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INTERNATIONAL CONFERENCE ON APPLIED LIFE SCIENCES (ICALS2012)

Konya, Turkey, September 10-12, 2012

ISALS

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Scientific Committee

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Preface

The organizing committee warmly welcomes our distinguished delegates and guests to the 2012 International Conference on Applied Life Sciences (ICALS 2012) held on September, 10-12, 2012 in Konya, Turkey. ICALS 2012 is organized by International Society for Applied Life Sciences (ISALS), and supported by ISALS Members and scholars from universities and institutes all around the world. The conference Program Committee is truly international, with membership from the Americas, Europe, Asia, Africa and Oceania. The main conference themes and tracks are Environment, Biology and Agriculture. The major goal of this event is to provide international scientific forums for exchange of new ideas in life sciences through discussions with international peers. This proceeding records the fully refereed papers presented at the conference.

The conference has gathered technical research submissions related to all aspects of main conference themes. All the submitted papers in the proceeding have been peer reviewed by the reviewers drawn from the scientific committee, external reviewers and editorial board depending on the subject matter of the paper. After the careful peer-review process, the submitted papers were selected on the basis of novelty, importance, and transparency for the purpose of the conference. The selected papers and additional late-breaking contributions to be presented as lectures will make an exciting technical program. The conference will therefore be a unique event, where attendees will be able to appreciate the latest results in their field of expertise, and to attain additional knowledge in other fields. We hope that all participants and other interested readers benefit scientifically from the proceedings and also find it motivating in the process.

With the best regards,

The Organizing Committee

September 10-12, 2012

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Greenhouse Experiments of Symbiotic Effectiveness of Acid-Aluminium Tolerance Bradyrhizobium Japonicum Strains on Soybean Plant

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Abstract

The aim of the research was to study the symbiotic effectiveness of seven strains of acid-aluminium tolerance *Bradyrhizobium japonicum* on soybean plant cultivar Slamet. The research conducted in the greenhouse and used complete randomized design with seven inoculation treatments, two controls and one reference strain which used the nutrient solution at pH 4.5. Each treatment had three replications. The all of parameters were measured at 37 days after planting (DAP). Result of the experiments showed that mutant Bj 11 (19) inoculated to soybean plant had the highest symbiotic effectiveness. The treatment of Bj 11 (19) could increase the dry weight of t upper crop (64,88%), N-uptake (190,88%), and symbiotic effectiveness (65,87%) better than treatments with and without nitrate control and the reference strain, USDA 110.

Keywords: soybean, acid-aluminium tolerance, Bradyrhizobium japonicum, symbiotic effectiveness.

1. Introduction

Availability of sufficient nitrogen is one of the keys to increase the productivity of soybean plants. Soybean plants generally take nitrogen from the air by root-nodule bacteria and then the bacteria convert nitrogen into ammonia that is needed for plant growth. *Bradyrhizobium japonicum* is one species of slow-growing nodule bacteria which is very important to uptake atmospheric nitrogen in soybean plant [1].

Acid soils usually cause problems in soybean production, such as consist of low phosphorus and high aluminium [2] that strongly inhibit the growth of symbiotic nitrogen fixation bacteria on soybean plants [3]. The failure of nodulation under acid soil conditions is common, especially in soils of pH less than 5.0 [4]. B. japonicum is more tolerant at low pH, around pH 4.0-4.5 than the fast growing nodule bacteria, such as Rhizobium [5]. Previous research by Endarini et al. [6] had managed to get acid aluminium tolerant B. japonicum from several locations in Indonesia [6]. The results showed that BJ 11 isolate has the highest tolerance on acid and had a good ability to grow on pH 4.5 media. Some of the strains showed more competitive than reference strain, USDA 110, in testing of the effectiveness symbiotic at greehouse. Furhermore, Wahyudi et al. [7] had constructed several strains of acid-aluminium tolerance B. japonicum with increased symbiotic effectiveness through transposon TN5 mutagenesis, such as Bj 11 (20) and KDR 15 (37). Some mutants showed the ability to form root nodules more than the wild-type strains viz. Bj 11 (5), Bj 11 (19), Bj 11 (20), and KDR 15 (37).

