TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 25

"Plant Productivity and Environmental Conservation"

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MALAYSIAN SOCIETY
OF PLANT PHYSIOLOGY

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

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Plant Productivity and Environmental Conservation

27th Malaysian Society of Plant Physiology Conference (MSPPC 2017)
Held at Pulai Springs Resort, Johor, Malaysia (21-23 August 2017)

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Organised by

Malaysian Society of Plant Physiology

Publisher

Malaysian Society of Plant Physiology
Locked Bag No. 282, UPM Post Office,
43409 UPM, Serdang, Selangor, Malaysia
URL: http//mspp.org.my

MSPP is a professional scientific body dedicated towards promoting research and development in tropical plant biology

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

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eISSN 2600-9595
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Plant Growth and Development

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Selenium and Organic Fertilizer on Growth, Yield and Nutrient Content of Choy Sum (*Brassica chinensis* var. *parachinensis)* Planted on Marginal Soil

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Introduction

Brassica chinensis var. *parachinensis* represented an interesting research project to produce it as a Selenium-enriched diet because *Brassica* could highly tolerate and accumulate Selenium (Se) when grown in soil treated with selenite (Jun et al., 2015). Therefore, foliar fertilization of Se is a good strategy to increase the Se content in *Brassica* to give additional health benefits (Hu et al., 2001), whereas organic fertilization is an important mean in improving soil fertility for sustainable crop production.

Scarcity of fertile and suitable land for vegetables nowadays and following years to come leads the necessities to improve soil fertility of existing marginal soil with poultry manure (PM). Sustainable concepts for increased food production are urgently needed to sustain with population growth, land forces conversion for urban development and depleting soil fertility land currently under agriculture use. PM has been recognized as the most desirable organic fertilizer. It improves soil fertility by adding essential nutrients and soil organic matter which improve moisture and nutrient retention (Adeleye et al*.,* 2010).

Se is an essential trace element and integral part of many antioxidant enzymes such as glutathioneperoxidase and selenoprotein in humans and animals. The recommended daily intake for Se is 6 to 41 µg day^{-1} for infants and adults, respectively in Malaysia (MOH, 2005) and toxicity occur when higher than 400 µg day^{-1} taken daily (Fordyce, 2005). There is increasing evidence that improving Se status without exceeding the toxic threshold may benefit long-term health, especially by lowering the risk of cancer (Finley, 2007). The consumption of Se-enriched plants is a good way to supplement Se, because of the higher bioavailability of the organic forms. Effective ways to increase Se levels in edible crops include spraying the plant with Se solution (Hu et al., 2001). Se has not been recognized as an essential micronutrient for higher plants (Seppänen et al., 2010). Se is incorporated into food chain mainly via crops and therefore the Se status of the food chain is dependent on Se level in soil. In areas where soils are naturally low in bioavailable Se, potential Se deficiencies may cause serious health problems (Hartikainen, 2005).

The objectives of this study are to determine the effect of PM and Se on the amount of Se in *B. chinensis* var. *parachinensis* and the growth and yield of *B. chinensis* var. *parachinensis*.

Materials and Methods

The field experiment was conducted at Intergrated Agriculture Training Centre, Department of Agriculture Sabah, Putatan, under a shelter (21 m x 11 m) with plastic roof and netted sides. The temperature was ranged between 26°C to 31°C. The soil of the experimental site was classified as Kelawat Family, District Cambisol soil series with sandy loam soil texture with a pH (KCl) of 7.40.

The experiment was laid out in two factors combination of treatments with six PM and Se concentration rates in randomized complete block design (RCBD) with three replications. Each replication was divided into six unit plots. There were six treatments distributed into these plots randomly. A total of 18 plots were prepared and each plot size was 4 m x 1.2 m (4.8 m^2) with inter row spacing of 0.6 m between plots. *Brassica'*s seeds were sown in a nursery tray and transplanted after eight days of sowing (DAS) to the plot with planting distance 15 cm x 20 cm. Transplanting was done in the late afternoon. There were six planting rows per plot with 27 plants per row or 162 plants per plot. The experimental plots were fertilized with PM furrowed into the soil at 10 t ha^{-1}, 20 t ha^{-1}, 30 t ha^{-1}, 40 t ha^{-1}, 50 t ha^{-1} and 60 t ha^{-1}, respectively. Foliar application of Se has been used for enrichment of agricultural products (Smrkolj et al., 2006). With this method a Se-containing solution is applied to the surface of the plant leaves by spraying. *Brassica* plants were sprayed at 21 DAS and 26 DAS with Sodium selenite ($NaSeO_3$) of analytical grade and produced by Acros New Jersey USA. The solutions containing 5 µL surfactant per litre water (TWEEN 20, Sigma-Aldrich, Copenhagen, Denmark). Se rates used were 0 mg L^{-1}, 20 mg L^{-1}, 40 mg L^{-1}, 60 mg L^{-1}, 80 mg L^{-1} and, 100 mg L^{-1}, respectively. Throughout the cultivation period of *Brassica*, treatments (Table 1) were applied.

Table 1: Treatments combination.

Treatments	Description
T1 (Control)	10 t ha^{-1} PM + 0 mg L^{-1} Se
T2	20 t ha^{-1} PM + 20 mg L^{-1} Se
T3	30 t ha^{-1} PM + 40 mg L^{-1} Se
T4	40 t ha^{-1} PM + 60 mg L^{-1} Se
T5	50 t ha^{-1} PM + 80 mg L^{-1} Se
T6	60 t ha^{-1} PM + 100 mg L^{-1} Se

The following parameters were taken, plant height was measured from the soil ground level to the tip of the *Brassica*, and the mean plant height was recorded in centimeters (cm) at 24, 30, 36, 40 DAS and yield fresh weight recorded at harvest (40 DAS). Total dry matter accumulation of two randomly selected sampled plants (g m^{-2}) was recorded at 24, 30, 36 and 40 DAS. The samples were kept in the oven at 103°C for 17 hours. Soil organic matter (SOM) was determined using Walkley Black method. Se in plant tissue was extracted using the dry ashing method and determined with inductively coupled plasma optical emission spectrometry (ICP-OES).

The data were subjected to one-way analysis of variance (ANOVA) using Statistical Program for Social Sciences (SPSS, Version 22 Software). The treatment means were then separated by using Duncan's multiple range test (DMRT) at 0.05 level of probability to determine whether the difference was significant or non-significant.

Results and Discussion

Foliar Se application showed increasing trend of Se in *Brassica* leaves (Figure 1). However, there were no significant differences on Se concentrations in the *Brassica* leaves after the treatments. Foliar application of Se fertilizer (10 mg L^{-1} Se) applied by foliar spraying in the form of sodium selenate was feasible and effective in St. John's wort (*Hypericum perforatum* L.) and resulted in Se-enriched nutritional supplements (Germ et al., 2009). The accumulation of selenium in a whole plant pak choi increased with increasing Se-supplemented concentrations up to 60 mg L^{-1} and could tolerate Se-supplemented concentration less than 120 mg L^{-1} in sand (Thosaikham et al., 2014).

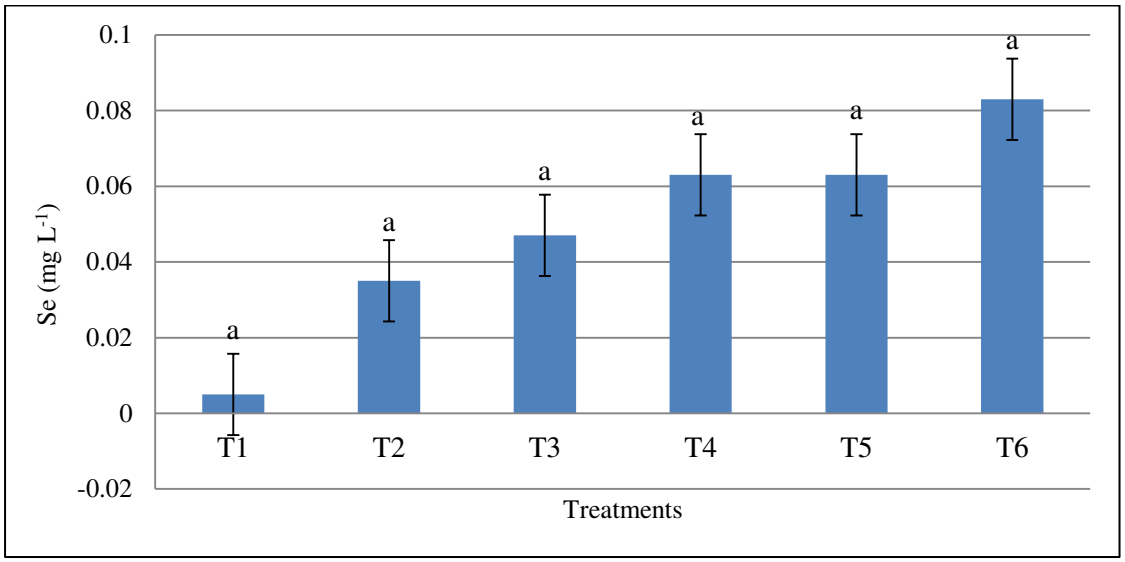

Figure 1: Effect of treatments on Se concentration in *Brassica chinensis* var. *parachinensis* leaves. Means with the same letter is not significantly different at P<0.05 by DMRT.

Plant height of *Brassica* at 24 DAS ranged from 8.50 cm to 10.92 cm and there were no significant differences between treatments (Table 2). There was rapid growth at T3 between 30 DAS, 36 DAS and 40 DAS plant heights ranged from 9.92 cm to 29.93 cm. There was a height increment of 20.01 cm (66%) from 24 DAS to 40 DAS. Significant differences between the treatments was only observed at 30 DAS, where T2 (15.67 cm) has the highest plant height compared to T5 (10 cm).

Table 2: Plant heights at four stages DAS of plant growth.

Treatments	24 DAS(cm)	30 DAS(cm)	36 DAS(cm)	40 DAS (cm)
T1	10.50	14.33^{ab}	22.50	26.59
T2	10.92	15.67^{a}	24.62	26.94
T3	9.92	13.40^{ab}	19.33	29.93
T4	9.75	15.00^{a}	17.00	19.91
T5	8.50	10.00^{b}	17.00	19.80
T6	9.75	11.33^{ab}	19.17	23.89
LSD (0.05)	0.530	0.098	0.613	0.532

Means followed by different letters within a column are significantly different at P<0.05 according to DMRT.

Significant difference in TDM was only observed at 36 DAS (Table 3) and T1 has the highest TDM of about 156.52 g m^{-2}. T1 showed increased of TDM at four stages DAS of plant growth.

Table 3: Total Dry Matter (TDM) at four stages DAS of plant growth.

Treatments	24 DAS(g m^{-2})	30 DAS(g m^{-2})	36 DAS(g m^{-2})	40 DAS(g m^{-2})
T1	7.72	24.61	156.52^{a}	242.78
T2	6.42	20.89	133.11^{ab}	182.39
T3	6.86	19.00	107.26^{ab}	194.06
T4	8.39	25.33	84.15^{b}	186.19
T5	5.14	11.11	81.78^{b}	193.45
T6	5.70	41.22	107.71^{ab}	175.86
LSD(0.05)	0.530	0.585	0.161	0.703

Means followed by different letters within a column are significantly different at P<0.05 according to DMRT.

There were significant differences of yield recorded and the highest yield was at T1 (9.67 t ha^{-1}). Yield reduction happened when PM application increased. Figure 2 showed yield reduction in T5 and T6. T4

(40 t ha^{-1}) is not significantly different with T2 and T3. Organic fertilizer as the sole source of nutrients can give yields that are higher or comparable to inorganic fertilizer. The optimum rate was about 30 t ha^{-1} of PM for leaf mustard, kangkong and lettuce and 36 t ha^{-1} for Chinese spinach on a clay loam soil (Lim and Vimala, 2012). Organic fertilizer as soil application in composted form of 20 t ha^{-1} improved soil health and fertility and increase tomato yield (Mohammad et al., 2013). Organic fertilizer could improve the physical and soil nutrients, it also could improve the physical and chemical property of soil, increasing the diversity of microbial community and improving the enzyme activity in soil (Singh et al., 2012). Several studies have been conducted to evaluate the efficacy of organic nutrient sources and rates on vegetable yields (Vimala et al., 2006; Vimala et al., 2010; Lim and Vimala, 2012).

