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PROCEEDING

INTERNATIONAL SYMPOSIUM ON SUSTAINABILITY SCIENCE

UNDERSTANDING CLIMATE CHANGE PHENOMENA FOR HUMAN WELL BEING

BANDUNG, SEPT 8TH – 10TH '14

CAMPUS OF UNIVERSITAS PADJADJARAN

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E-mail: insys2014@gmail.com

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Postgraduate Programme on Environmental Studies
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PLENARY SESSION DISCUSSION NOTE

Date: 8th September, 2014

Keynote Speaker : Prof. Dr. P.K. Ramachandran Nair (University of Florida, USA)

Title : Sustainability Science: Beyond Environmental Sciences

Time of presentation : 10.30-11.00 WIB

Question and Answer

Titi (Social Department)
Q: Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs. Actually, what standards we use to define those needs? Because each country has different standards, how do we define equality of life or standard of living?

A: One size is not fit to all. That is hard for us to equalize living standards across the country. There are many standards, but equality is not the concept or goal of sustainable development. The important point is how to keep the quality of life is maintained.

Gunawan (Universitas Padjadjaran)
Q: Agroforestry is a traditional way in managing natural resources. In Indonesia, the management of technology often fail. Sometimes local knowledge become good solutions. Why technology and local knowledge is never in sync? In context of sustainable development, what exactly is the traditional solution? To change the use of technology or for conservation efforts?

A: There is confusion about the concept of technology. Technology is the application of science to improve the livelihood income. An example is the mobile phone. A technology usually created based on the suitability of human needs. The key to sustainable development is the concept of basic human needs and limitations that can be supported with technology.

Dede Tresna (Anthropology lecturer, Universitas Padjadjaran)
Q: How is your opinion of women role in meeting the needs of the household?

A: Women play an important role in various aspects, including in agroforestry. Sustainable land use systems, often played by women. Dependence and the role of women in the use of forest resources is greater than men. It is characterized by the increasing women workload around the forest, both in domestic work and management of forest resources.

Nurrohman (Institut Teknologi Bandung)
Q: How the implementation of agroforestry to sustainable development?

A: Agroforestry has close connection with climate change as it relates to economic, social and many other aspects. The most important thing is agroforestry produce agricultural products that can improve the quality of human life.

Keynote Speaker : Prof. Hans Bressers (Twente University, the Netherland)
Title : Sustainability Governance in a Glocalized World: Governance Qualities to meet the challenges
Time of presentation : 11.00-11.30 WIB

Question and Answer

Hertu (Ministry of Law and Human Rights)
Q: How we can use sustainable energy for transportation sector?

A: Energy which can be used for transportation is renewable energy. Nevertheless, it should be identified on the part of energy which are suitable for transportation.

Dede Tresna (Anthropology lecturer, Universitas Padjadjaran)
Q: How we can open their eyes about the effects when they cut down trees?

A: It is too naive if we blame them for cutting the trees. So we must thinking about issue of equality, we need to think about alternatives behavior, change their livelihood to support them and their families. The alternative plan is to meet the solution through the power of institutions, funding, policy and sustainable alternative. It is our collective responsibility to maintain the sustainability of forests.

Dara Manuruk (Universitas Indonesia)
Q: Base on your presentation about collaborative strategic, when it can be applicate?

A: The collaborative strategic can be applied in mitigation or prevention policies. Collaborative strategy is adaptation and prevention policies that can generate action or collaborative action.

Adela (Universitas Padjdjaran)
Q: What should we do if we want to do bottom-up or top down approach to people who are not educated such as farmer??

A: You can do the combination of it, so the local farmer not as an individual but groups that representing the local authorities. The important is to create not just policy document but also policy act as a complete of them.

Keynote Speaker : Prof. Takao Yoshimatsu (MIE University, Japan)
Title : Climate Change and Its Impact on Aquaculture
Time of presentation : 13.30-14.00 WIB

Question and Answer

A B Susanto (Universitas Diponegoro)
Q: Which one is the important issue? Aquaculture in Terrestrial or Seawater?

A: Both are important. For instance, a big storm coming in from the outside of the ocean can be caused by ocean acidification.

Juli (Universitas Padjadjaran)
Q: How the concept of aquaculture can avoid environmental degradation and extinction of several marine organisms?

A: Currently aquaculture research and development are very limited. Therefore, it is need more research and scientific reports.