The efforts to obtain potential strains are still wide open in agricultural research. The purpose of the research was aimed to study the symbiotic effectiveness of seven strains of acid-aluminium tolerance Bradyrhizobium japonicum on soybean plant cultivar Slamet.

2. Materials and Methods

2.1. Materials

Acid tolerant isolates *B. japonicum* were used in the study viz. Bj 11 (wt), KDR 15 (wt), Bj 13 (wt), Bj 11 (5), Bj 11 (19), Bj 11(20), KDR 15 (37), and USDA 110. All bacteria were collected at IPB Culture Collection, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University. Soybean seeds cultivar Slamet were obtained from Research Institute for Food Crops and Genetic Resources, Bogor, Indonesia.

2.2. Methods

Experimental Design. All the data collected in greenhouse were analyzed using complete randomized block design using Statistical Analysis System (SAS) and the means at p (0.05) level of significance. The experiment was arranged into seven inoculation treatments, two controls and one standard strain which used the nutrient solution at pH 4,5. Each treatments were made in three replications. Growth parameters such as height of plant, dry-weight of upper crop, dry-weight of nodules, number of nodules, N uptake of plants, nitrogenase activity of root nodule, and symbiotic effectiveness were determined.

Medium and Inoculants Preparation. *Bradyrhizobium japonicum* isolates were grown on Yeast Mannitol Agar (YMA) for 7-8 days at room temperature. The YMA media consist of mannitol (10 g/L), K_2HPO_4 (0.5 g/L), $MgSO_4.7H_2O$ (0.2 g/L), NaCl (0.2 g/L), yeast extract (0.5 g/L), added with 0.0025% congo red and rifampicin (50 μg/ml). The isolates were resistant to rifampicin [7] & [8]. Then they were subcultured into Yeast Mannitol Broth (YMB) and incubated for seven days with 125 rpm at room temperature.

Soybean Seed Inoculation. Soybean seeds were selected based on size and healthiness (able to shoot). Seed surface were sterilized using 95% alcohol for 10 seconds and 5% H_2O_2 for five minutes then rinsed seven times using sterilized water. The seeds are germinated in a petri dish at room temperature without light. Two days old sprouts grown in Leonard jar, pots filled with sand [7] which filled with N-free nutrient solution of pH 4.5 [9]. Each sprout was inoculated with 10^8 cell. ml^{-1} of *B. japonicum*. N-free nutrient solution and nutrient solution contained KNO_3 as control was added every second day. All treatment plants were maintained until 37 DAP.

Plant Maintenace at Greenhouse. During soybean plants growing in the greenhouse regularly added with sterile nutrient solution into the bottom of Leonard bottle. Since 10 days after planting (DAP), plants sprayed with fungicide (1g/L) once a week. Harvesting plants were done by cutting plants at the cotyledon to the former boundary separating the top and plant roots. The roots are removed from the bottle and carefully cleaned of sand and charcoal then washed.

Test of Nitrogenase Activity. Nitrogenase activity was measured by acetilen reduction using gas chromatography. Each of root and nodule soybean plant put into incubation bottle and then sealed with a rubber cover. The next stage was to capture gas from the bottle as much as 2 ml and replaced it with the injection of 2 ml acetylene gas. The bottles were incubated for 30 minutes and then 0.1 ml was taken for gas injected into the gas chromatography Shimadzu 17A. There are three triplicates for each treatment. Ethylene gas produced was calculated based on peak areas on paper chromatograms. Nitrogenase activity was defined as the total amount of ethylene formed per number of plants per hour in units of μmol [8].

Symbiotic Effectiveness Test. Symbiotic effectiveness values (SE) was obtained by formula of Gibson [10] SEN (Symbiotic Effectiveness N) = percentage of dry weight of plants inoculated test strain to dry weight of plants treated with KNO_3 and SER (Symbiotic Effectiveness R) = percentage of dry weight of plants inoculated test strain to dry weight of plants treated with reference strain, USDA 110.

Test of N Total Plant. N Total number of plant referred to the N total number of the canopy. Amount of N content was determined by Kjeldahl method [8].