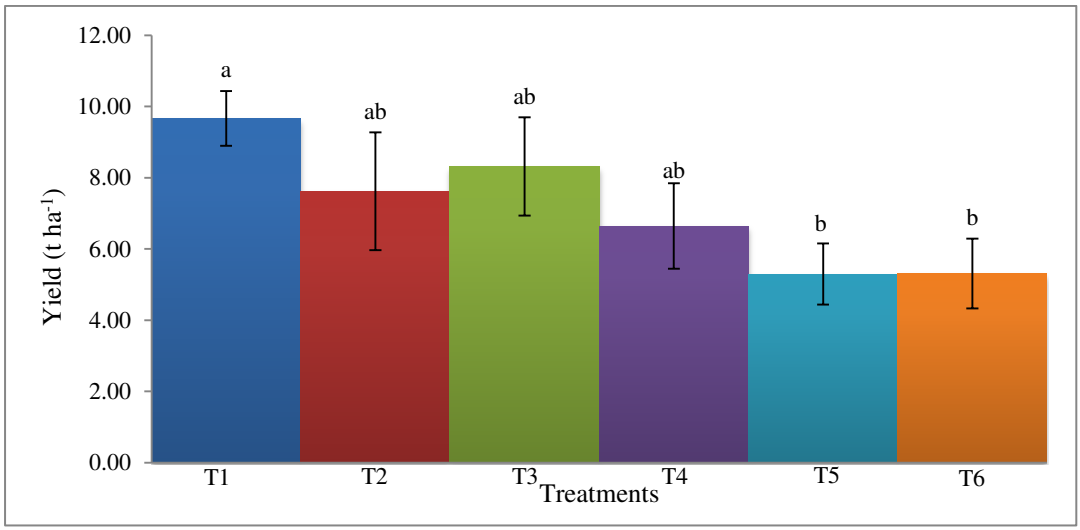

Figure 2: Effect of treatments on yield of *Brassica chinensis* var. *parachinensis*. Means with the same letter(s) are not significantly different at P<0.05 according to DMRT.

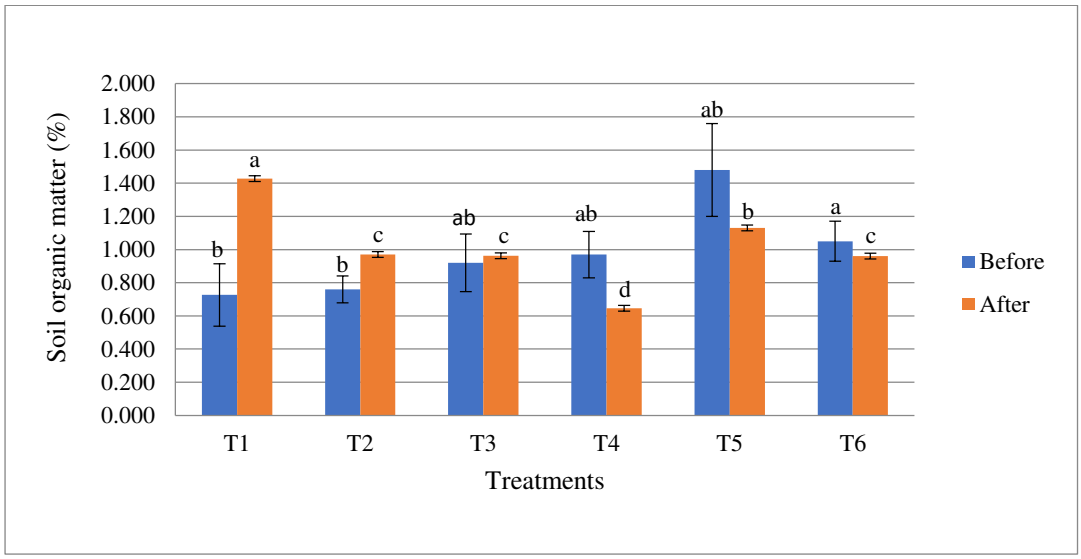

Figure 3: Effect of treatments on SOM. Means with the same letter(s) are not significantly different at P<0.05 according to DMRT.

SOM increased significantly at 10 t ha^{-1} PM compared to other treatments (Figure 3). PM at 10 t ha^{-1} application produced the highest yield (9.67 t ha^{-1}). It also increased nutrient uptake, growth and yield of yam significantly (Adeleye et al., 2010).

Conclusions

The highest yield of *B. chinensis* var. *parachinensis* was from T1 (9.67 t ha^{-1}) with 10 t ha^{-1} PM application. Plant heights at 30 DAS and total dry matter at 36 DAS showed significant difference, respectively. SOM increased significantly at 10 t ha^{-1} PM application (T1). Results showed that Se concentrations in *B. chinensis* var. *parachinensis* highest as selenite rates increased to 100 mg L^{-1}.T1 provided the best plant growth and yield of *B. chinensis* var. *parachinensis.* Further research need to be done to determine optimum absorption of Se in *B. chinensis* var. *parachinensis.*

Acknowledgements

The authors thankful for the support provided by colleagues' Jabatan Pertanian Sabah, Malaysia and Universiti Malaysia Sabah, Malaysia.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Sucker Pruning Effects on Mother Palm and Succession Sucker of Sago Palm (*Metroxylon sagu* Rottb.) Growth Performance

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Introduction

Sago palm (*Metroxylon sagu* Rottb.) is a perennial plant that grows vigorously in the coastal region and flood plains of rivers in the swampy area of South East Asia and New Guinea Island (Flach, 1997; Ehara, 2015). In Sarawak, sago palms are found extensively grows in the low-lying mineral or alluvial soils and swampy forest of Mukah and Betong Division, along the lower stretches of the Balingian, Mukah, Oya, Igan, Lupar, Saribas and Rimbas Rivers (Figure 1).

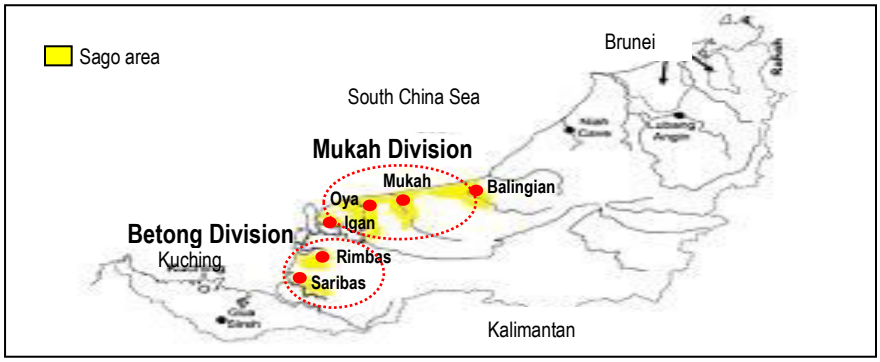

Figure 1: Sago area in Mukah and Betong Division, Sarawak.

This palm belongs to the Arecaceae family, genera *Metroxylon* and species *sagu* which is both hapaxanthic (once- flowering) and soboliferous (suckering) (Flach, 2005; Ehara, 2015). On average, depending on the soil type, sago palm has a life cycle of 10 to 15 years (Hassan, 2002). Flach (1997) reported that sago palm can reached harvestable stage in 8 to 10 years on mineral soil, while Johnson and Raymond (1956) observed that sago palm can only be harvested after 15 to 17 years on peat soil. Sago starch accumulated in the palm's trunk have been extracted and produced for both export and domestic consumption and is one of the major commodities for Sarawak. Although Sarawak is not the world's largest producer, however it is the sole exporter of world sago starch (PELITA, 2013). World demand on sago starch especially for food industries have increased tremendously but the supply is insufficient.

Despite having the potential as commercial starch producing palm, agronomical studies on sago palms are quite limited as compared to other main commodity crops. Information on the proper agronomical practices for sago palm was scarce and as a result, palms are left to grow without any proper and systematic maintenance, resulting in low and inconsistent sago log productivity with longer harvesting intervals experienced by both sago plantation and smallholder (Ipor et al., 2005). Observations on normal sago palm growth have found out that the ability to produce suckers have an adverse effect on the palm growth performance. Without any system for sucker regulation, it may eventually resulting in a dense cluster of palms with different growth stages. These palms will compete for nutrients, light and growing space which are essential for optimum growth (Pasolon et al., 2001; Irawan et al., 2015). Study done by Ecosol Consultancy Sdn. Bhd., a Sarawak based agriculture consultant, showed that

sago palms grown in the plantation without any proper sucker regulating system had a poor growth performance with low trunk productions and starch yield (Ecosol Report, 2005). Nabeya et al. (2012) on the other hand, stressed that suckers control will not only regulated cluster density but also positioning the trunk to ease trunk harvesting.

Currently, there is no intensive study done to observe the growth performance for both mother palm and succession suckers in response to sucker pruning activities. Having every individual palms achieving optimum growth performance within the cluster is essential to ensure sustainability production of sago log. Hence, study on the effect of sucker regulation system on sago palm growth and its physiological characteristics are critical to improve sago palm trunk formation and reducing the harvesting interval in order to increase and sustain sago log productivity.

The objective of this study was to determine the growth performance of both mother palm and succession suckers in response to different sucker pruning treatments. The outcome of the study will determine the suitable sucker regulating practices to be included in the sago palm agronomical practices standard for both plantations and smallholders which might improve trunk formation besides sustain sago log production and starch yield.

Materials and Methods

Palm materials and experimental location

The study was conducted in a three hectares sago palm block, planted in July 2007 using rooted sago suckers nursed using rafting system in Sungai Talau Research Station, CRAUN Research, Mukah, Sarawak (N 02° 49’ 31.8” E 111° 55’ 13.1”). The sucker pruning plot was established in the same year and the first sucker pruning treatment started one year later.

Treatments and experimental design

There were five sucker pruning treatments (Table 1) designed for the study which was arranged using Randomized Complete Block Design (RCBD) in five blocks. There were five replicates for each treatment in each block. All sago clusters within the study plot underwent the same palm maintenance activities which include weeding, fertilizer (applied three times a year with a total of 25 kg NPK 12:12:17+TE) and water level was maintained at the range of 40 to 50 cm throughout the study. Sucker pruning treatments were carried out three times annually and the period for retaining succession sucker was based on the given treatment. Each retained succession suckers were selected from a healthy sucker located at least 100 cm from the mother palm and other previously retained succession suckers. Other suckers which were not retained were pruned at the height of at least 20 cm from their base.

Table 1: Description of the sucker pruning treatments.

Treatment	Description
T0	All existing suckers to be retained (Control).
T1	Sucker pruning started after 12 months from field planting with one succession sucker retained every 12 months interval.
T2	Sucker pruning started after 12 months from field planting with one succession sucker retained every 18 months interval.
T3	Sucker pruning started after 36 months from field planting with one succession sucker retained every 12 months interval.
T4	Sucker pruning started after 36 months from field planting with one succession sucker retained every 18 months interval.

Growth performance-related parameters

Growth performance-related parameters for eight years old sago cluster including palm developmental stages, number of trunking palms, palms base girth circumference and trunk height were recorded for both mother palms and succession suckers.

Statistical analysis

Analysis of variance (ANOVA) using Statistical Analysis System software version 9.3 (SAS 9.3) was used to analyse the significance of different sucker pruning treatment on number of trunking palms, palms base girth circumference and trunk height for both sago mother palm and succession sucker. Duncan New Multiple Range Test (DNMRT) at $P = 0.05$ was used to compare the means for all growth performance parameters.

Results and Discussion

Trunking stage indicated by visible trunk formation was only observed in the early part of the fifth year for mother palm and sixth year for succession sucker, as also been observed by Flach (1997). Overall observation showed that pruned sago clusters had more mother palms and succession suckers achieved trunking stage with taller and broader trunk than sago clusters without any sucker pruning being done (Figure 2).

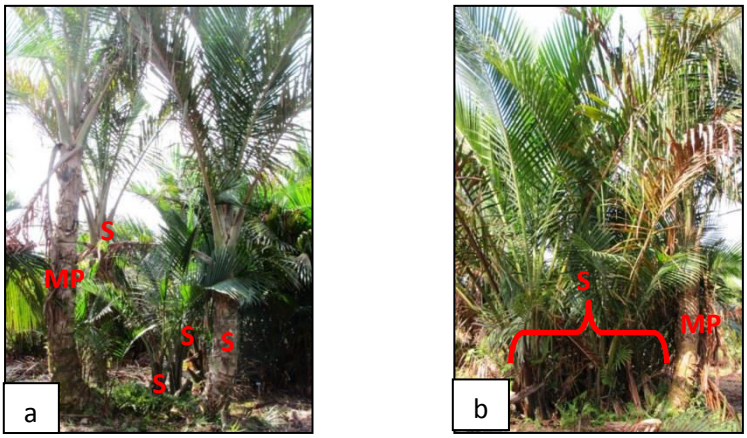

Figure 2: Differences of sago palm growth performance between pruned sago cluster (a) with un-pruned sago cluster (b) (MP: Mother palm; S: Sucker).