Anwar Syarif
Q: Climate change can have a negative impact on aquaculture. What should we do besides reducing CO_2?

A: CO_2 is not a major problem. Climate change is a natural phenomenon that has lasted long time and the increasing of CO_2 caused by human activity. Therefore we need change the people to environment-friendly behavior.

Zahra
Q: Which is more important, the development of freshwater or marine aquaculture?

A: Both of them are important and need scientific report to conduct more detail.

Kamia Handayani (PT. PLN)
Q: Which one is more danger? Temperature rice or acidification?

A: Both are equally dangerous.

Date: 9th September, 2014

Keynote Speaker : Dr. Osamu Saito (United Nations University, Japan) Title : Sustainability Science in the Context of Biodiversity and Ecosystem Services Time of presentation : 09.00-09.20 WIB

Indra (Universitas Padjadjaran)
Q: What do you think about *pekarangan* which planted in monoculture?

A: Not good due to lack of diversity. It is better to have more different plants.

Elisabeth (RDI)
Q: Can Saotomi and Satoyama adapt to urban/city society?

A: Yes. It is a part of international agenda. .

Yeri(Universitas Padjadjaran)
Q: Which one is better, village sustainability or city sustainability?

A: Both were good. It necessary to evaluate dependency between town and country, because both are affect to each other.

Keynote Speaker : Parikesit Ph.D (Deprtment Biology & Graduate Programme on Environmental Studies, Universitas Padjadjaran)

Title : Towards a New Generation of Sustainability: The Needs for Trans-disciplinary Learning Process in Higher Education

Time of presentation : 09.20-09.55 WIB

Indra (Universitas Padjadjaran)
Q: How should education about sustainability science be taught? Teach it separately or integrated with existing studies?

A: Depending on the circumstances. In Unpad, incorporate with relevant discipline.

Muhammad (Universitas Padjadjaran)
Q: Do we have to distinguish between a generation and start sustainability science education on the next generation? Or we can start now?

A: Sustainability science should be taught in formal and informal education. Sustainability science should be part of our daily live.

Souphaline (Intitut Teknologi Bandung)
Q: Are organic farming and GMO was solution for sustainable agriculture?

A: Integrated sustainable agriculture in agricultural science is the key.

BREEDING, CLONAL PROPAGATION AND APPLICATION OF MOLECULAR MARKER ANALYSIS OF *Phalaenopsis*

Dewi Sukma[1], Sandra A. Aziz[1], Sudarsono[1], Atra Romeida[2], Aline Sisi Handini[3] and Erick Raynalta[3]

[1]*Bogor Agricultural University, Faculty of Agriculture, Department of Agronomy and Horticulture, Bogor, Indonesia.*
[2]*Bengkulu University, Faculty of Agriculture, Department of Agrotechnology, Bengkulu, Indonesia*
[3]*Plant Breeding and Biotechnology Program, IPB Graduate Program, Department of Agronomy and Horticulture, Bogor, Indonesia*
dsukma70@yahoo.com

***Abstract* — *Phalaenopsis* is one of famous orchid with high economic value. Breeding is needed to develop a new population as material selection for a new variety which adaptive to climate change. Molecular marker is useful for diversity analysis and molecular assisted breeding in plants. *Phalaenopsis* has long juvenile stage for flowering, in that case, molecular marker could be developed for early selection of progenies which have specific character. Clonal propagation is needed to provide the propagules of selected varieties. The research was carried out to collect and characterize some species and hybrids of *Phalaenopsis*, develop some hybrid population, clonal propagation or micropropagation protocol and apply molecular marker for diversity analysis of species and hybrids.In this article, morphological and molecular characterization of TSW 1102 *Phalaenopsis*hybrid population (*Phal. bellina* x *Phal.'Salu Spot'*) and clonal propagation of TSW 1103*Phalaenopsis*hybrid population (*Phal. 'Sogo Diamond'* x *Phal. 'Jin Bao Red Rose'*)are reported. The results of the research showed that 123 progenies of TSW 1102 population were clustered into main group with the level of similarity about 50.or diversity 50%. Molecular marker analysis by using 3 locus of SNP marker which was developed from Chalchone Synthase Gene (CHS) Sequence could separate the population into 3 group with the level of similarity 80-86%. Clonal propagation of TSW 1103 population carried out usingaxillary branching.Murashige and Skoog Medium withaddition of 10-30 ppm of benzylaminopurine (BAP)gave the best result.**

