict the responses of the testing set. The PLS-DA is applied once more by selecting another four sets as training set and the other one as testing set. After each set is once selected as testing set, the whole process is repeated using number of components *q* + 1.

Prediction Error Sum of Square (PRESS) using number of components *q* for efficacy group *k* is calculated as:

$$PRESS(q)_k = \sum_{i=1}^{n} \left(y_{ik} - \hat{y}_{(-i,k)q}\right)^2$$

where $\hat{y}_{(-i,k)q}$ denotes the prediction of the efficacy *k* for Jamu *i* using the PLS-DA model obtained without Jamu *i*, i.e. *i* is within the testing data, and using number of components *q*. The plot of this statistic against number of component *q* is shown in Figure 4. The plots are almost constant starting from *q* = 10 onward for all nine efficacy groups. Thus, the number of components of PLS-DA model for the original Jamu data set (and also for all 1000 permutation rounds) is set to 10. Analyzing PLSDA using 10 components on the original Jamu data set, we obtain the percent variation accounted for predictors and responses are equal to 5.5% and 40.5%, respectively.

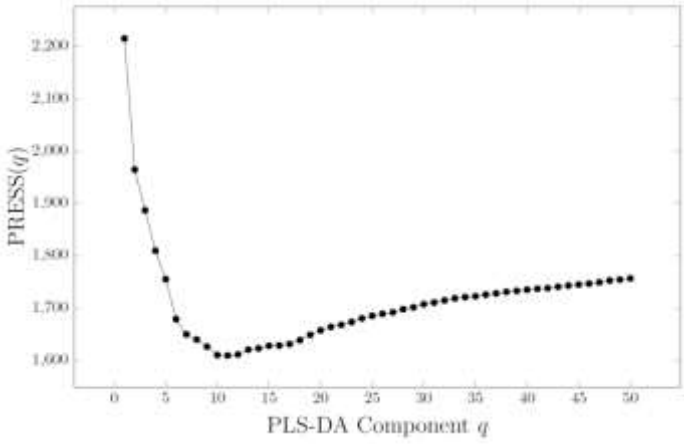

Figure 4. PRESS plot of 5–fold cross–validation.

Figure 5 gives illustration of the coefficient distribution under null hypothesis obtained from the permutation process. In this illustration, both plants are evaluated due to their usage for Jamu that useful for efficacy URI. The mean of the two distributions (and also for all other distributions) are very close to 0, as expected, indicating that the distributions are generated under null hypothesis. The normal curves were sketched on both distributions to show that not all permutation distributions can be approached with normal distribution. This result supports the p value calculation using empirical distribution as formulated in Eq (6). Using significance level α = 5%, we can conclude that *Phellodendron chinense* is significantly affecting the Jamu efficacy URI, whereas *Foeniculum vulgare* is not.

The results of the significancy testing of all plants used in each efficacy are shown in Table 1. Note that one plant may be used for more than one efficacy. From the testing, we observed 234 plants (50.3% among all 465 plants) showing no significant status for all 9 efficacies; whereas the other 231 plants have significant status which comprise of 189 plants (40.6%) are significant only for 1 efficacy, 38 plants (8.2%) are significant for 2 efficacies, and the other 4 plants (0.9%) are significant for 3 efficacies.

Besides testing the plants usage statistically, furthermore, we also check from scientific paper the usage of significant plants on their corresponding efficacy. The results are shown in Table 1. We obtained that most of the testing results are supported by scientific paper.