The number of trunking mother palms and succession suckers from sago clusters undergoes different sucker pruning treatment in the eighth year after planting was shown in Figure 3. There were a significantly higher number of trunking mother palms from pruned sago clusters (T1, T2, T3 and T4) as compared to un-pruned sago clusters (T0). Sago clusters experienced T2 and T4 demonstrated the highest mean number of trunking mother palms of 3.80. Nevertheless, there was no significant different among the pruned sago clusters.

Throughout the eight years of observation, only three levels of retained succession sucker, succession sucker 1 (S1), succession sucker 2 (S2) and succession sucker 3 (S3) had achieved trunking stage. Sago clusters under T1, T2, T3 and T4 showed their S1 achieved trunking stage, but only sago cluster under T4 exhibited both its S2 and S3 achieved trunking stage. However, none of the three levels of succession suckers from sago cluster under control treatment formed any trunk. Pruned sago clusters with only certain number of palms to grow at a given period of time might optimised nutrient uptake, sunlight interception and spacious growing area that enabling palms to obtain optimum trunk

formation and elongation. This explained the high number of trunking mother palm and succession sucker shown by pruned sago clusters as compared to sago clusters without any sucker pruning.

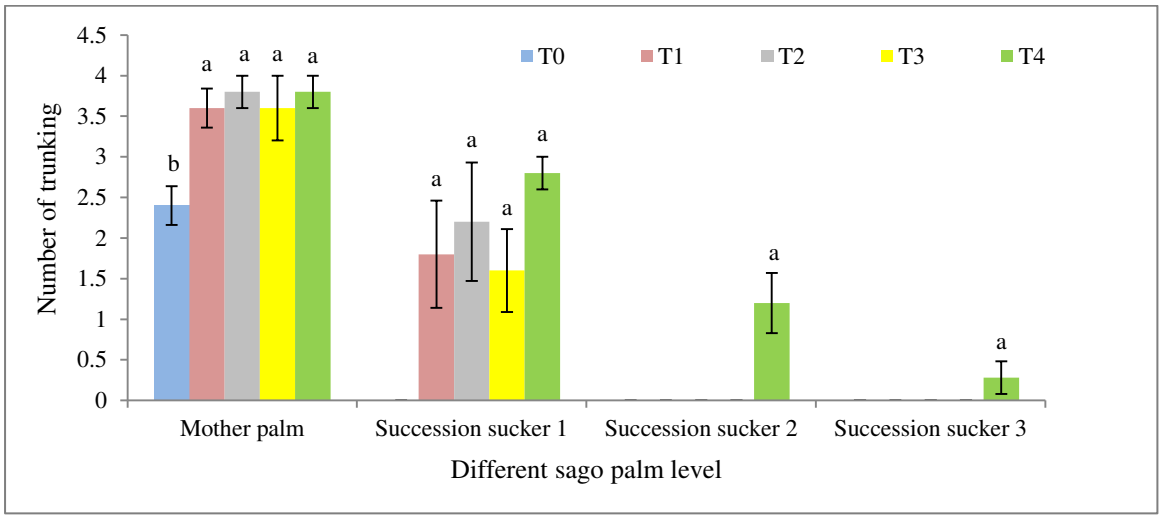

Figure 3: Mean number of trunking mother palms and succession suckers in different sucker pruning treatment for eight years old sago clusters. Note: Bars with the same letters indicates the absence of a significant difference ($p<0.05$) according to DNMRT.

Trunk height for both mother palms and succession suckers showed a similar pattern as of the trunking number as shown in Figure 4. Trunking mother palms from pruned sago cluster demonstrated significantly taller trunk as compared to sago cluster without any sucker pruning. However, there were no significant difference for mother palms and succession suckers trunk height among the pruned sago cluster. Trunking palms from sago clusters adopting T2 and T4 were among the tallest trunk with those under T4 showed the highest mean of 3.51 m. The results showed that less number of growing palms within the pruned sago cluster had a positive effect on the trunk growth similarly to the observation reported by Pasolon et al. (2001) and Irawan et al. (2015).

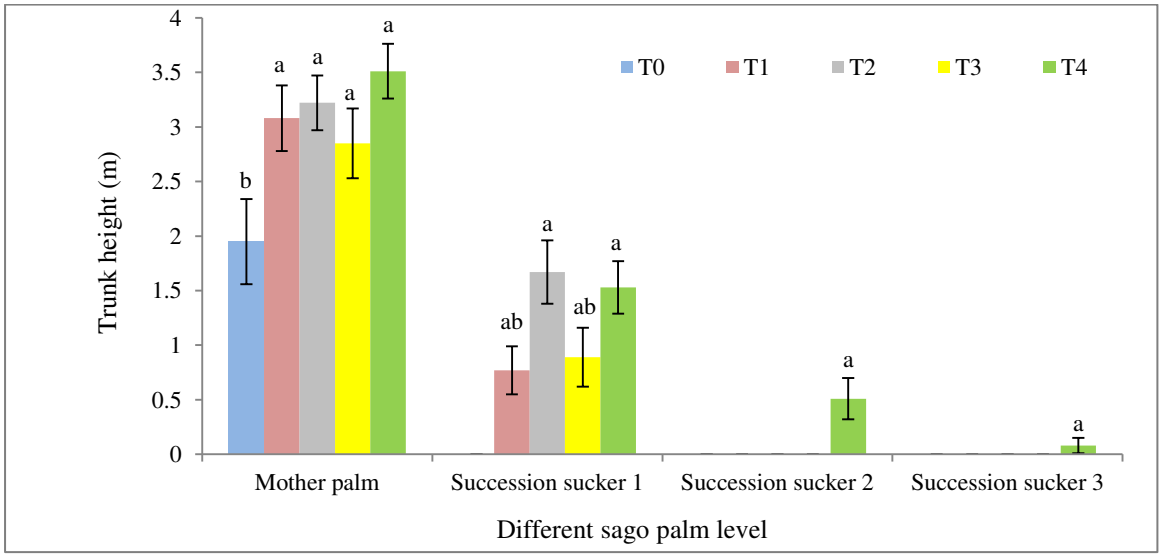

Figure 4: Mean trunk height for mother palms and succession suckers in different sucker pruning treatment for eight years old sago clusters. Note: Bars with the same letters indicates the absence of a significant difference ($p<0.05$) according to DNMRT.

As shown in Figure 5, sago mother palms from pruned sago clusters showed significantly broader palm base as compared to mother palm from un-pruned sago cluster. Mother palm from the cluster experiencing T3 demonstrated much broader palm base with the mean of 203 cm, however there was no significant difference among the mother palm from pruned sago cluster. Different pattern of results were observed among the different levels of succession suckers. Succession sucker 1 from pruned sago clusters showed significantly broader palm base as compared to those form sago cluster under control treatment. Succession sucker 2 under T1 and T4 showed significantly broader palm base, whereas sago clusters under T1 and T3 exhibited significantly broader palm base for S3.

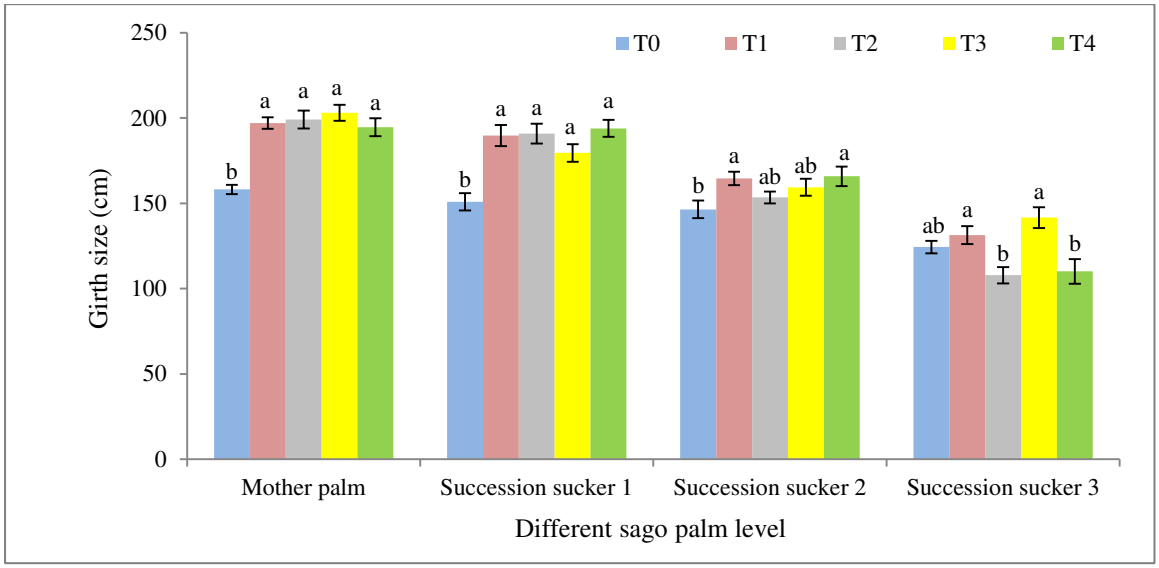

Figure 5: Mean base girth circumference for mother palms and succession suckers in different sucker pruning treatment for eight years old sago clusters. Note: Bars with the same letters indicates the absence of a significant difference ($p<0.05$) according to DNMRT.

Depending on the treatment description, similar succession suckers level might not have a similar rate of physical development. Different timing for initiation of each sucker pruning treatment and sucker retaining period had a significant effect on sago palm growth performance. The development of palm base girth was observed to be delayed when constantly pruned previously but the development would start vigorously once it was retained. When retained earlier with adequate nutrients, light interception and spacing, assist these suckers for base enlargement which is essential for the preparation of trunk initiation and formation. Hypothetically, succession suckers from pruned sago clusters should constantly having better growth performance than those equivalent succession suckers from sago clusters without any sucker pruning.

Conclusions

Results showed that different sucker pruning treatments influenced sago palm growth performance differently. Generally, better growth was exhibited by both mother palms and succession suckers from pruned sago clusters as compared to those from sago clusters without any sucker pruning. Positive growth responses by the succession suckers toward sucker pruning activities would ensure chronological development of succession suckers in each cluster for subsequent staggered harvestings. Result of this study indicated that palms from sago clusters being treated using T2 and T4 outshone other palms from clusters under other treatments in almost all growth parameters studied. Both treatments have less number of palms within the cluster, which ensured adequate nutrient distribution, optimum light interception for photosynthesis activity and spacious growing area. However, T4 showed much better growth performance for both mother palms and succession suckers. Therefore, in

order to improve sago palms growth performance and sustainable productivity, sucker pruning activities should be included in the agronomical and cultural practices standard for sago palm. The sucker pruning should be initiated after the sago cluster reached the third year of field planting with one succession sucker to be retained at the interval of 18 months which was relevant to T4 in this study. Further studies to relate sago palms growth to different sucker pruning treatment under different type of soil is needed to further improve sago palm maintenance programme.

Acknowledgements

The authors would like to thank CRAUN Research for the research fund (ID S11/P13/000671) and Faculty of Agriculture and Food Sciences, UPMKB for the technical advises.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Collection and Propagation of *Chromolaena odorata* L. for Future Production of Quality Planting Materials

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Introduction

Chromolaena odorata L. commonly known as Siam weeds or in Malaysia is known as 'pokok kapal terbang', 'pokok Jerman', 'rumput Jepun' or 'rumput Siam', belongs to family Asteraceaea. The plant is native in North America and has been introduced to South America, Tropical Asia, West Africa and parts of Australia (McFadyen and Skaratt, 1996). It is a semi-woody shrub and able to reach 3.0-7.0 meter in height. It is very common on the road side, open areas, forest clearings, and abandoned gardens and near the beach (Syed, 1979). It is reported that *C. odorata* is an aggressive competitor that suppresses young plantation, agriculture crops and grows on other vegetations (Azmi, 2002). However, there was research showing that the species has positive contribution to the agricultural sector. The leaves of *C. odorata* are used as an ingredient for formulating animal feeds especially in rabbit's diet where the nutrient profile is similar to a concentrated feed (Bamikole et al., 2004). The leaves are also claimed to have high nutritive values and have potential to be used as protein supplements to ruminants (Apori et al., 2000).