Keywords — ***Axillary branching, micropropagation, molecular characterization, morphological characterization, Phalaenopsis orchids, similarity, SNAP marker.***

I. INTRODUCTION

Indonesia is famous for its huge plant biodiversity including orchids (*Orchidaceae*). There are around 5000 species of orchid lives in Indonesian tropical forest including *Phalaenopsis*. *Phalaenopsis*, one of the most popular genera in Orchidaceae family, is widely distributed across Southeast Asia and includes South India, Sri Lanka, southern China to Taiwan, Indonesia, Thailand, Myanmar, Malaysia, the Philippines, Papua New Guinea, and northern Australia. Sweet (1980) wrote about 47 species while Christenson (2001) had identified about 62 species in this genus. Lately, global climate change, deforestation by human activity and natural disaster such as earthquake and volcanic eruption cause loss of plant germplasm including orchids.

Phalaenopsis is included in the most commercial orchids in global market. Many valuable varieties hadbeen developed especially by Taiwan breeder which bring Taiwan to become a big exporter for *Phalaenopsis*. Breeding and development of new varieties is an important strategies to keep Taiwan as the leading producer of *Phalaenopsis* in the world (Tang and Chen, 2007). In Indonesia, breeding and developing of new varieties is also performed by breeder or researcher. In relation to climate change, breeding of *Phalaenopsis*could use native species of Indonesia which were well adapted to tropical climate. The species could be crossed to novelty hybrid that was introduced from Taiwan to get offspring with new superior hybrid characters.

One constrain in *Phalaenopsis* breeding is long juvenile periods of plant before flowering which cause new variety selection could be carried about 3-4 years after crossing. An early efficient of selection method should be developed to speed up of plant variety development. Molecular marker is an alternative to evaluate genetic diversity or similarity and to develop molecular marker which associated with certain plant character which may be useful for early selection of new variety.

There are many constrain in developing orchids industry in Indonesia, one is the availability of the propagules. Although some new hybrid varieties of *Phalaenopsis*has been found by Indonesian breeder, but clonal propagation is still not well developed. Breeder or plant propagator could not fulfill the grower demand for plantlets. As a consequence, most*Phalaenopsis* grower still imported plantlets or propagules from Taiwan.

Clonal propagation by tissue culture is an alternative way to provide *Phalaenopsis*propagules of varieties including F1 hybrid (*Phal. Sogo Diamond x Phal. Jin Bao Red Rose*). Mode of regeneration *in vitro* could be axillary branching or *protocorm like bodies* (plbs) formation. Success in *in vitro* clonal propagation of *Phalaenopsis* was still varied. The main

aspect of clonal propagations are concentration of plant regulator which could enhanced shoot or *plbs* initiation and multiplication.

The aims of the research were to analysemorphological and molecular diversity of F1 population (*Phal. bellina x* Phal. 'Salu Spot'), medium composition for clonal propagation F1 population of *Phal.bellina x* Phal. 'Salu Spot').

II. METHODOLOGY

The research were consisted of three sections :a) morphological characterization of TSW 1102 (F1 hybrid population *Phal. bellinax Phal. 'Salu*Spot') in vegetative stage.b) Similarity analysis of TSW 1102 population based on molecular marker Single Nucleotide Polimorphism (SNP) and c)Axillary shoot induction of TSW 1103 (F1 hybrid population of *Phal. 'Sogo Diamond' x Phal. 'Jin Bao Red Rose'*) onsome medium composition. Both of hybrid population above wereproducedby TjuntjunSalehWijaya, a collector of orchids.

In the first section, morphological characters were observed at the vegetative stage (one year-old plants after acclimatization). The characters evaluated were leaf shape (lanset or oval), leaf colour (green or purplish green), leaf edge colour (green or purplish green), leaf tip shape (blunt or taper), root tip colour (green or purplish green) and shape of young leaf edge (flat or serrated). The data were converted to biner data and analysis by using NTSYS software.

In the second section (molecular analysis), genomic DNA were isolated from young leaves of TSW 1102 population. Two parents and 40 F1 progenies were used in this research. DNA isolation followed CTAB method (Doyle & Doyle, 1987) which was modified by Das *et al.* (2009). Quality and quantity DNA was determined through *Cole Parme*® electrophoresis on agarose gel 1% in SB Buffer 1x with 100 Volt for 60 minutes. DNA standard (*ladder* 100bp) used and DNA visualization were performed by *gelred* staining and the agarose gel were documented with digital camera.