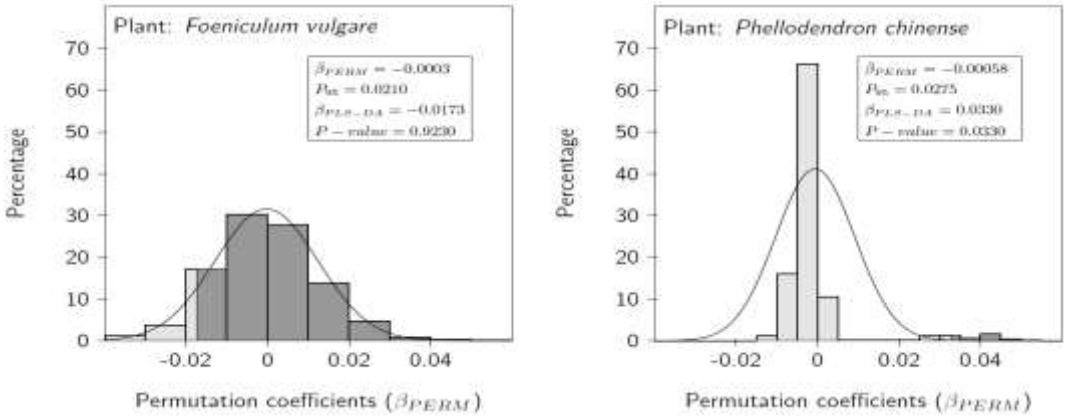

Figure 5. Illustration of the coefficient distribution under null hypothesis obtained using permutation process.

Table 1. The number of significant plants resulting from permutation testing and their support status from scientific papers

Efficacy	Number of plants used in the corresponding efficacy	Significant plants		
		Total	Support from scientific paper	
URI	80	20	19	(95.0%)
DOA	148	21	21	(100.0%)
DMB	47	12	11	(91.7%)
GST	290	26	26	(100.0%)
FML	182	40	39	(97.5%)
MSC	270	40	39	(97.5%)
PIN	183	39	38	(97.4%)
RSP	105	36	35	(97.2%)
WND	120	43	43	(100.0%)

Conclusion

PLS-DA model is used to establish relationship between plants composition in Jamu and Jamu efficacy to investigate which plants act as main ingredients and which are supporting by checking the plants significance. Permutation testing is used in the testing by generating the coefficients distribution under null hypothesis from which p value is calculated and then compared with significance level α. By performing this permutation process 1000 times and α = 5%, we found,

over all efficacies, 231 out of 465 plants are significant. Moreover, from literature review, among these 231 plants, the usages of 226 plants on the assigned efficacy are supported by scientific paper.

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	:	Syamsul Komar (IndoBIC)

List of Participants

No	Name of Participant	Institution
1.	Abdul Munif	Faculty of Agriculture, Bogor Agricultural University, Indonesia
2.	Agus Rachmat	Research Center For Biotechnology, Indonesian Institute of Sciences, Indonesia
3.	Agus Sutanto	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
4.	Ahmad Anwar Ismail	University of Malaysia, Kelantan, Malaysia
5.	Ahmad Rifqi Fauzi	Department of Agronomy, Faculty of Agriculture, Universitas Negeri Papua, Indonesia
6.	Akihiro Fujii	Graduate School of Agriculture, Tohoku Univ., Miyagi, Japan
7.	Akihiro Suzuki	Saga University, Japan
8.	Akiho Yokota	Nara Institut of Science and Technology, Japan
9.	Akira Yamauchi	Graduate School of Bioagricultural Science, Nagoya University, Nagoya 464-8601, Japan ayama@agr.nagoya-u.ac.jp
10.	Ali Asgar	Indonesian Vegetables Research Institute Jl. Tangkuban Perahu No. 517 Lembang 40791 Tel.(022)2786245, PO BOX 1586 Bandung, Indonesia asgar1957@yahoo.com
11.	Alia Astuti	Department of Biology, Bogor Agricultural University, Indonesia
12.	Anggayudha Ananda Rasa	Bandung Institute of Technology (ITB), Indonesia anggayudhaa@gmail.com
13.	Anggiani Nasution	Indonesian Center for Rice Research, ICRR, Sukamandi, Indonesia
14.	Anugoolprasert Ornprapa	Mie University, Japan
15.	Ardha Apriyanto	School of Life and Sciences, Bandung Institute of Technology, Bandung, Indonesia
16.	Ari Susilowati	Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sebelas Maret. Jl. Ir. Sutami No. 36A, Kentingan, Surakarta, Indonesia 57126
17.	Arief Sabdo Yuwono	Dept. of Civil and Environmental Engineering (SIL), Bogor Agricultural University, Bogor, Indonesia
18.	Arum Sekar Wulandari	Faculty of Forestry, Bogor Agricultural University, Indonesia