Besides that, the leaves and other parts of *C. odorata* also provide a lot benefits especially in traditional medicine. A decoction of the leaves is used as a cough remedy while the flower is used as tonic. The leaves are also traditionally applied onto cuts or wounds to stop bleeding and promote healing (Muhamad and Mustafa, 1994). Scientific findings on *C. odorata* leaf extract towards wound healing show positive results especially in helping to form new tissue cells (Chee, 2012). In addition, fresh leaves or decoction are used to treat leech bite, soften tissue wounds, burn wounds and skin infection (Phan et al., 2001). Due to its vast benefits in pharmaceutical aspects, the species has high potential to be commercialized in the market.

Thus, Plant Improvement Programme from Forest Research Institute Malaysia (FRIM) has taken an initiative to grow this species in a selected area by establishing a germplasm which consisted mother plants from various populations in Peninsular Malaysia. This germplasm will provide basic materials used to initiate a breeding programme for the species. Through plant breeding, plants from different origins planted in the germplasm can be improved to produce superior clones. It is also one of the conservation effort and a method to sustain the production of quality planting materials in the future. Therefore, this paper highlights the process of sample collection, propagation technique and method of plot establishment of *C. odorat*a as its first stage of the breeding programme to produce improved and high quality plant materials.

Materials and Methods

Collection of mother plants

A total of 150 mother plants were collected from five populations in Peninsular Malaysia and labelled with different code numbers. All mother plants were wrapped and transported back to FRIM. The five populations were coded as RKT (from Mata Ayer, Perlis), JKT (from Kota Tinggi, Johor), MKT (from Jasin, Melaka), CKT (from Maran, Pahang) and TKT (from Setiu, Terengganu). The topographic information such as coordinates, altitudes and dates of assessment were also recorded. The locations of origin were also tagged using Global Positioning System (GPS) for records (Table 1). Samples of seeds, stumps and stems of each mother plants were collected from each of the populations. The morphological characteristics of all mother plants such as number of clumps, height, diameter, leaf length, leaf width, crown diameter and number of nodes were measured (Table 2).

Table 1: Topographic information of five *Chromolaena odorata* populations.

Population	Altitude (m)	Point (X)	Point (Y)	Date of survey
RKT	53.95	250791.55	732232.78	1-3 March
JKT	31.68	638250.52	199172.32	15-18 May
MKT	31.04	491942.81	254241.37	16-20 Feb
CKT	45.18	536110.36	400435.74	17-20 April
TKT	11.38	564747.03	597568.38	3-7 April

Table 2: Morphological characteristics of mother plants for *Chromolaena odorata* from five populations.

Population	Number of clumps	Height (m)	Diameter (mm)	Leaf Length (cm)	Leaf Width (cm)	Crown diameter X (cm)	Crown diameter Y cm)	Number of nodes
RKT	1-7	1.11-2.28	3.5-9.4	4.8-10.6	2.3-7.2	15-73	12-65	8-24
JKT	2-5	1.38-2.35	5.2-12.3	9.4-12.5	4.5-7.2	15-48	20-57	9-23
MKT	2-5	1.32-2.50	5.6-12.6	8.0-19.8	3.5-9.0	10-55	9-45	8-24
CKT	1-7	1.35-2.74	3.2-12.3	8.7-13.4	4.7-7.9	21-55	24-72	9-27
TKT	2-5	1.06-2.90	5.0-14.3	7.9-14.4	3.8-9.8	11-15	16-65	9-29

Analysis of soil composition at five populations of Chromolaena odorata

The soils were collected from the five different populations. Soil inspection was done at the depth of 0 cm to 50 cm using auger at each plant. Several chemical and physical property analysis was done at the Soil Chemistry Laboratory, FRIM and using standard soil chemistry laboratory analysis method.

Propagation of Chromolaena odorata by seeds

Seeds of *C. odorata* is small, numerous and fragile. Thus, it is easily spread to distance once the seeds mature. The mature seeds normally turn into brown colour. Thus, seeds collected from the five populations were carefully covered with tissue paper and packed in plastic bags before they were transported back to FRIM in Kepong, Selangor. Upon arrival, the seeds were stored in Seed Laboratory to retain their viability. Germination of seeds was conducted at both laboratory and nursery.

Nursery condition

The layout of the experiment was designed according to randomized complete block design (RCBD). A total of 450 mature seeds were sown in sand media in germination trays with 30 seeds each, and replicated 15 times in the nursery. This media used for the germination test was a standard media used in FRIM nursery and it is assumed suitable for all species (Aminah et al., 1995). Seed germination was carried out under 50% direct sunlight with an average/ambient temperature of about 30ºC. Continuous moist condition was maintained by regular watering, i.e. in the morning and late afternoon. Germination was recorded daily and germinant were counted once cotyledons emerged above the media (Bahuguna et al., 1987).

Laboratory condition

This test consisted of 4 replicates with 50 seeds per replicate. The seeds were placed in glass petri dishes with tissue paper as media. Frequent watering with distilled water was conducted when necessary. The seeds were germinated at room temperature 28ºC with RH of 90% in minimal light condition. Daily observation and record of germination were made. A seed was considered to have germinated when the radical and/or conical shoot become visible. This experiment was terminated when there was no more germination observed. The parameters observed were germination percentage, mortality percentage and the germination period.

Propagation of Chromolaena odorata by cuttings

An experiment using stem cuttings of *C. odorata* was carried out in nursery of FRIM in April 2017. Each stem was cut into three portions, i.e. top, middle and bottom. The length of each cutting was 11 cm each. The base of the cutting was treated with commercial powdered hormone, Seradix 1 (0.1% IBA). A total of 90 cuttings were used and they were arranged randomly in 3 blocks with 30 cuttings per block. These cuttings were planted in cleaned river sand in a propagation tray. Observation on cuttings was made weekly and the experiment was terminated at week 7 since most of them had rooted. Variables collected were the number of rooted, unrooted and dead cuttings and root length. Data were subjected to analysis of variance using a statistical package for social study (SPSS) version 22.0 (International Business Machines Corporation, New York, USA). This was followed by Duncan's Multiple Range Test (DMRT) to examine the effect of different cutting parts on rooting.

Raising of planting materials at FRIM's nursery

Stumps collected from the origins, seedlings from germination and rooted cuttings were raised in polybags in the nursery. A total of 180 rooted stem cuttings and seedlings arranged in RCBD with 3 replicates were further tested for growing media experiment. Three growing media used were i) soil: leaf compost: sand (2:3:1); ii) soil: leaf compost: cocopeat: sand (2:2:1:1) and iii) soil: peat grow: sand (2:3:1). Variables recorded were survival rate (%) and root length (cm). The data were analysed using ANOVA (SPSS Version 22.0) followed by Duncan's Multiple Range Test (DMRT) to see the effect of different growing media on the growth of the plants.

Whereas for stumps, they were planted in 10' x 12' polybags containing top soil: cocopeat: sand (2:3:1) as growing media. A total of 150 stumps collected from five populations were raised under 50% shade until new stalks and leaves were produced. The stumps were used as sources of planting materials for the establishment of germplasm at FRIM's Research Station located in Maran, Pahang.

Establishment of Chromolaena odorata germplasm

FRIM's Substation in Maran, Pahang was selected as a location for the germplasm establishment. The site was selected due to its suitable soil condition which is categorized as clay loam. At the initial phase of establishment, the selected area was cleared and ploughed to further improve the soil condition. Planting distance used was 1.0 m x 1.0 m. A total of 150 stumps of *C. odorata* from five populations were planted. The plot was covered with 50% shade netting and irrigated with sprinkler system. The planting plots were also covered with silver shine plastics for weed control. The established germplasms will be used for future breeding programme for the species.

Results and Discussion

Propagation of Chromolaena odorata by seeds

Figure 1 shows that germination percentages of *C. odorata* seeds under laboratory and under nursery condition were 19% and 74%, respectively. Germination in the laboratory started on day 4 and completed on day 14 (week 1-2). Under nursery condition, the germination period was longer between one (day 7) to three weeks (day 22). According to Williamson and Jackson (1994), under ideal conditions, emergence will likely occur during a two weeks' period beginning from 7-10 days after sowing. It was found that *C. odorata* seeds under nursery condition performed better than under laboratory condition. External factors such as temperature and light could be the factors affecting the seed germination of *C. odorata* in nursery and laboratory condition thus resulted in different percentages of germination (Roberts, 1972).

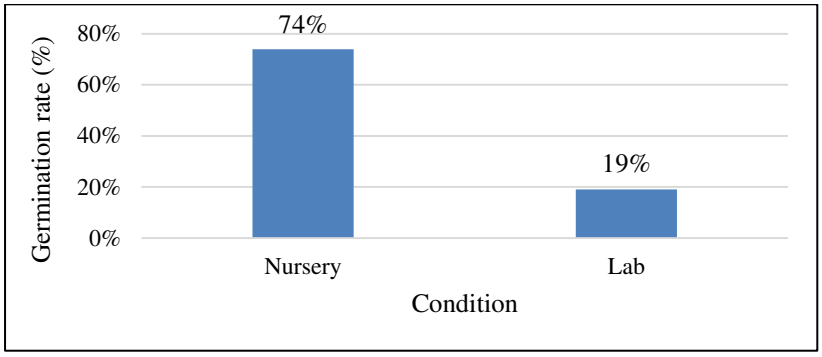

Figure 1: Percentage of germination for seeds of *Chromolaena odorata* under different conditions.

Propagation of Chromolaena odorata by cuttings

Analysis of variance on variables taken on 7 weeks after planting showed that there was significant difference among the positions of cuttings. The cuttings taken from top showed higher percentage of rooted cutting and higher survival than those of middle and bottom part as presented in Table 3. For root length, top part showed the highest measurement (11.47 ± 1.37cm) while bottom part showed poor performance of root length (4.02 ± 0.91 cm).

Table 3: Effect of cutting position on rooting ability of *Chromolaena odorata* on 7 weeks after cutting.

Cutting position	Rooted cuttings (%)	Unrooted cuttings (%)	Dead cuttings (%)	Mean root length±SEM (cm)
Top	90.0[a]	NA	10.0[b]	11.47±1.37[a]
Middle	63.3[b]	NA	36.7[a]	6.61±1.30[b]
Bottom	60.0[b]	NA	40.0[a]	4.02±0.91[b]

Means followed by the same letters in the same column are not significantly different at P<0.05.
SEM: Standard error of means.

The difference in rooting percentages with the position of cutting could be due to the different degree of juvenility along the stem. This assumption is based on the diameter of cuttings where the top cutting had significantly smaller diameter and was more juvenile than the middle and bottom parts (Figure 2). The juvenility of stem reduces from top to bottom thus give different response to the rooting percentage. This finding is in line with Otiende et al. (2016) who studied on the effects of cutting position (top, middle and bottom) of *Rosa hybrida* rootstocks. Porlingis and Therois (1976) also supported that juvenile cuttings rooted faster and in higher percentages compared to adult cuttings of leafy olive.

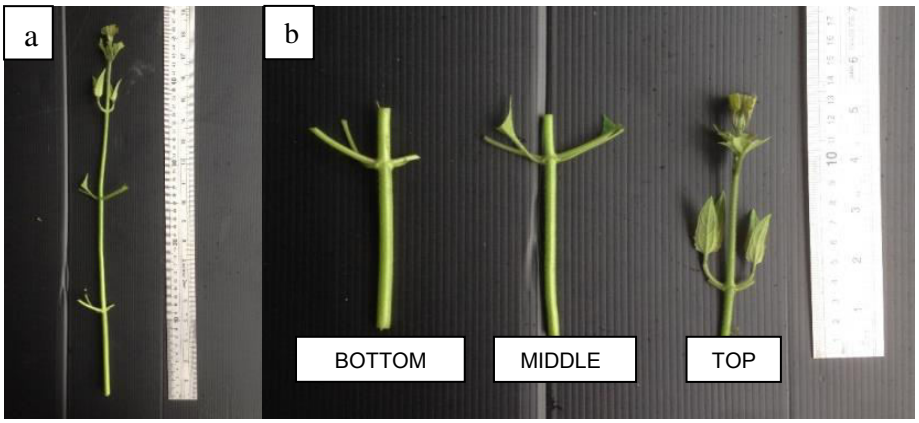

Figure 2: *Chromolaena odorata* L. stem (a); bottom, middle and top (b) cuttings.