3. Results and Discussion

3.1. Results

Bacterial Isolate Growth. Isolates were able to grow on YMA which were added with 0.0025% congo red and 50 μg/ml rifampicin after 7 days incubated on room temperature. Morphology of *B. japonicum* colonies were mucoid, not quite able to absorb congo red, and curve elevated (Fig.1)

Fig 1. The growth of Bradyrhizobium japonicum Bj 11 (19) on YMA media + 0.0025% congo red + 50 μg/ml rifampicin ten days after inoculation.

Number and Dry-Weight of Nodules. Inoculation of variuos strains of *B. japonicum* on soybean cultivar Slamet showed variation number of nodule between 9-21 nodule per plant. The highest number of nodule was found in the plant inoculated with mutant strain, Bj 13 (wt) (Table 1). Most of nodules were located on the secondary roots. The range of nodule dry-weight was 0.0089-0.0440 g per plant. The highest nodule dry-weight presented in soybean plant inoculated by BJ 11 (5) (Table 1).

Height of Plant and Dry-weight of Upper Crop. All treatments were inoculated with *B. japonicum* strains showed height plant higher than control N without inoculation, except Bj 11 (20) and KDR 15 (37). In general, the dry-weight of upper crop showed significantly different with control without inoculation and without added with 0.05% KNO_3.

Nitrogenase Activitity. Inoculation of various strains of *B. japonicum* on soybean cultivar Slamet were significantly influence on the activity of nitrogenase. Mutant strain Bj 11 (5) had a higher nitrogenase activity (12,79 μmol 2 plant^{-1}) and significantly different than the other strains except Bj 11 (wt) and reference strain, USDA 110 (Table 1).

Symbiotic Effectivenes. The highest symbiotic effectiveness was found in the plant inoculated with mutant strain Bj 11 (19) of 165,87% compared to control N effectivenes and 156.78% compared to USDA 110. While strain KDR 15 (37) showed the lowest symbiotic effectivenes only 97,30% (Table 1).

N Uptake. In general, N uptake in plants inoculated with *B. japonicum* strains were significantly different with control treatments, except strain KDR15 (37). Maximum N uptake of the plant was noticed with the strain Bj 11 (19) up to 20,10 mg N plant^{-1} and significantly different with control treatments (Table 1).

3.2. Discussion

The symbiotic interaction between soybean and root nodule bacteria played an important role in increasing the plant growth of soybean plant. Effectivity of a root nodule bacteria in fixing nitrogen were affected by the compatibility between bacteria and the soybean plant [11]. Data on the effect of inoculation acid-aluminium tolerance *B. japonicum* on nodulation and vegetative growth of soybean plant (Table 1) showed that inoculation of root nodule bacteria could increase height of could increase plant, and dry weight of upper-crop up to 37 DAP. Increasing in nodule dry weight could increase N fixination and the plant growth [12]. The nodule dry weight was positively correlated with the ability of plants to fix N and dry weight of the shoot. In the study, three strains viz. Bj 11 (wt), Bj 11 (5), and Bj (19) showed the best on height of plants, dry weight of upper crop, and dry weight of nodule. The highest symbiotic effectiveness, dry-weight of upper crop, and N uptake was found in the soybean plant inoculated with Bj 11 (19) compared to plant inoculated with other strains and the reference strain, USDA 110. Bj 11 (19) was proposed to be useful isolate for soybean plant on acid soil pH 4.5. The success or failure of inoculation depends on the competitive nodulation ability against indigenous bradyrhizobia under natural conditions [4].