19.	Asma Sembiring	Indonesian Vegetable Research Institute (BALITSA) Jl.Tangkuban Perahu No.517 Lembang, Bandung-40395, Indonesia Tel : +62 22786245; fax : 62 22786416 asma_h_1999@yahoo.com
20.	Asmini Budiani	Indonesian Biotechnology Research Institute for Estate Crops, Bogor, ndonesia
21.	Astri S	Perkindo/Perkin Elmer
22.	Atsushi Kato	Laboratory of Plant Development and Molecular Morphogenesis, Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), Nara 630-0101, Japan
23.	Aweng a Mr. /l Eh Rak	University of Malaysia Kelantan (UMK), Malaysia
24.	Baiq Nurul Hidayah	Assessment Institute for Agricultural Technology (AIAT) Nusa Tenggara Barat, Indonesia
25.	Bambang Pujiasmanto	Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia
26.	Bambang Sapto Purwoko	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor Indonesia
27.	Bayu Dwi Apri Nugroho	The United Graduate School of Agricultural Sciences, Ueda 3-18-34, Morioka, Iwate, Japan 020-8550. Tel.: +81 19 643 3408; fax: +81 19 641 9296; u0410004@iwate-u.ac.jp
28.	Cailin Lei	Japan International Research Center for Agricultural Sciences (JIRCAS)
29.	Cassandra	Monash University, Australia
30.	Cecep	Bogor Agricultural University, Indonesia
31.	Chengyun Li	Japan International Research Center for Agricultural Sciences (JIRCAS)
32.	Chisato Sugiyama	Graduate school of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo183-8509, Japan Tel: +81 42 367 5672; Fax: +81 42 367 5671; 50011531016@st.tuat.ac.jp
33.	Darman M Arsyad	Central Institute for Agricultural Technology Assessment and Development, Bogor, Indonesia
34.	Deddy	Crop Life Indonesia
35.	Dedeh Hartati	Bogor Agricultural University, Indonesia

36.	Delima H A	Center of Agricultural Research and Development, Ministry of Agriculture, Bogor, Indonesia
37.	Dewi Indriyani Roslim	Department of Biology, Bogor Agricultural University, Bogor, Indonesia
38.	Dewi Sukma	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor Indonesia
39.	Dhika Amanda	School of Science and Technology, Bandung Institute of Technology, Bandung, Indonesia
40.	Dian Catur P	PT. BISI International Tbk.
41.	Dwi Praptomo	Assessment Institute for Agricultural Technology (AIAT) Nusa Tenggara Barat, Indonesia
42.	Dwinita W Utami	The Agency of Crop Biodiversity and Genetic Resources, Center of Agricultural Research and Development, Ministry of Agriculture, Bogor, Indonesia
43.	Elis Nina Herliyana	Bogor Agricultural University, Indonesia
44.	Elly Kesumawati	Faculty of Agriculture, University of Syiah Kuala, Banda Aceh, Indonesia
45.	Endang Yuniastuti	Faculty of Agriculture, Sebelas Maret University, Indonesia
46.	Endro Gunawan	Center of Agricultural Research and Development, Ministry of Agriculture, Bogor, Indonesia
47.	Eri Maai	Graduate School of Bioagricultural Sciences, Nagoya University, Aichi, Japan
48.	Eries Dyah Mustikarini	Jurusan Agroteknologi FPPB, Universitas Bangka Belitung. Balunijuk, Merawang, Bangka Kepulauan Bangka Belitung, Indonesia
49.	Etsushi Kumagai	NARO Tohoku Agricultural Research Center, 4 Akahira, Shimokuriyagawa, Morioka, Iwate 020-0198, Japan
50.	Etti Swasti	Faculty of Agriculture, Andalas University. Padang, Indonesia
51.	Eva Erdayani	Research Center for Biotechnology, Indonesian Institute of Science, Cibinong, Indonesia
52.	Fadhillah	PT Pandu
53.	Fadlila Dewi R	Syngenta
54.	Farit Mochamad Affendi	Graduate School of Information Science, Nara Institute of Science and Technology, Ikoma, Nara 630-0101, Japan. Tel.: +81-743-72-5952; fax: +81-743-72-5953; farit-a@is.naist.jp