Growth performances of Chromolaena odorata at nursery

Growth performance of *C. odorata* plants in terms of survival and root length produced from two propagation methods grown in three different growing media of i) soil: leaf compost: sand (2:3:1); ii) soil: leaf compost: cocopeat: sand (2:2:1:1) and iii) soil: peat grow: sand (2:3:1) were monitored at one month after transplanting into polybags. It was found that plants produced from cutting (100%) gave higher rate of survival compared to plants produced from seeds (94.4%) (Figure 3).

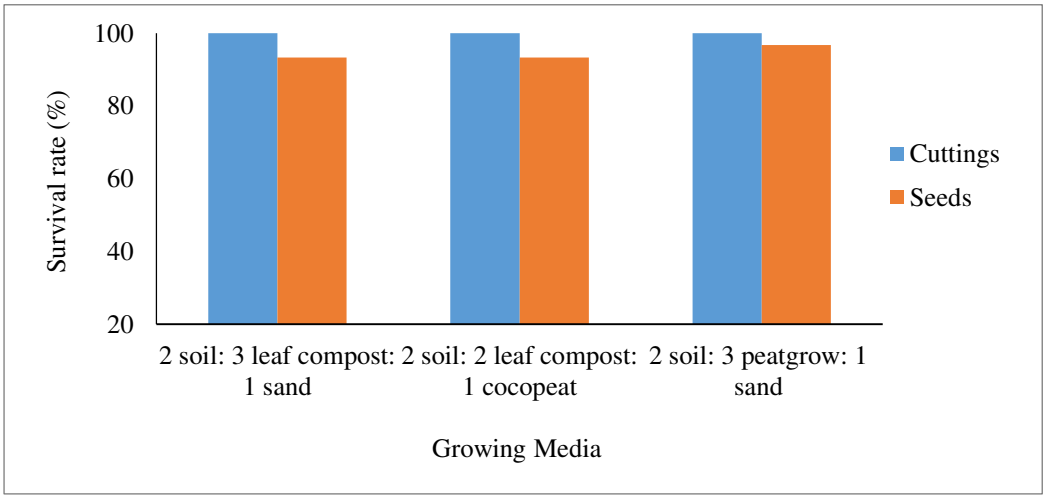

Figure 3: Percentage of survival of *Chromolaena odorata* produced from cuttings and seeds grown in three different growing media.

In terms of root length, cutting materials had no significant difference with all treatments (Figure 4). However, for seedlings, it was found that treatment 1 (2 soil: 3 leaf compost: 1 sand) gave the highest value (11 cm) compared to treatment 2 and 3. In general, all treatment media gave good growth

performance for *C. odorata* regardless their origin from seeds or cuttings. The addition of organic substance such as leaf compost and peat grow in the growing media might influence the growth of this plant as supported by Chen and Aviad (1990).

Table 4: Mean root length of *Chromolaena odorata* plants produced from cuttings and seedlings grown in three different growing media.

Treatments	Mean root length (cm)	
	Cuttings	Seedlings
T1 – 2 soil: 3 leaf compost: 1 sand	16.5[a]	11.0[a]
T2 – 2 soil: 2 leaf compost: 1 cocopeat	14.8[a]	9.1[ab]
T3 – 2 soil: 3 peatgrow: 1 sand	17.1[a]	8.2[b]

Means followed by the same letter are not significantly different at significance level of 0.05.

Establishment of Chromolaena odorata germplasm

A germplasm of *C. odorata* was established at FRIM's Substation in Maran, Pahang. The size of the plot is about 0.02 hectare with planting distance at 0.3 m x 0.3 m (Figure 4). This germplasm will provide basic materials used to initiate a breeding programme for the species. Through plant breeding, plants from different origins planted in the germplasm can be improved to produce superior clones. It is also one of the conservation effort and a method to sustain the production of quality planting materials in the future.

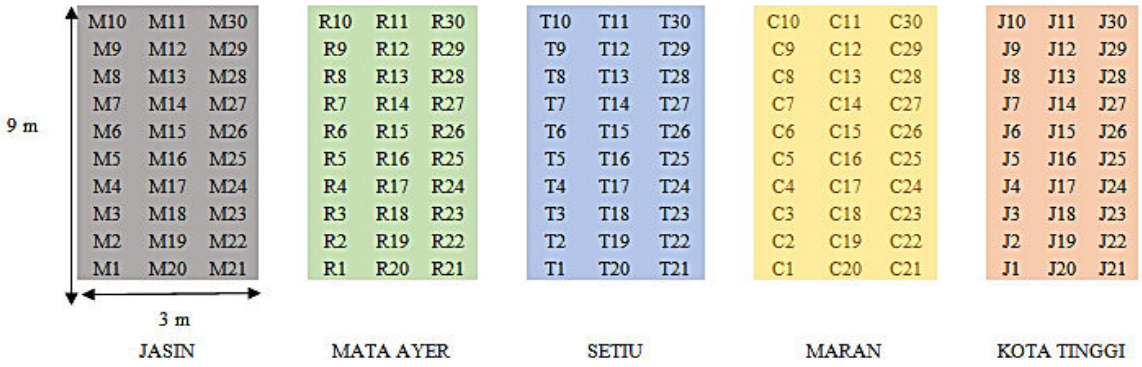

Figure 4: Layout of planting plot of *Chromolaena odorata* germplasms.

Conclusions

Chromolaena odorata can be easily propagated using seeds and cutting technique for production of planting stocks. The established germplasm is useful for conducting breeding strategy of the species in future such as for selecting superior mother plants and conducting clonal/progeny trials.

Acknowledgements

Financial support for fieldwork was provided by the Rancangan Malaysia (RMK) Ke-11 fund for research grant entitled *Pemantapan Bank Germplasma.* Facilities and equipments for viability and germination seeds are provided by FRIM's Seed Technology Laboratory.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Accession Characterization and Evaluation of Yard Long Bean (*Vigna unguiculata* (L.) Walp.) Germplasm

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Introduction

Vigna unguiculata is a multipurpose crop belonging to the family Fabaceae and sub-family Fabiodeae. It is also known as the yardlong bean, bara, cowpea, asparagus bean and snake bean. This species is originated from central West Africa where a large genetic diversity of wild types occurs throughout the continent. Today it is cultivated extensively in many countries in Southeast Asia as a good source of protein. In West Africa, this crop is often called as 'poor man's meat', where people use it as the substitute of animal protein. The grains are containing in average 22.0% protein, 1.4% fat, 59.1% carbohydrate, and 3.7% ash (Ajit et al., 2014). These crops also fix atmosphere nitrogen through symbiosis with nodule bacteria to improve the poor soils. It can be included in crop rotations to build up soil nitrogen (Mullen, 1999).

Its utilization is majorly as grain crop, vegetable and fodder for livestock. In Malaysia, it was cultivated mainly for it fruits which are consumed in fresh form as green vegetable. The production of yard long bean was estimated at 63,473 metric tonne in year 2016. This crop has been reported as one of the 10 selected vegetables and cash crops that have contributed to the sufficient domestic supply (DOA, 2016).

The aim of the study is to assess the extent of the genetic variability especially on the quantitative and qualitative traits as the genetic diversity has been loss dramatically for many cultivated species (Wilkes, 1983). Only crops with better yielding are adopted by farmers. This situation causes the genetic base to be narrow and increases the potential for weakness to biotic and abiotic stress. The present day cultivar also exhibits lower productivity and non-synchronous flowering and fruiting. Therefore, there is an urgent need to generate more information on variability among the existing germplasm and also broadening the gene pool of the crop to initiate an effective selection for the development of more improve varieties for adaptation to specific constrain. Previous works have reported the genetic variability among different varieties of *V. unguiculata* (Animasaun et al., 2015; Kumar and Shrikant, 2016).

Materials and Methods

Plant materials

A total of 16 accessions of *V. unguiculata* were used in this study. All the seeds were conserved in the Seed Bank of MARDI Jerangau. The seeds have been grown from January to July 2017 at the experiment plot located in MARDI Jerangau. The list of accession used in the present study were DINO 04-1469, 08-0004, NTH 08-0070, NTH 08-0094, NTH 08-0095, NTH 08-0132, EST 09-0140, EST 09-0144, KTN 13-0013, KTN 13-0035, KTN 13-0039, KTN 13-0042, KTN 15-0036, KTN 15-0038, TRG 16-0004 and PHG 16-0043.

Morphological evaluation

Data were collected on five randomly selected plants for qualitative and quantitative characteristics during vegetation period. Qualitative characters considered in this study were flower colour, leaflet shape, pod curvature, immature pod pigmentation, immature pod colour, seed shape and seed colour. For the quantitative characters, days to flowering, leaflet length, leaflet width, mature pod length, mature pod width, number of locules per pod, pod weight per 50 pods, number of pods per peduncle, seed length, seed width, seed thickness and weight of 50 seeds have been considered.

Fruit quality evaluation

The total soluble solid content (˚Brix) was recorded for each accession during the immature pod stage to compare the sweetness between accessions. To evaluate the acceptance of fruits by consumers, a sensorial testing was conducted by 25 consumer panel that compared the quality of the 16 *V. unguiculata* accessions. The tester was asked to taste and record their assessment on Hedonic scale 10-point scale. The panel rated the score from 1 (dislike very much) to 10 score (like very much).

Results and Discussion

Morphological characteristic

Flower colour ranged from violet, white to purple (Figure 1). Most accession had violet flower colour (62%). NTH 08-0095, KTN 13-0013, KTN 13-0035 and KTN 15-0036 had purple flower (in total 25%). Only two accessions (13%) exhibited white flower colour. Those were KTN 15-0038 and NTH 08-0070.*Vigna unguiculata* accessions had three forms of terminal leaflet shape: ovate, ovate lanceolate and lanceolate. Most accessions had ovate lanceolate to lanceolate shaped leaves. On the basis of this character, we can easily distinguish long bean genotypes in the field. Pod curvature trait varied from straight, slightly curve to the curve. Slightly curve pod character was the dominant pod shape in *V. unguiculata* accession (50%) compared to other traits. The pigmentation pattern of the immature pods of the accession was characterized by the presence or absence of pigment with various transitional forms. Of total accessions, 11 accessions (69%) had immature pod without pigmentation, which means that they were green. Three accessions (19%) exhibited pigmented tip and another two accessions (12%) were uniformly pigmented. The 16 accessions of *V. unguiculata* also showed variability in immature fruit colour which clearly distinguished two groups of *V. unguiculate*; those produced green colour fruits, and those produced purple colour fruits.

Figure 1: Variation for flower colour among accessions: KTN 13-0035 (top left), DINO 04-1469 (top centre left), KTN 15-0036 (top centre right), EST 09-0144 (top right), KTN 13-0039 (bottom left), KTN 13-0042 (bottom centre left), NTH 08-0070 (bottom centre right) and KTN 15-0038 (bottom right).

Kidney seed shape was most frequently observed (87%), which can be seen in Table 1 and Figure 2. Only two accessions had ovoid seed shape; they were accession NTH 08-0094 and KTN 15-0036. Seed colour ranged from brown, brown with whitish at one end, black and black with whitish at one end (Figure 2). Brown colour seed was dominant (63%) compared to other seed colour.

Figure 2: Variation for seed shape and colour among accessions: TRG 16-0004 (top left), KTN 15 0038 (top centre left), NTH 08-0070 (top centre right), NTH 08-0094 (top right), KTN 13-0013 (bottom left), 08-004 (bottom centre left), EST 09-0140 (bottom centre right) and KTN 15-0036 (bottom right).

Table 2 shows the quantitative characteristics recorded during the study. KTN 15-0038 and KTN 13-0042 were the earliest for flowering (33 days). This character made it possible to categorize the genotype into early flowering group and late flowering group. However, the trait cannot be used as reliable trait for genotype because it can also be affected by the environmental effect. The lowest value for the terminal leaflet length was recorded in accession KTN 15-0038 and the highest value was in accessions NTH 08-0132. Terminal leaflet width measurements showed that the accessions KTN 15-0038 had the narrowest terminal leaflet width (5.32 mm) and the broadest was observed in accession KTN 13-0039 (10.01 mm). Among accessions, NTH 08-0094 recorded the lowest pod length (23.50 cm), PHG 16-0043 recorded the highest pod length (57.20 cm) whereas highest pod width was noticed in KTN 13-0039. The pods of KTN 13-0035 had the maximum number of locules in a pod (22) and the maximum 50 pod weight of 874.0 g was recorded in accession NTH 08-0070.