SNAP primer were developed from PhalaenopsisChalchone Synthase (CHS) Gene sequence from NCBI GeneBank with accession number AY282575 and U88077. Non-synonymous SNP site from CHS sequence were determined through alignment analysis of DNA sequence and amino acid prediction by using Genious Pro 5.6.6. Software (Biomatters, USA). Primers were designed based on non-synonymous SNP site by using WebSNAPER software that provided at http://ausubellab.mgh.harvard.edu/.

PCR reaction were performed in 12.5 µl PCR reaction that contain KAPA2G™ PCR Kit (KapaBiosystem Inc., USA). The composition of PCR reaction were 5.0 µl 5 X PCR buffer (contain 1.5 mM Mg^{2+}, 0.5 µl MgCl2 25 mM, 0.5 µl dNTPs 10 mM), 1.0 µl of 10 µM *forward* and*reverse* primers), 30 ng genomic DNA, 0.1 µl Taq DNA polymerase (5 U μl^{-1}) and 15.4 µl ddH_2O. PCR were carried by GeneAmp® PCR System BioRad T-100 machine followed optimum temperature for each primer. DNA template denaturation was at 95 °C for 3 minutes, followed by 35 cycle at 95 °C for 10 seconds, 60 to 62°C for 10 seconds and 72°C for 3 seconds. PCR was finished by one cycle at 72°C for 10 minutes. PCR products were visualized and separated through electrophoresis on agarose gel 1% on electrophoresis machine at 80 volt for 25 minutes. The electrophoregramwere scoredand converted to binary data and phylogenetic analysis was performed using NTSYS ver2.02 software (Exeter Software, New York, USA).

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In the third section, clonal propagation through axillary branching induction were carried on a half strength of Murashige and Skoog (1/2MS) medium (1962) with addition of Naphthalene Acetic Acid (NAA) and Benzylaminopurine (BAP). Plant material used were11/2 year-old plantlet of TSW 1103 population.The list of medium composition were 1).½ MS, 2). ½ MS+ 10 ppm BAP, 3).½ MS+ 20 ppm BAP, 4).½ MS+ 30 ppm BAP, 5). ½ MS+ 1 ppm NAA, 6).½ MS+ 1 ppm NAA+ 10 ppm BAP, 7).½ MS+ 1 ppm NAA+ 20 ppm BAP, 8).½ MS+ 1 ppm NAA+ 30 ppm BAP, 9).½ MS+ 2 ppm NAA, 10).½ MS+ 2 ppm NAA+ 10 ppm BAP, 11).½ MS+ 2 ppm NAA+ 20 ppm BAP and 12).½ MS+ 2 ppm NAA+ 30 ppm BAP.Observation were performed at shoot initiation and number of shoots at 2, 4 and 8 weeks after treatment (WAT).

III. RESULT AND DISCUSSION

a. Morphological characterization of F1 hybrid population (TSW 1102)

The parents of F1 hybrid population (TSW 1102) are *Phal. bellina* as female parent and *Phal. 'Salu Spot'* as male parent. *Phal. bellina* is a species which have oval leaf shape, green leaf colour, green leaf edge colour, blunt leaf tip shape, green root tip colour, and flat young leaf edge. *Phal. 'Salu Spot'* is a hybrid which have lanset leaf shape green leaf colour, green leaf edge colour, taper leaf tip shape, purplish green root tip colour, and flat young leaf edge. Cluster analysis by using NTSYS gave the dendogram that was provided in Figure 1.

Figure 1 showed that progenies were distributed into twenty class of characters which level of similarity varied between 50-*100* % or diversity between 0-50%. Most of progenies had the hybrid characters of the parents. There were only eight progenies (F-48, F-140, F-138, F25, F-130, F-163, F-27 and F-35) which showed 100% similarity with P1 (*Phal. bellina*) and two progenies (F1-47 and F1-137) which have

100% similarity with P2 (*Phal. 'Salu Spot'*). In the 50% of similarity, parents and progenies were distributed in two group where P1, P2 and 98 progenies were in first group and 25 progenies were in the second group. The dendogram also showed that the second group are hybrids which had 50% similarity to the parents.