55.	Fatichin	The United Graduated School of Agricultural Sciences, Kagoshima University, Japan
56.	Futoshi Kato	Faculty of Agriculture, Shinshu University. 8304 Minamiminowa, Nagano 399-4598, Japan. Tel.: +81 265 77 1409 ; fax: +81 265 77 1409; fkato@shinshu-u.ac.jp
57.	Futoshi Sasaki	Interdisciplinary Graduate School of Science and Technology, Shinshu University, Minamiminowa, Japan
58.	Hadiwiyono	Faculty of Agriculture, University of Sebelas Maret (UNS), Surakarta Jl. Ir. Sutami No.36A Kentingan Surakarta (57126), Indonesia Tel/Fax: (0274) 637457; hadi_hpt@yahoo.com
59.	Hamda Fauza	Faculty of Agriculture, Andalas University, Kampus Limau Manis 25163 Padang, Indonesia
60.	Happy Widiastuti	Indonesian Biotechnology Research Institute for Estate Crops, Bogor, Indonesia
61.	Haruna Tanaka	Mie University, Japan
62.	Hassan Y. Al Ayied	Natural Resources and Environment Research Institute (NRERI)
63.	Hayati Minarsih	Indonesian Biotechnological Research Institute for Estate Crops, Bogor, Indonesia
64.	Hiroshi Ehara	Graduate School of Bioresources, Mie University Japan
65.	Hiroshi Miyake	Nagoya University, Furo-cho, Chikusa, Nagoya, Aichi, 464-8601, Japan.
66.	Hiroyuki Shimono	Faculty of Agriculture, Iwate University, 3-18-8, Ueda, Iwate, 020-8550, Japan. Tel.: +81 19 621 6146; fax: +81 19 621 6146; shimn@iwate-u.ac.jp
67.	Hiroyuki Tsuji	Laboratory of Plant Molecular Genetics, Nara Institute of Science and Technology, Japan
68.	Indrastuti Apri Rumanti	Indonesian Center for Rice Research (BB Padi) Jl Raya 9 Sukamandi, Indonesia
69.	Inez HS Loedin	Research Center for Biotechnology, Indonesian Institute of Science, Bogor, Indonesia And IRRI Manila, Philippine
70.	Iskandar Lubis	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
71.	Iswari S Dewi	ICABIOGRAD Jl. Tentara Pelajar no 10A Bogor, Indonesia iswari.dewi01@gmail.com
72.	Jinbin Li	Japan International Research Center for Agricultural Sciences (JIRCAS)

73.	Jonathan Niones	Nagoya University, Japan
74.	Jose Maria AO	Bogor Agricutural University, Indonesia
75.	Jun-ichi Sakagami	Japan International Research Center for Agricultural Sciences (JIRCAS)
76.	Kazunobu Toriyama	Japan International Research Center for Agricultural Sciences (JIRCAS)
77.	Keisuke Katsura	Graduate School of Agriculture, Kyoto University, Japan
78.	Kentaro Kawaguchi	National Agriculture and Food Research Organization (NARO)
79.	Khay Sathya	Japan International Research Center for Agricultural Sciences (JIRCAS)
80.	Kohtaro Iseki	Graduate School of Agriculture, Kyoto University, Kyoto, Japan
81.	Koki Homma	Graduate School of Agriculture, Kyoto University, Kyoto, Japan
82.	Kulvinder S Gill	Department of Crop and Soil Science, Washington State University
83.	Kuniyuki Saitoh	Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan
84.	Kustiariyah	Faculty of Fisheries & Marine Sciences, Bogor Agricultural University, Indonesia
85.	Lailatul Badriyah	School of Life and Sciences, Bandung Institute of Technology, Bandung, Indonesia
86.	Latifah K Darusman	Biofarmaka, Bogor Agricultural University, Indonesia
87.	Lia Hadiawati	Assessment Institute for Agricultural Technology (AIAT) Nusa Tenggara Barat, Indonesia
88.	Loida M Perez	Japan International Research Center for Agricultural Sciences (JIRCAS)
89.	Mai Goto	Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan
90.	Maya Melati	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
91.	Md. Ansar Ali	Japan International Research Center for Agricultural Sciences (JIRCAS)
92.	Michihiko Fujii	Faculty of Education, Shizuoka University, Japan