PHG 16-0043 recorded the highest number of pods in a peduncle with average four. However, two pods in a peduncle was the dominant character for this plant. In the observation, the higher number of pods in a peduncle resulted in higher yield that we can harvest at a time for that accession. Accession NTH 08-0094 had the lowest value for seed length trait (8.35 mm) while the highest seed length was with accession PHG 16-0043 (12.38 mm). Seed width was the lowest in accession KTN 15-0038 and the highest was in accession 08-0004. EST 09-0144 had the maximum value of seed thickness (4.55 mm). The combination of seed length, seed width and seed thickness can be used to determine the size of seed for each accession. The maximum 50 seed weight of 11.0 g was recorded in accession NTH 08-0132. Black colour of seed trait hadsignificantly higher size and weight compared to other colour traits.

Fruit quality

The total soluble solids content (TSS), measured in ˚Brix, are directly related to *V. unguiculata* sweetness. Variability was observed for the average TSS, ranging from 5.5˚Brix in EST 09-0144 and EST 09-0140 to 8.0˚Brix in accessions KTN 13-0013 (Figure 3). Result shows that accessions with purple immature fruit colour had higher reading of TSS compared to green immature fruit colour except in accession KTN 13-0013.

Table 1: Qualitative characters among accessions.

	Seed colour	Seed shape	Leaflet shape	Flower colour	Immature pod pigmentation	Immature pod colour	Pod curvature
NTH 08-0094	Brown	Ovoid	Ovate	Violet	None	Green	Slightly curve
KTN 15-0038	Brown with whitish at one end	Kidney	Ovate lanceolate	White	None	Green	Slightly curve
08-0004	Black	Kidney	Ovate lanceolate	Violet	Pigmented tip	Green with red tip	Curve
NTH 08-0095	Brown	Kidney	Ovate	Purple	Uniformly pigmented	Purple	Slightly curve
TRG 16-0004	Brown	Kidney	Ovate lanceolate	Violet	None	Green	Slightly curve
NTH 08-0070	Black with whitish at one end	Kidney	Lanceolate	White	None	Green	Slightly curve
KTN 13-0013	Brown	Kidney	Lanceolate	Violet	None	Green	Curve
DINO 04-1469	Brown	Kidney	Ovate	Purple	Uniformly pigmented	Purple	Slightly curve
KTN 13-0042	Brown	Kidney	Ovate lanceolate	Violet	Uniformly pigmented	purple	Straight
KTN 13-0035	Brown	Kidney	Lanceolate	Purple	None	Green	Straight
KTN 13-0039	Black	Kidney	Ovate	Violet	None	Green	Slightly curve
KTN 15-0036	Brown	Ovoid	Lanceolate	Purple	None	Green	Curve
PHG 16-0043	Brown	Kidney	Lanceolate	Violet	None	Green	Straight
NTH 08-0132	Black	Kidney	Ovate lanceolate	Violet	None	Green	Straight
EST 09-0144	Black	Kidney	Ovate lanceolate	Violet	Pigmented tip	Green with red tip	Straight
EST 09-0140	Brown	Kidney	Lanceolate	Violet	None	Green	Slightly curve

Table 2: Qualitative characters among accession.

	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Weight/ 50 seeds (g)	Leaflet length (cm)	Leaflet width (cm)	Days to flowering	Mature pod length (cm)	Mature pod width (mm)	Number of locules per pod	Number of pods per peduncle	Pod weight/ 100 pods
NTH 08-0094	8.35	5.61	3.99	10.0	10.60	6.54	43	23.50	7.40	17	2	508
KTN 15-0038	8.78	5.11	3.58	5.5	10.20	5.32	33	45.96	7.00	18	2	739
08-0004	12.12	6.13	3.93	10.5	16.35	9.60	40	46.70	6.76	20	2	738
NTH 08-0095	11.45	5.55	3.93	8.0	10.47	8.38	40	39.20	8.68	16	2	805
TRG 16-0004	9.91	5.91	4.18	7.0	12.50	8.30	44	26.60	7.64	16	2	615
NTH 08-0070	11.21	5.38	3.90	8.0	11.44	7.08	40	46.40	7.93	19	2	874
KTN 13-0013	10.73	5.82	4.31	8.0	12.24	6.44	41	29.40	7.43	16	3	540
DINO 04-1469	10.66	5.52	4.49	8.0	11.30	8.10	43	34.00	7.82	16	2	581
KTN 13-0042	11.34	5.91	4.01	9.0	12.06	7.22	33	36.56	7.51	19	2	527
KTN 13-0035	11.28	5.71	3.89	7.5	13.22	7.74	40	44.80	7.99	22	2	866
KTN 13-0039	12.08	5.34	4.65	10.0	13.99	10.01	43	44.51	9.66	16	2	475
KTN 15-0036	9.27	5.80	3.87	7.5	14.19	8.18	48	27.75	7.02	15	2	469
PHG 16-0043	12.38	5.93	3.36	8.5	14.12	6.56	40	57.20	7.40	20	4	767
NTH 08-0132	12.02	5.90	4.33	11.0	12.37	8.43	38	44.85	7.44	17	2	638
EST 09-0144	11.14	5.60	4.55	10.5	12.08	7.33	38	31.13	7.87	15	2	613
EST 09-0140	11.40	5.51	3.60	7.5	13.98	8.61	47	38.62	6.92	9	2	750

The total soluble solids content, measured in ˚Brix is directly related to *V. unguiculata* sweetness. The variability was observed for the average soluble solids content, ranging from 5.5˚Brix in EST 09-0144 and EST 09-0140 to 8.0˚Brix in accessions KTN 13-0013 (Figure 3). Result shows that accessions with purple immature fruit colour relatively have higher reading of TSS compared to green immature fruit colour except in accession KTN 13-0013.

Figure 4 shows the overall fruit quality acceptance of the taste tested. The accession with the highest score in this test was KTN 15-0038 followed by NTH 0-0070 and KTN 13-0013 while the lowest score was EST 09-0140, KTN 13-0039 and NTH 08-0095. Result shows the highest score of overall acceptance was the green colour of fruit. That accession was much appreciated among consumers compared to purple colour fruits. TSS was also not correlated with the overall acceptance. Higher overall acceptance was with fruits having TSS between 5.9-6.7 ˚Brix.

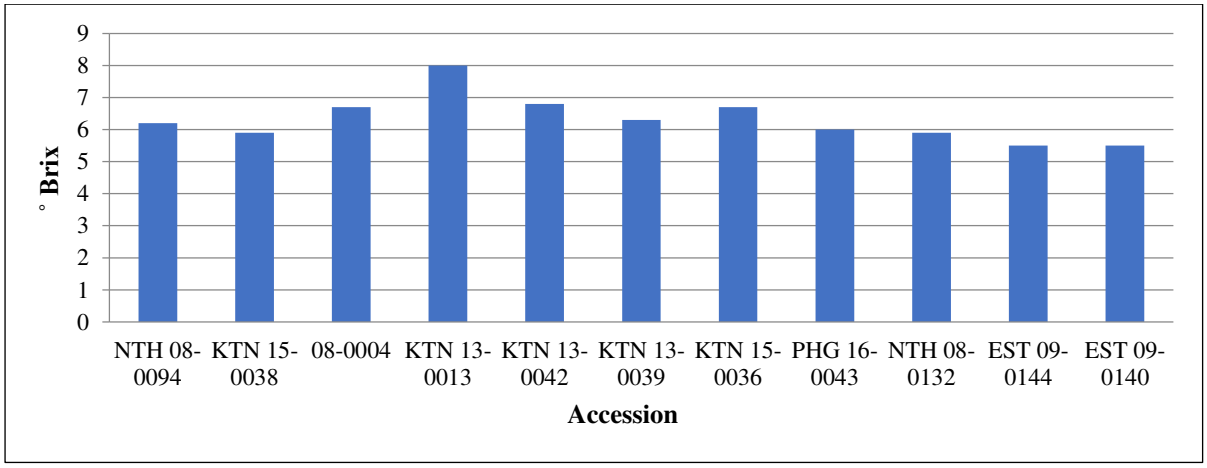

Figure 3: Average total soluble solid content (˚Brix) of fruits from 16 *V. unguiculata* accessions.

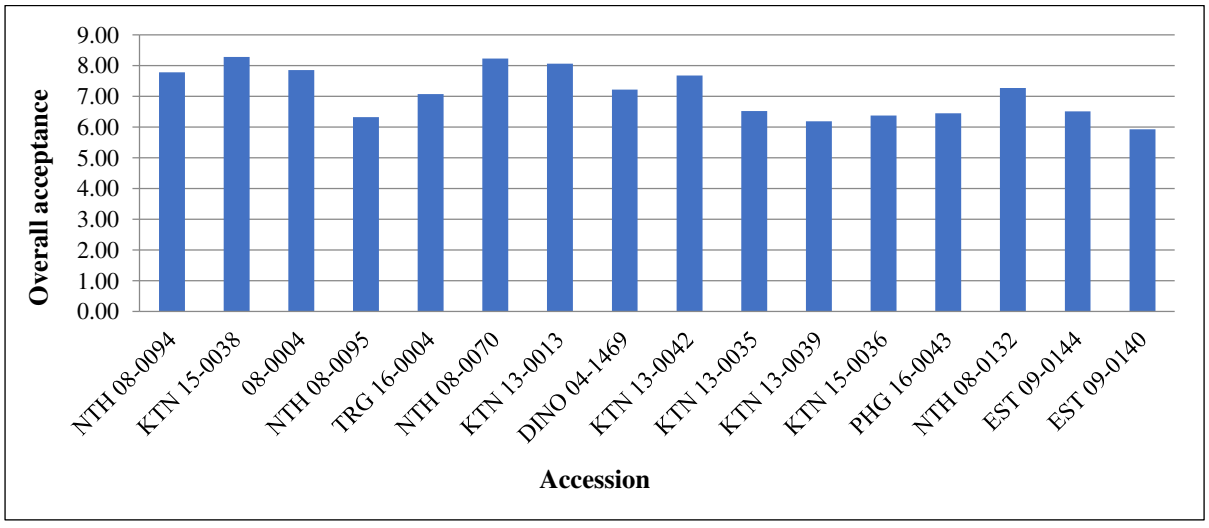

Figure 4: Overall acceptance of 16 *V. unguiculata* accessions.

Conclusions

Vigna unguiculata accession can clearly be grouped into two; those produced green colour fruit, and those produced purple colour fruit. Green colour fruit was much appreciated among consumers compared to purple colour fruits. Variabilities in days to flowering, pod and seed characters were high

and played important role in genotype differentiation to be used for the improvement of the cultivars. The performances of the accessions are highly influenced by the environmental factors. For crop improvement, each character of interest should be specifically selected and screened.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Performance of Selected Rice Varieties Grown under Organic Farming in Kahang

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Introduction

One of the scopes outlined in the national food security policy to produce safe food is the awareness use of chemical agricultural inputs (Malaysian Ministry of Health, 2013). According to Zainalabidin et al. (2016), the presence of chemical content in food can lead to excessive risk of toxicity. One approach to prevent these risks is through the practice of organic farming (Ahmad Zakaria, 2017). Therefore, study on developing organic technology should be conducted. As a first step, evaluation of some selected rice (national ample food) varieties which have potential as organic production must be carried out.

Materials and Methods

The study was conducted from January to December 2016 (main and off season) in Kahang, Johor. A total of 40 varieties (Table 1) were evaluated. Experimental design used was a randomized complete block (RCBD). Parameters evaluated included days required to achieve 50% heading, plant height, number of tillers, grain sterility per panicle, and grain yield. All varieties were transplanted at 21 days after sowing (DAS).

Table 1: List of varieties tested.