Treatment	Number of nodule (nodule plant^{-1})		Dry-weight of nodule (g plant^{-1})		Height of plant (cm)		Nitrogenase activity (μmol 2 plant^{-1} hour^{-1})		Dry-weight of upper crop (g plant^{-1})		N uptake (mg N plant^{-1})		SEN(%)		SER (%)	
Bj 11 (20)	17	abc	0,0338	abc	54,4	d	10,44	b	0,7407	bc	16,03	c	137,01	bc	124,85	ab
Bj 11 (19)	16	abc	0,0377	ab	69,3	ab	10,17	b	0,9083	a	20,10	a	165,87	a	156,78	a
Bj 11 (5)	10	c	0,0440	a	67,8	ab	12,79	a	0,8317	ab	18,65	ab	151,48	ab	144,05	ab
Bj 11 (wt)	17	abc	0,0397	a	71,4	a	12,54	a	0,8447	ab	16,88	ab	155,37	ab	144,55	ab
Bj 13 (wt)	21	a	0,0262	bc	60,7	c	9,79	b	0,7963	b	16,08	bc	145,86	ab	136,60	ab
KDR 15(37)	9	c	0,0089	d	48,8	d	9,38	b	0,5342	e	7,72	d	97,30	d	92,19	b
KDR 15(wt)	20	ab	0,0373	ab	63,6	ab	10,23	b	0,8031	ab	18,12	ab	146,54	ab	140,35	ab
USDA 110	12	bc	0,0241	c	63,0	b	12,21	a	0,6164	d	13,63	c	114,92	cd	100,00	ab
Control N	0	d	0	d	46,0	d	0	c	0,5509	de	13,58	c	100,00	cd	96,26	ab
Control NO	0	d	0	d	37,3	e	0	c	0,4561	e	6,91	d	83,88	d	77,55	b

Tabel 1. Effect of inoculation of B. japonicum on soybean cultivar Slamet at 37 DAP using N-free solution at pH 4,5 + A150 μM

Numbers on the same column followed by the same letter were not significantly different based on Duncan Multiple Range Test (α = 0.05). 0 =no detection, N:without inoculation consist of KNO_3 0,05%, N0: without inoculation and without KNO_3 0,05%, Symbiotic Effectiveness (SE) against N/R

4. Conclusions

Inoculation of acid-aluminium tolerant *Bradyrhizobium japonicum* lead to good nodulation, vegetative growth, and symbiotic effectiveness of soybean cultivar Slamet at pH 4.5. Mutant of *Bradyrhizobium japonicum* strain Bj 11 (19) could increase the dry weight of the upper crop (64,88%), the

N-uptake (190,88%), symbiotic effectiveness (65,87%) better than treatments with and without nitrate and the standard strain, USDA 110.

5. Acknowledgements

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A Gravity Model Analysis of Egypt's Trade and Some Economic Blocks

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Abstract

This paper aims to study the economic effects of trade flows between Egypt and some economic blocs, where study confine to AFTA agreement particularly a Arab interface business and agreements of each bloc COMESA and EU generally because of strength opportunities for these blocs by using descriptive analysis and Gravity Model (GM). The major results confirmed the efficiency of the model in explaining Egyptian trade flow for the three previous blocs. Based on the above results, the study recommends continuing to increase the volume of foreign trade, in addition to reducing the constraints faced by Intra-Arab trade.

Keywords: Economic Effects of Trade Flows, Economic Blocks, Gravity model approach

1. Introduction

Trade among Arab countries (ACs) has been consistently weak in spite of several efforts to engage into different forms of regional economic integration. The most important attempts to achieve Arab economic integration were the agreement of 1953 on Transit Trade, the Common Market attempt of 1964, and the agreement of 1981 on the facilitation and development of trade, all signed under the auspices of the Arab League. These attempts, in addition to about 135 bilateral trade-related agreements, were not capable of stretching inter-trade beyond its peak of 10 percent of the total trade of ACs [1].

In 1994, the Intra-trade of ACs as percentage of their total exports was around 8.3 percent. This rate compares unfavorably with the corresponding rates of many regional groupings from both Developed and Developing countries. The latter ratios were 69.9 percent for APEC, 61.7 percent for EU, 47.6 percent for NAFTA, and 11.6 percent for EFTA. For regional groupings from the Developing countries these rates were 18.2 percent for MERCOSUR (Latin America), 12.0 percent for UEMOA (West Africa), and 21.2 percent for ASEAN (South-East Asia).(1)

These rates are not strictly comparable across groupings. Difference in the degree of development, size, and weight in international trade of the different countries of the groupings, explain to a great extent the observed variation between these regional groups. This can be said, however, the extent of Intra-Arab trade is arguably weaker than what it should have been given the common historical, religious, social, cultural, and language characteristics shared by these countries.

Many factors were presented to explain the weakness of Intra-Arab trade and the obvious failure of previous Arab regional agreements to stimulate trade among Arab countries. These factors

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