93.	Michio Kawasaki	Faculty of Agriculture and Life Science, Hirosaki University, Aomori, Japan
94.	Miftahudin	Department of Biology, Bogor Agricultural University, Bogor, Indonesia
95.	Mikio Nakazono	Graduate School of Bioagricultural Sciences, Nagoya University, Japan
96.	Mikio Sekimuna	Interdisciplinary Graduate school of Science and Technology, Shinshu University, Japan
97.	Mituhiro Obara	Japan International Research Center for Agricultural Sciences (JIRCAS)
98.	Mochamad Arief Soleh	Graduate School of Agricultural Science, Tohoku University, Japan
99.	Motonobu Yoshida	Dept of Agricultural Science, Kinki University, Nakamachi 3327-204, Nara, Japan
100.	Muhammad Addip Novianto	BMKG
101.	Muhammad Syafii	Bogor Agricultural University, Bogor, Indonesia
102.	Muhammad Yunus	The Agency of Crop Biodiversity and Genetic Resources, Center of Agricultural Research and Development, Ministry of Agriculture, Bogor, Indonesia
103.	Munif Ghulamahdi	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
104.	Nagao Hayashi	Japan International Research Center for Agricultural Sciences (JIRCAS)
105.	Naomi Asagi	Ibaraki University, Japan
106.	Naoya Nakamura	Graduate school of Biological Sciences, Nara Institute of Science and Technology (NAIST), Ikoma, Nara 630-0101, Japan
107.	Narasimham Upadyayula	Monsanto Singapore Co (Pte) Ltd Singapore
108.	Nguyen Thi Bich Yen	Asian Natural Environmental Science Center, The University of Tokyo, Japan
109.	Nguyen Thi Lang	Japan International Research Center for Agricultural Sciences (JIRCAS)
110.	Nguyen Thi Thanh Thuy	Japan International Research Center for Agricultural Sciences (JIRCAS)
111.	Nguyen Van Kien	Division of Genebank Management, Plant Resources Center An Khanh, Hoai Duc, Ha Noi, Vietnam
112.	Niken TM Pratiwi	Faculty of Fisheries & Marine Sciences, Bogor Agricultural University

113.	Niken Trisnaningrum	PT. BISI International Tbk.
114.	Nishe Fransiska	Bogor Agricutural University, Indonesia
115.	Nobuhiro Noda	Mie university nobu0witch3@yahoo.co.jp 514-8507 Mie, Tsu-shi, 1577 Kurimamachiya-cho
116.	Nurliani Bermawie	BALITTRO Bogor, Indonesia
117.	Nurul Khumaida	Department of Agronomy and Horticulture, Bogor Agricultural University, Indonesia
118.	Ohn Mar Khin	Faculty of Agriculture, Kyushu Univ., Fukuoka, Japan
119.	Panca Dewi Manu Hara Karti	Faculty of Animal Husbandry, Bogor Agricultural University, Bogor Indonesia
120.	Paul PS Teng	National Institute of Education, Nanyang Technological University, SIngapore
121.	Phoumi Inthapaya	Japan International Research Center for Agricultural Sciences (JIRCAS)
122.	Poonsak Mekwatanakarn	Japan International Research Center for Agricultural Sciences (JIRCAS)
123.	PV Vara Prasad	Department of Agronomy, Kansas State University, Manhattan, Kansas 66506, USA
124.	Raed A Haleem	University of Duhok (UoD), Faculty of Agriculture and Forestry, Kurdistan region, Iraq
125.	Rahmat H	CENTRAS, LPPM, Bogor Agricultural University, Indonesia
126.	Randy Hautea	SEAsia Center Director and ISAAA Global Coordinator International Service for the Acquisition of Agri-biotech Applications (ISAAA)
127.	Renih Hayati	Fac. of Agriculture, Sriwijaya University, Palembang, Indonesia
128.	Renny Fatmyah Utamy	Faculty of Agriculture, University of Miyazaki, Japan
129.	Retno Lestari	Universitas Indonesia
130.	Rika Fithri Nurani B	PT SMART Tbk.
131.	Rika Raffiudin	Department of Biology, Bogor Agricultural University, Bogor, Indonesia
132.	Rinda Kirana	Institution Vegetables Research (IVEGRI), Indonesia