Number	Varieties	Number	Varieties
1	MR123	21	Malinja
2	MR288	22	Mahsuri
3	MR263	23	Bahagia
4	MR253	24	Murni
5	MR292	25	Sri Malaysia 1
6	MR297	26	Sri Malaysia 2
7	MR269	27	Pulut Malaysia 1
8	MR232	28	Setanjung
9	MR185	29	Sekembang
10	MR211	30	Pulut Siding
11	MR127	31	Manik
12	MRQ74	32	Muda
13	MRQ76	33	Seberang
14	MRQ88	34	Makmur
15	Masria	35	MR84
16	Sekencang	36	MR103
17	Ria	37	MR106
18	Jaya	38	PulutHitam 9
19	MRQ50	39	MR159
20	Kadaria	40	MR167

Results and Discussion

Analysis of variance (ANOVA) in Table 2 indicated that all parameters were significantly affected by variety. There was no significant interaction between season and variety.

Table 2: Mean square of ANOVA of selected rice varieties grown under organic farming in Kahang.

Sources of Variances	Parameter				
	Days to 50% heading	Plant height (cm)	Number of tillers per plant	Grain sterility per panicle	Grain Yield (kg/ha)
Season	1465.20**	1191.27**	1.35	36.68362	360220.02
Rep	395.26	10.45	1.95	84.15769	4076780.66
Rep (Season)	29.45**	0.4	0.0125	5.171162	4355506**
Variety	674.97**	704.89**	5.95**	249.43**	2170724.66**
Season x Variety	0.53	0.55	0.27	2.35006	37333.72
Grand mean	104.19	92.51	14.81	35.92	2596.83
C.V. (%)	1.15	3.42	6.7	15.3	13.42

**significant at p<0.05; ** significant at p<0.01.*

Mean comparison of all parameters in Table 3 shows the effect of varieties on days required to achieve 50% heading, plant height, tiller number, percent grain sterility and grain yield.

Flowering

According to Table 3, variety Bahagia significantly showed the longest period to achieve 50% heading which was 120 days compared to Sekencang which was only 87.8 days. The difference was 36.67%. Akhter et al. (2007) observed that super Basmati required 127 days and the difference was 4.76 and 30.31% compared to Bahagia and Sekencang, respectively. Ranawake and Amarasinghe (2014) also concluded that heading trait in rice affected yield production and other traits.

Plant height

Malinja was observed to obtain the highest plant height of 128.18 cm as compared to Masria which had the lowest plant height of 73.23 cm. The difference was approximately 75%. Oladosuet et al. (2014) discovered that variety played a role in paddy height. Liu et al. (2006) concluded that plant height was controlled by genetic factor. However, according to Beser and Genctan (1999), plant height was affected by planting approach, density and fertilizer application.

Number of tillers

Apart from attaining significant highest height, Malinja also produced significant highest tiller number which was 16.3 tillers per plant. Lowest tiller number was observed for variety MR123 which was 12.3 and it was 32.5% lower as compared to Malinja. According to Ndouret et al. (2016), tiller number was significantly affected by planting date and genotype individually. Oladosu et al. (2014) observed that tiller number also varied between varieties. According to Ranawake and Amarasinghe (2014), 55% traditional cultivars in Sri Lanka produced less than 5 tillers. This may indicate that tiller number could be largely controlled by genetic factor.

Table 3: Mean comparison of all parameters according to varieties.

Number	Varieties	Days to 50% heading		Plant height (cm)		Number of tillers per plant		Grain sterility per panicle		Grain yield (kg/ha)	
1	MR123	88.2	o	91.20	d-i	12.3	g	47.8	abc	2,336.3	g-j
2	MR288	100.8	jk	95.22	def	13.2	d-g	28.7	g-k	1,665.3	no
3	MR263	99.7	jk	91.02	d-i	14.0	b-g	31.9	e-k	2,554.3	e-m
4	MR253	93.7	lm	97.97	d	14.2	a-g	35.3	c-k	2,431.8	f-n
5	MR292	99.0	k	92.95	d-h	15.2	a-e	27.3	h-k	2,904.2	c-g
6	MR297	92.2	mn	80.50	mn	15.3	a-d	30.6	e-k	3,927.7	a
7	MR269	100.3	jk	92.08	d-h	14.8	a-e	48.6	a	2,220.2	h-j
8	MR232	99.7	jk	86.32	h-m	13.2	e-g	41.3	a-f	2,607.3	e-m
9	MR185	89.7	on	91.82	d-h	16.2	ab	39.3	a-g	3,293.5	a-e
10	MR211	76.5	p	82.12	klm	15.7	abc	34.5	d-k	2,865	d-j
11	MR127	93.3	lm	89.57	e-i	15.0	a-e	36.6	a-k	2,530.2	e-m
12	MRQ74	92.2	mn	88.72	f-k	16.0	ab	32.8	e-k	1,930.3	mno
13	MRQ76	100.3	jk	87.83	g-k	14.8	a-e	32.6	e-k	2,592.8	e-m
14	MRQ88	95.7	l	84.70	i-m	15.2	a-e	37.6	a-j	1,957.3	lmn
15	Masria	95.5	l	73.23	o	14.2	a-g	40.8	a-g	2,101	j-n
16	Sekencang	87.8	o	89.67	e-i	15.5	abc	40.2	a-g	2,545	e-m
17	Ria	101.0	jk	91.33	d-i	16.2	ab	45.9	a-d	2,640.5	e-m
18	Jaya	100.5	jk	73.32	on	13.0	eg	42.3	a-e	3,067.3	b-g
19	MRQ50	102.2	j	93.53	d-h	15.8	abc	36.0	b-k	2,071.2	j-n
20	Kadaria	92.2	mn	95.38	def	16.0	ab	34.8	d-k	3,557.8	a-d
21	Malinja	118.8	ab	128.18	a	16.3	a	39.3	a-g	2,784.7	d-j
22	Mahsuri	113.0	def	116.50	b	15.2	a-e	24.4	k	1,146.2	o
23	Bahagia	120.7	a	108.57	c	12.5	fg	40.4	a-g	3,124.2	b-g
24	Murni	113.2	def	97.18	d	13.7	d-g	36.4	a-k	3,720.3	ab
25	Sri Malaysia1	116.2	bc	80.70	lm	15.0	a-e	29.8	e-k	2,204.3	i-j
26	Sri Malaysia 2	105.2	i	96.13	ed	15.2	a-e	33.0	e-k	2,769.5	d-k
27	Pulut Malaysia 1	114.2	cde	91.73	d-i	15.2	a-e	48.2	ab	1,971.2	lmn
28	Setanjung	114.3	cde	94.50	d-g	15.5	abc	39.2	a-h	1,971.2	lmn
29	Sekembang	116.7	bc	87.80	g-k	16.0	ab	37.6	a-j	3,148	a-f
30	Pulut Siding	111.7	e-h	87.73	g-m	14.7	a-f	29.2	f-k	2,373.5	f-n

Means followed by different letters are significantly different at $p<0.05$ according to Tukey's Studentized Range Test.

Table 3: Mean comparison of all parameters according to varieties – cont.

Number	Varieties	Days to 50% heading		Plant height (cm)		Number of tillers per plant		Grain sterility per panicle		Grain yield (kg/ha)	
31	Manik	116.5	bc	99.80	ed	14.5	a-g	39.3	a-h	3,014.7	b-h
32	Muda	117.5	b	116.40	b	15.3	a-d	38.4	a-h	2,448.5	f-n
33	Seberang	110.8	fgh	106.48	c	14.8	a-e	26.0	ijk	3,699.7	abc
34	Makmur	110.8	fgh	93.83	d-g	15.3	a-d	29.6	f-k	3,037	b-g
35	MR84	112.5	d-g	93.08	d-h	15.2	a-e	25.3	jk	2,819	d-j
36	MR103	110.7	fgh	96.40	ed	14.7	a-f	29.3	f-k	2,746	e-k
37	MR106	109.7	h	87.45	h-m	14.3	a-f	32.0	e-k	1,863.2	mno
38	PulutHitam 9	110.2	gh	82.42	j-m	14.7	a-f	30.5	e-k	3,101.2	b-g
39	MR159	110.2	gh	87.68	h-m	15.0	a-e	46.9	a-d	1,977	k-n
40	MR167	114.5	d	82.52	j-m	14.0	b-g	37.1	a-j	2,154.7	i-j

Means followed by different letters are significantly different at p<0.05 according to Tukey's Studentized Range Test.

Percent grain sterility

The results also showed that MR269 had the highest sterility which was 48.6% compared to lowest sterility observed for Mahsuri with only 24.4%. The difference was 99.1%. According to Ndour et al. (2016), interaction of both genotype and planting date significantly affected sterility in rice. Nevertheless, yield loss due to sterility can be caused by extreme high temperature (Nurbaiti, 2016).

Grain yield

Significant highest yield was observed for MR297 which was 3,927.7 kg/ha and the lowest yield was observed for Mahsuri which only managed to produce 1,146.2 kg/ha. The difference was approximately 242%. However, study carried out by Ahmad Arif et al. (2014) discovered that yield was significantly affected by interaction between season and variety. In another study, Ndour et al. (2016) observed that rice yield was significantly affected by interaction of genotype with planting date. All these findings suggest that variety is an important factor contributed to rice yield.

Correlation analysis in Table 4 shows that none of the parameters recorded had significant positive association with yield. Other traits or parameters need to be included in future study for a better understanding on how different traits could affect the yield.

Table 4: Correlation analysis among parameters.

	Days to 50% heading	Plant height	Number of tillers per plant	Grains sterility per panicle	Grain yield
Days to 50% heading	1	0.43631 **	-0.0554 ns	-0.06995 ns	-0.05102 ns
Plant height		1	0.13478 *	-0.09786 ns	0.01523 ns
Number of tillers per plant			1	-0.09338 ns	0.02248 ns
Grains sterility per panicle				1	-0.07156 ns
Grain yield					1

Conclusions

Several rice varieties such as MR297, MR185, Kadaria, Murni, Sekembang and Seberang may have the potential to be introduced as potential varieties to be planted in organic rice system in Kahang, Malaysia since their yield are at par. However, further study needs to be carried out.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Growth and Chlorophyll Contents of Oil Palm Seedlings Inoculated with *Ganoderma boninense* and Planted on Five Different Soil Types

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Introduction

Ganoderma boninense is the most pathogenic species to cause basal stem rot (BSR) disease of oil palm among the other *Ganoderma* species isolated from BSR-infected oil palms – *G. zonatum, G. miniatocinctum,* and *G. tornatum* (Idris, 1999). It is reported that BSR causes approximately US$0.5 billion economic losses annually in Malaysian and Indonesian oil palm industries (Hushiarian et al., 2013). Various soil factors, such as pH, soil conductivity, water table, and nutritional status, were also suggested to be predisposing factors for *Ganoderma* infection (Gurmit, 1991). Certain acid sulphate and non-acid sulphate coastal soils were also reported to have differences in *Ganoderma* incidences, survival duration and life expectancy of diseased palms (Chen et al., 2017). The objectives of this study were to determine the effects of five different soil series, namely Parit Botak and Jawa (Acid sulphate soils), Bungor (inland soil), Blenheim (coastal shell deposit soil), and Kabu (limestone-derived, coarse sandy soil) on the growth and chlorophyll contents of oil palm seedlings inoculated with and without *G. boninense*.

Materials and Methods

Five soil series were selected in this study: Bungor (*Typic Kandiudults*, inland, fine sandy clay) (coordinate: 3°16'13.36'' N; 101°27'18.62'' E), Jawa (*Typic Sulfaquepts*, coastal, clayey) (coordinate: 3°10'55.76'' N; 101°22'06.52'' E) soils both collected from Tuan Mee Estate in Selangor while Parit Botak (*Typic Sulfaquepts*, coastal, clayey), Blenheim (*Typic Quartzipsamments*, shell deposits, coastal, sandy clay loam) soils from Blenheim Estate (coordinate: 3°55'39.40'' N; 100°48'48.58'' E) and Kabu soil (*Typic Paleudults*, coarse sandy loam, limestone) from Menglembu Estate, (coordinate: 4°33'40.77'' N; 101°02'07.39'' E) in Perak. The five soil types were used in artificial inoculation of *Ganoderma boninense (G. boninense). Ganoderma*-inoculated rubber wood blocks (RWBs) were prepared according to the protocols outlined in Kok et al. (2013), with slight modifications, i.e., smaller RWBs with the size of 6 cm x 6 cm x 6 cm were used. However, the most aggressive *G. boninense* isolate (G10) (Kok et al., 2013) was used. Vegetative growth parameters, namely height (cm), diameter of girth (cm), and leaf area (LA) (cm^2) were measured 1, 3, an 5 months after transplanting (MAT) for the seedlings inoculated with and without *G. boninense*. Furthermore, chlorophylls were extracted and three chlorophyll parameters (Sim et al., 2015; Lichtenthaler and Buschmann, 2001), namely chlorophyll A, chlorophyll B, and carotenoids, were recorded at 5 MAT. Treatments and controls on different soil types were replicated 8 times, except for Kabu soil with only 6 replicates. Leaf and rachis samples from *Ganoderma*-inoculated seedlings were harvested and sent to Advanced Agriecological Research Sdn. Bhd. (AAR) Chemistry Laboratory for foliar nutrient analyses of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and boron (B). Means for all the chlorophyll components at 5 MAT were normally distributed, whereas, the means for all the vegetative growth parameters at 1, 3, and 5 MAT were not normally distributed under Anderson-Darling normality test (Minitab 16). ANOVA-Fisher test was used to analyse means of all the chlorophyll components, whereas, non-parametric test – Kruskal-Wallis followed by Mann-Whitney test at $P = 0.05$ was adopted for analyses of non-parametric data obtained from the vegetative growth measurements.