Figure 1. Cluster analysis based on morphological charactersof TSW 1102 population.

Table 1.The primers used for SNAP marker analysis generatedbase on CHSgene sequence variations

Loci	Primer type	*Forward 5'-3'*	*Reverse 3'-5'*	Amplicon size (bp)	TA (°C)
SNP1	*Ref*	GTGCTGAAGCAAGGGTGTGC	GAGCCTTGAATCCATCAAGAAGG	358	60.0
	Alt	TGTGCTGAAGCAAGGGTCTGA			47.0
SNP2	*Ref*	GTAAGGCAAGAGGTTGCTATACGC	GCAAATAAAGCCTGGGTCACAA	371	62.0
	Alt	GTAAGGCAAGAGGTTGCTATGAGG			61.7
SNP3	*Ref*	GAGCTGGTAATCAGCACCAGGTATGG	TTCAACGCATCTGTAAGTCATCTCAATT	375	62.0
	Alt	GAGCTGGTAATCAGCACCAGGTGAGT			61.7

Ta = Temperature annealing

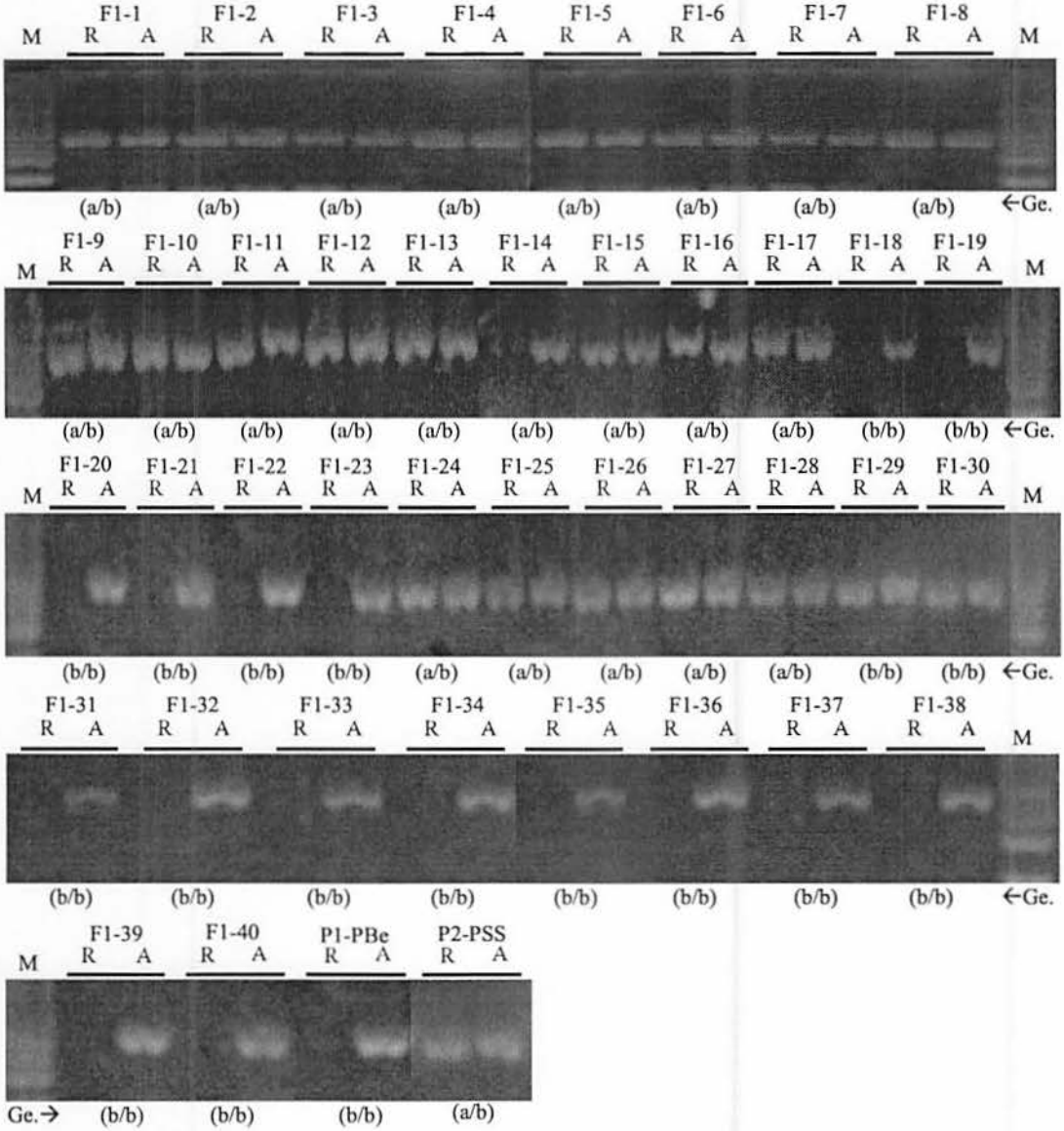

Figure 2. Representativeresults of SNAP marker analysis at locus SNP3_CHS of 40 hybrid progenies and their parents and their predicted genotype. R: PCR product using referenceprimer andA: PCR product using alternateprimer. M: DNA size marker - 100bp ladder DNA.Ge. – predicted individual genotypes.

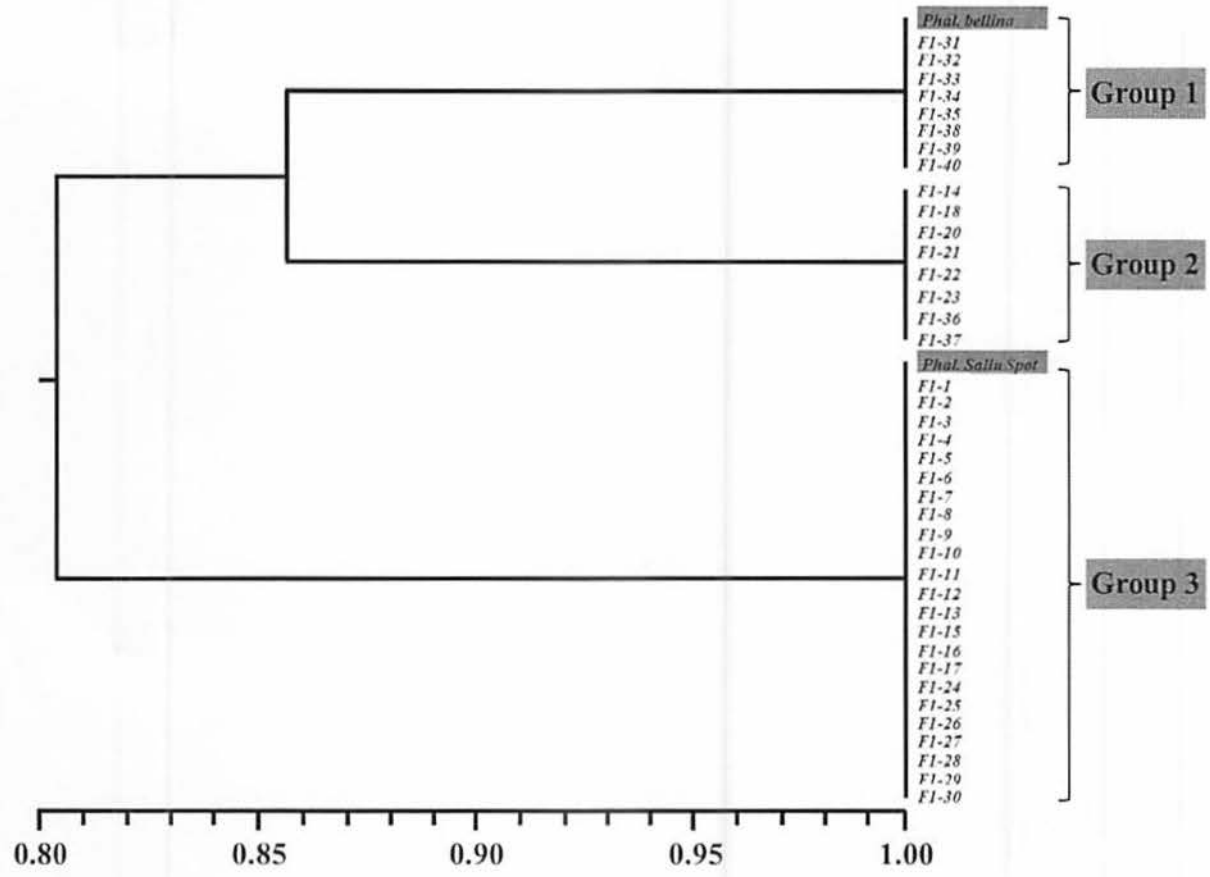

Figure 3. Cluster analysis of *Phalaenopsis* hybrid progeny population (TSW 1102) of a cross between *Phal. bellina* x *Phal.* 'Salu Spot' and their parents based on SNAP marker.