133.	Ritsuko Hara	Graduate School of Agriculture, Kyoto University, Japan
134.	Rudiyanto	Research Center for Biotechnology , Indonesian Institute of Science, Bogor, Indonesia
135.	Rujito Agus Suwignyo	Faculty of Agriculture, Sriwijaya University, Palembang, Indonesia
136.	Ruth Martha Winnie	Bogor Agricultural University, Bogor, Indonesia
137.	Samanhudi	Department of Agrotechnology, Faculty of Agriculture, Sebelas Maret University Jl. Ir. Sutami 36A, Surakarta, Indonesia 57126
138.	Sandra Arifin Aziz	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
139.	Santoso	Indonesian Center for Rice Research, Jln Raya 9, Sukamandi, Subang 41256, West Java, Indonesia
140.	Sara	PT ITS
141.	Satoko Yasumoto	Biomass Production and Processing Research Team, Production Systems Research Area, National Agricultural Research Center Tsukuba, Japan
142.	Satoshi Nakamura	School of Food, Agricultural and Environmental Sciences, Miyagi University, Sendai, Miyagi, Japan
143.	Satriyas Ilyas	Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University, Bogor, Indonesia
144.	Shao-Hui Zheng	Coastal Bioenvironment Center, Saga University, Karatsu, Saga 840-0021, Japan
145.	Shigeki Yokoyama	Japan International Research Center for Agricultural Sciences (JIRCAS)
146.	Shigeru Shigeoka	Department of Advanced Bioscience, Faculty of Agriculture, Kinki University, Japan
147.	Shinsuke Kasa	Graduate School of Bioresourches and Bioenvironmental Science, Kyushu University, Japan
148.	Shintaro Kobayashi	Japan International Research Center for Agricultural Sciences, Tsukuba, Japan
149.	Shinya Kasajima	Faculty of Bioindustry, Tokyo University of Agriculture, Hokkaido, Japan
150.	Shiro Mitsuya	Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan
151.	Shunsuke Adachi	Tokyo University of Agriculture and Technology, Japan
152.	Sintho Wahyuning Ardie	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia

153.	Sobir	Bogor Agricultural University
154.	Sony Suhandono	School of Life and Sciences, Bandung Institute of Technology, Bandung, Indonesia
155.	Sri Rahayu	Bogor Botanical Gardens, Indonesian Institute of Sciences, Bogor, Indonesia
156.	Stanis Malangen	United Graduate School of Ehime University, Matsuyama, Ehime, 790-8566, Japan
157.	Suharsono	Department of Biology, Bogor Agricultural University, Bogor, Indonesia
158.	Sulandjari	Faculty of Agriculture , Sebelas Maret University, Surakarta, Indonesia
159.	Suliman Ali Al-Khateeb	Environment and natural resources Department, King Faisal University, Kingdom of Saudi Arabia. P.O.Box 420, Alhofof 31982. skhateeb@kfu.edu.sa
160.	Supijatno	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
161.	Susumu Arima	Faculty of Agriculture, Saga University, Saga 840-8502, Japan
162.	Suwarni Tri Rahayu	Institution Vegetables Research (IVEGRI) Indonesia
163.	Suwarno	Indonesian Center for Rice Research, Jln Raya 9, Sukamandi, Subang 41256, West Java, Indonesia
164.	Tadashi Hirasawa	Tokyo University of Agriculture and Technology, Japan
165.	Tadashi Sato	Japan International Research Center for Agricultural Sciences (JIRCAS)
166.	Taiichiro Ookawa	Tokyo University of Agriculture and Technology, Japan
167.	Takao Oi	Graduate School of Bioagricultural Sciences, Nagoya University, Aich, Japan
168.	Takashi Homma	The College of Agricuture, Ibaraki University, 3-21-1, Ami, Ibaraki 300-0393, Japan Tel: +81 29 888 8556; fax: +81 29 888 8556 homma@mx.ibaraki.ac.jp
169.	Takasi Hashimoto	Graduate School of Biological Science, Nara Institute of Science and Technology, Japan
170.	Takehiro Mukouyama	Tokyo University of Agriculture and Technology, Japan
171.	Takeshi Kano	Japan International Research Center for Agricultural Sciences (JIRCAS)