Table 2. PIC, Ho, and He value on SNP1_CHS and SNP3_CHS from TSW 1102 (*Phal. bellina x Phal.* 'Salu spot') population

Materi	Lokus	PIC	Ho	He
Hibrida	SNP1_*CHS*	0.173	0.214	0.194
TSW 1102	SNP3_*CHS*	0.325	0.413	0.571

Note: PIC= *Polymorphic Information Content,* Ho= *Observed hereozigosity,* He= *Expected heterozigosity*

Table 3.Effects of various media and plant growth regulator treatments on average number of proliferated shoots from explant of *Phalaenopsis* TSW1103.

Treatments	Average number of proliferated shoots		
	2 WAT	4 WAT	8 WAT
½ MS	0.00	0.00	0.00
½ MS+ 10 ppm BAP	0.11	1.33	0.83
½ MS+ 20 ppm BAP	0.11	0.33	0.67
½ MS+ 30 ppm BAP	0.11	0.00	1.67
½ MS+ 1 ppm NAA	0.00	0.00	0.28
½ MS+ 1 ppm NAA+ 10 ppm BAP	0.00	0.67	1.67
½ MS+ 1 ppm NAA+ 20 ppm BAP	0.00	0.00	0.78
½ MS+ 1 ppm NAA+ 30 ppm BAP	0.00	0.00	1.00
½ MS+ 2 ppm NAA	0.00	0.00	0.00
½ MS+ 2 ppm NAA+ 10 ppm BAP	0.11	0.00	1.22
½ MS+ 2 ppm NAA+ 20 ppm BAP	0.00	0.33	0.44
½ MS+ 2 ppm NAA+ 30 ppm BAP	0.00	0.00	0.50

Note: BAP – benzylamino purine, NAA – naphthalene acetic acid, WAT – week after treatment.

b. Similarity analysis of TSW 1102 population based on molecular marker Single Nucleotide Polimorphism (SNP)

CHS gene is a specific gene that determine flower colour. It codes general key enzyme in flavonoid biosynthesis pathway that produced anthocyanin (Johnson et al. 2001). Most of anthocyanin gives a pink, magenta, purple and red colour(Schwinn and Davies, 2004). Another pigment which contributed flower colour is carotene. Carotene gives a red or yellow colour (Cuttriss and Pogson, 2004). Developing molecular marker based on CHS sequences are important to help plant breeder to determine or predict flower colour of progenies at the early stage of growth (seedling).

In this research, three primers that develop from CHS sequence, could amplify the genomic DNA of 40 progenies of *Phal. bellina* x *Phal.* 'Salu Spot. Profile of the primers could be seen in Table 1. The amplified fragment for SNP1_CHS was 358 bp, SNP2_CHS was 371 bp and SNP3_CHS was 475 bp. SNP1_CHS and SNP3_CHS were polymorphic while SNP2_ was monomorphic. Representative of electrophoregramfor SNP3_CHS locus of 40 hybrid progenies, their parents and their predicted genotype were shown in Figure 2. *Phal.bellina* resulted PCR product only in alternate primer, indicated it had thehomozygote genotype while *Phal.* 'Salu spot' gave amplification product on reference and alternate primer, indicated it had heterozygote genotype.

Dendogram of similarity analysis of 40 progenies is shown in Figure 3. The progenies were grouped into three cluster with 86% level of similarity. The first cluster comprises of 8 progenies which have high similarity to female parent (Phal. bellina). The progenies were F1-31 to F1-35, and F1-38 to F1-40). The second cluster consisted of 9 progenies (F1-14, F1-18 to F1-23, and F1-37, showed hybrid character of their two parents. The third cluster had 23 progenies (F1-1 to F1-13, F1-15 to F1-17, and F1-24 to F1-30) which had high similarity to their male parents *Phal.* 'Salu Spot'.

Table 2 showed the PIC, Ho and He value of TSW 1102 based on two SNP_ CHS locus. The PIC for SNP1_CHS and SNP_1 CHS were 0.173 and 0.375. According to Sardou *et al.* (2011), highly polymorphic markers had the PIC value above 0.5.High polymorphic markers exhibited the highest gene diversity which could separate the genotypes better. Boonsrangsom*et al.* (2008) noted that high He value indicated the high locus diversity and more informative in plant identification.Ho and He value were also an indication for gene diversity. Ho and He value of SNP3_CHS (0.518) was higher than SNP1-CHS, suggested that SNP3-CHS had more heterezygote allele than SNP1-CHS. It also showed that SNP3_CHS had high diversity in the population used. It needs to explored more polymorphic locus and number of progenies in order to get high PIC, He and Ho value and separate progenies in the population for their specific characters effectively.

c. Clonal propagation through axillary branching

Phalaenopsis is a monopodial orchids which only have one terminal shoot tip. According to Chug *et al.* (2009), *in vitro* shoot tip culture in sympodial orchids is an efficient system for reproduction of large numbers of plantlets in a short period of time. Propagation of orchids through shoot tip culture has been also successful inAnacamptis, Anoectochilus, Arudina, Cymbidium, Dendrobium, Paphiopedilum, Phaius, Phalaenopsis, Vanda and Vanilla (Morel 1970, Huang 1988, Nagaraju andParthasarathy 1995*in* Mondal *et al.* 2013, Devi et al. 1997, Seeni and Latha (2000), Roy and Banerjee(2003), Subramanium and Taha (2003), Ket et al. (2004), Kalimuthu et al. (2006),Roy et al. (2007). *In vitro* axilary shoot induction was failed at the BAP concentration 1-3 ppm (data not published). In this experiment, BAP was increased until 30 ppm. The high concentration of BAP did not show the toxic sympton on the explant.

Shoot initiation was detected visually at 2 WAT on medium composition with addition of 10, 20 and 30 ppm of BAP and medium with combination 2 ppm of NAA and 10 ppm of BAP. All medium composition with addition of BAP or combination of NAA and BAP could induced axillary shoots at 8 WAT. Control medium (½MS, without addition of NAA or BAP), and medium which only contain NAA as plant regulator could not induce axillary shoots until 8 WAT. The highest number of shoots was got on ½MS+ 30 ppm BAP and ½MS + 1 ppm NAA + 10 ppm BAP. This results showed the important role of BAP (cytokinine) for axillary shoot formation. The increasing of NAA concentration in the medium tended to decrease the number of shoots.

The effect of plant growth regulator especially BAP and NAA was reported by Mondal*et al.* (2013) on *Doritispulcherrima*Lindl. The highest formation of axillary shoots were recorded in the medium containing 2 mg/l of BAP, resulted 1-2 shoots per explant. The higher concentration of BAP showed inhibitory effects on axillary shoot formation. There are differences between *Phalaenopsis*and *Doritis*eventhough both of them are included in sub family *Epidendroideae*and tribe *Vandaeae. Phalaenopsis*are monopodial while *Doritispulcherrima*are sympodial. It is suspected that sympodial orchids has lower apical dominant than monopodial orchids. As a consequence, monopodial orchid need higher concentration of cytokinin for induction of axillary shoots.

IV. Conclusion

Based on the morphological characters on the vegetative stage, it could be conclude that hybrid population of TSW 1102 (*Phal. bellinax Phal.* 'Salu Spot') had high diversity. Progenies were grouped into two main clusters with the level of similarity around 50%.

Molecular analysis of 40 progenies and their parents by using SNP1-CHS and SNP3_CHS resulted three main group of population. The first group was more similar to *Phal. bellina,* the second groupwas more similar to hybrid of *Phal. bellina*and *Phal.* 'SaluSpot', and the third group was more similar to *Phal.* 'Salu Spot'. The level similarity of progenies in their group was 100%, while between groupswere between 80-85%. The results suggested the low diversity of the locus. Exploring more locus and progenies were needed to develop effective marker for identification of progenies.

In the section of clonal propagation, BAP could induce axillary shoot which detected visually at 4 WAT. The maximum number of shoots were found on ½ MS medium with addition of BAP 30 ppm or ½ MS medium with addition of NAA 1 ppm and BAP 10 ppm, but it still need more experiment to evaluate of rate of shoot multiplication, the development of shoot to plantlet and shoot and plantlet quality.

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