172.	Takunari Kono	Graduate school of Biological Sciences, Nara Institute of Science and Technology (NAIST), Ikoma, Nara 630-0101, Japan
173.	Takuya Koyama	Department of Biosphere Resources Science, Nagoya University, Nagoya, Japan
174.	Tantono Subagyo	Syngenta Indonesia, Perkantoran Hijau Arkadia, Tower C, 8th fl, Jakarta
175.	Tati Kristianti	School of Science and Technology, Bandung Institute of Technology, Bandung, Indonesia
176.	Thelma F Padolina	Japan International Research Center for Agricultural Sciences (JIRCAS)
177.	Titi Rahayu	USA Embassy, Jakarta
178.	Tito Deski	PBS, IFPRI
179.	Tohru Kobata	Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu-cho, Matsue, 690-8504, Japan
180.	Tomohito Hayasi	Graduate School of Bioagricultural Sciences Nagoya University, Nagoya, Japan
181.	Tomomi Abiko	Japan International Research Center for Agricultural Sciences (JIRCAS)
182.	Tomoyuki Katsube-Tanaka	Graduate School of Agriculture, Kyoto University, Kitashirakawa, Kyoto 606-8502, Japan
183.	Tony Liwang	Plant Production and Biotechnology Division, PT SMART Tbk, BII Plaza Tower 2, 10th floor. Jl. MH Thamrin 51 Jakarta, Indonesia
184.	Toshiaki Kokubo	United Graduate School of Agricultural Sciences, Ehime University, Japan
185.	Toshihiro Mochizuki	Faculty of Agriculture, Kyushu University, Japan
186.	Toshiki Yamamoto	Mie University, Japan
187.	Totok Hesterianoto	Faculty of Fisheries & Marine Sciences, Bogor Agricultural University, Indonesia
188.	Tri Lestari	University of Bangka Belitung, Bangka Belitung, Indonesia
189.	Tri Muji Ermayanti	Research Center For Biotechnology, Indonesian Institute of Science, Bogor, Indonesia
190.	Tsutomu Ishimaru	Japan International Research Center for Agricultural Sciences (JIRCAS)
191.	Utut Widyastuti	Department of Biology, Bogor Agricultural University, Bogor, Indonesia

192.	Wahyudi	Department of Forestry, Palangka Raya University, Palangka Raya, Indonesia
193.	Wisnu Adi Wicaksono	PT SMART Tbk
194.	Woro Umayi Ananda	Syngenta
195.	Xin Zhao	The United Graduated School of Agricultural Sciences, Kagoshima University, Japan
196.	Yasuhiro Tsujimoto	Japan International Research Center for Agricultural Sciences (JIRCAS)
197.	Yasumoto Satoko	Biomass Production and Processing Research Team, Production Systems Research Area, National Agricultural Research Center Tsukuba, Japan
198.	Yasuyuki Ishii	Faculty of Agriculture, University of Miyazaki, Japan
199.	Yohei Kawasaki	Graduate School of Agriculture, Kyoto University, Japan
200.	Yohei Koide	Japan International Research Center for Agricultural Sciences (JIRCAS)
201.	Yoshihiro Nishihara	Faculty of Agriculture, Saga University, Saga, Japan
202.	Yoshimichi Fukuta	Japan International Research Center for Agricultural Sciences (JIRCAS)
203.	Youji Nitta	Ibaraki University 300-0393 Japan
204.	Yudiwanti Wahyu E Kusumo	Department of Agronomy and Horticulture, Bogor Agricultural University, Bogor, Indonesia
205.	Yukari Isobe	The Colledge of Agriculture, Ibaraki University, Ami, Ibaraki, Japan
206.	Yushi Ishibashi	Crop Science Lab., Faculty of Agriculture, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan
207.	Zaitun	Faculty of Agriculture, University of Syiah Kuala, Banda Aceh, Indonesia

List of Organizers

Acknowledgements