Results and Discussion

The heights of oil palm seedlings were significantly lower (22.48 cm versus 24.02 cm at 3 MAT and 26.79 cm versus 30.41 cm at 5 MAT) in *G. boninense*-inoculated treatments at three and five months after transplanting, compared to the uninoculated control (Table 1). The leaf areas were also significantly lower (35.79 cm versus 42.08 cm at 3 MAT and 52.39 cm versus 74.76 cm at 5 MAT) in the inoculated treatments compared to the uninoculated control (Table 1). Similar observations were reported by Goh et al. (2014) where leaf area, height, and diameter of girth were significantly lower in the treatment with highly aggressive *G. boninense* isolates compared to less aggressive isolates and control. Furthermore, the chlorophyll contents, namely ChlA, ChlB, and carotenoids, for the seedlings were significantly higher in the non-inoculated treatment (Table 1). Haniff et al. (2005) reported a reduction in relative leaf chlorophyll content and a few other physiological parameters in *Ganoderma*-infected old mature palms at age 17-years old as compared to the healthy palms. For those treatments without *G. boninense,* the growths of oil palm seedlings were slightly better in Parit Botak, Jawa, and Bungor soils as compared to Blenheim and Kabu soils. This could be due to better soil fertility with relatively high soil nitrogen and potassium contents, as well as cation exchange capacity (CEC) (Goh et al., 2017), and better foliar nutrient status (Table 2 and 3). Better vegetative growth for those seedlings planted on coastal soils (Parit Botak and Jawa soils) could be due to their higher soil fertility (relatively high soil nitrogen, potassium, and CEC), and better water holding capacity (Goh et al., 2017) as well as higher proportion of clay contents compared to Blenheim and Kabu soil series. Furthermore, root volume was larger in Parit Botak, Jawa, and Bungor soils (Unpublished data). Similarly, leaf nitrogen (N), phosphorus (P), and potassium (K) levels were higher in seedlings planted on the former soil series compared to the latter. Rachis K levels were higher in Jawa, Bungor, and Parit Botak compared to Blenheim and Kabu. On the contrary, leaf calcium (Ca) for seedlings planted on Blenheim and Kabu were higher compared to other soil series. Rachis Ca levels were also higher in Blenheim and Kabu compared to other soil series. This could be due to the high soil Ca in Blenheim and Kabu soils.

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Table 1: Chlorophyll readings and the growth of oil palm seedlings inoculated with or without *Ganoderma boninense* and planted on five different soil types.

					Vegetative growth measurements*†								
		Chlorophyll at 5 MAT*‡			1 MAT			3 MAT			5 MAT		
Treatments	Soil types	ChlA	ChlB	Carotenoids	Height (cm)	Girth (cm)	LA (cm^2)	Height (cm)	Girth (cm)	LA (cm^2)	Height (cm)	Girth (cm)	LA (cm^2)
Without *Ganoderma*	Bungor	0.197^{c}	0.105^{c}	0.221^{c}	19.96^{a}	0.588^{a}	29.47^{a}	23.76^{bc}	0.931^{a}	39.76^{b}	32.28^{a}	1.169^{a}	89.1^{a}
	Jawa	0.22^{c}	0.109^{c}	0.232^{c}	20.13^{a}	0.569^{a}	29.15^{a}	26.38^{a}	0.981^{a}	47.84^{a}	32.23^{a}	1.138^{a}	80.89^{a}
	Blenheim	0.397^{b}	0.209^{b}	0.433^{b}	20.99^{a}	0.619^{a}	31.10^{a}	22.51^{bc}	0.9^{a}	35.75^{b}	29.79^{a}	1.138^{a}	67.16^{ab}
	Parit Botak	0.495^{a}	0.251^{a}	0.526^{a}	20.68^{a}	0.594^{a}	31.69^{a}	25.68^{ab}	0.938^{a}	51.03^{ab}	32.39^{a}	1.106^{a}	88.58^{a}
	Kabu	0.382^{b}	0.200^{b}	0.413^{b}	20.33^{a}	0.608^{a}	30.58^{a}	21.78^{c}	0.9^{a}	36.02^{ab}	25.35^{b}	1.05^{a}	48.09^{b}
	Mean	0.3382	0.1748	0.365	20.418	0.596	30.398	24.022	0.93	42.08	30.408	1.1202	74.764
With *Ganoderma*	Bungor	0.131^{b}	0.0695^{b}	0.1301^{b}	20.26^{a}	0.594^{a}	28.09^{a}	22.56^{a}	0.9^{a}	35.83^{a}	28.18^{abc}	1.263^{a}	65.66^{abc}
	Jawa	0.157^{ab}	0.0699^{b}	0.1361^{b}	19.26^{a}	0.621^{a}	28.94^{a}	22.64^{a}	0.914^{a}	35.49^{a}	24.56^{bc}	1.08^{a}	41.09^{bc}
	Blenheim	0.337^{a}	0.1704^{a}	0.3541^{a}	20.26^{a}	0.619^{a}	29.31^{a}	23.06^{a}	0.913^{a}	36.84^{a}	27.92^{b}	1.242^{a}	53.6^{b}
	Parit Botak	0.273^{ab}	0.136^{ab}	0.2941^{ab}	19.13^{a}	0.55^{a}	27.58^{a}	21.7^{a}	0.944^{a}	37.17^{a}	30.65^{a}	1.188^{a}	70.06^{a}
	Kabu	0.223^{ab}	0.1122^{ab}	0.2295^{ab}	19.08^{a}	0.592^{a}	28.32^{a}	22.45^{a}	0.9^{a}	33.6^{a}	22.65^{c}	1.025^{a}	31.56^{c}
	Mean	0.2242	0.1116	0.2288	19.598	0.5952	28.448	22.482	0.9142	35.786	26.792	1.1596	52.394
Significant Difference‡‡		*	*	*	ns	ns	ns	*	ns	*	*	ns	*

**Means for all the chlorophyll components at 5 MAT (month-after-transplanting) were normally distributed, whereas, the means for all the vegetative growth parameters at 1, 3, and 5 MAT were not normally distributed.*

‡Each chlorophyll components at 5 MAT within the respective treatments (with and without Ganoderma) was analyzed separately. Means within each column of the respective chlorophyll components, namely ChlA (chlorophyll A), ChlB (chlorophyll B), and Carotenoids followed by the same letter are not significantly different at P = 0.05 after ANOVA-Fisher test.

†Each individual vegetative growth parameters at the respective individual time-points for both treatments (with and without Ganoderma) was analyzed separately. Means within each column of the vegetative growth parameters (height, girth of the bole, and LA – leaf area) for the respective time-points (1, 3, and 5 MAT) followed by the same letter are not significantly different at P = 0.05 after Kruskal-Wallis test followed by Mann-Whitney test.

*‡‡Means derived from each chlorophyll components at 5 MAT and the respective vegetative growth parameters at 1, 3, and 5 MAT for all the five soil types between two treatments (with and without Ganoderma) were subjected to t-test for each individual chlorophyll component at 5 MAT and Mann-Whitney test for the respective vegetative growth readings at three different time-points. * and ns indicate significantly different and non-significantly different at P = 0.05, respectively, after t-test or Mann-Whitney test.*

Table 2: Leaf nutrient analysis results for oil palm seedlings planted on five different soils with *Ganoderma boninense* at 5 month-after-transplanting.

	Major elements (% Dry matter)					Minor elements (mg/kg)
Soil types*	N	P	K	Ca	Mg	B
Bungor	1.55	0.196	1.38	0.93	0.21	10.9
Jawa	1.6	0.2	1.68	0.74	0.31	10.7
Blenheim	1.43	0.145	0.98	1.35	0.22	10.4
Parit Botak	1.5	0.14	1.6	0.53	0.35	9.87
Department of Agriculture	1.59	0.15	0.94	1.65	0.19	12

**Leaf samples for the respective soil types were bulked into one single sample for leaf analysis.*

Table 3: Rachis nutrient analysis results for oil palm seedlings planted on five different soils with *Ganoderma boninense.*

	Major elements (% Dry matter)					Minor elements (mg/kg)
Soil types*	N	P	K	Ca	Mg	B
Bungor	0.51	0.36	1.92	0.49	0.16	7.66
Jawa	0.45	0.432	2.16	0.48	0.16	8.02
Blenheim	0.43	0.16	1.34	0.68	0.16	7.47
Parit Botak	0.42	0.188	1.8	0.38	0.19	7.5
Kabu	0.47	0.325	1.68	0.75	0.15	7.04

**Rachis samples for the respective soil types were bulked into one single sample for leaf analysis.*

Conclusions

Ganoderma-inoculated seedlings were smaller in vegetative parameters, vigour, and colour, as well as chlorophyll readings compared to the non-inoculated control. Soils from different origins and having different soil chemical as well as physical properties (different proportion of clay contents, soil nitrogen and potassium contents, cation exchange capacity, as well as soil water holding capacity) were observed to affect oil palm growth, chlorophyll readings, and foliar nutrients differently. This information will be useful for future studies on aetiology and epidemiology of *G. boninense* disease in oil palms. The stipulated results required further studies for tolerance screening which can be extrapolated to other soil types, particularly if vegetative growth is used as the main parameter.

Acknowledgements

The authors would like to thank the Estate Managements of Tuan Mee, Blenheim, and Menglembu Estates, namely Mr. Mohmad Azman Abd Majid, Mr. Poh Syee Wha and Mr. Teoh Ken Boon, respectively, for their assistance in soil sample collection in their respective estates. We also thank the valuable technical assistances from Mr. Muhammad Al Qayyum Hassan Basri and Mr. Rahaizul bin Rakman. We thank AAR Principals, Boustead Plantations Berhad and Kuala Lumpur Kepong Berhad for funding this research and for their permission to publish the data.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Evaluation of Selected Bitter Gourd Accessions for Yield and Related Traits

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Introduction

Bitter Gourd (*Momordica charantia* L.) is also known as balsam pear, bitter melon, bitter cucumber and African cucumber (Heiser, 1979). It is one of the important cucurbitaceae family vegetable in the world, including Malaysia. The demand for bitter gourd appears constantly rising and the production in 2016 was 17,413 metric tonnes (Anon, 2016). Among the cucurbits, it is considered a valuable vegetable because of its rich in phytonutrients including dietary fibre, minerals, vitamins and anti-oxidants. It is also grown as an ornamental plant and used as a folk medicine for lowering blood sugar in people with type II Diabetes (AVRDC, 2011). Within the population of Malaysia, *Momordica charantia* L. is among the traditional vegetables or generally referred to as "ulam" and is locally known as Peria Katak. Although its importance and diversified use, very little attention has been made for improvement in horticulture traits. A few varieties and hybrids have been grown for commercial planting in Malaysia and most of the seeds are imported. Thus, this study was conducted to evaluate twelve selected bitter gourd accessions based on yield and related traits. This assessment is to ensure the best accession that can be used as a parent for breeding purposes or directly selected as introduced variety.

Materials and Methods

P10, P11, P13, P92, P93, P94, and PK12 are the local bitter gourd varieties collected, together with Indian collections (P96, P97, P98 and P101) and local check accession Peria Katak (PK) were used in this study. This study was carried out at Vegetable Research Plot at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. The experiment was laid out using randomized block design with three replications each. Each accession was represented by 10 plants in each replication; with a total of 30 plants for each accession. The seedlings were raised in nursery for about 30 days before they were transplanted on single-row beds at a planting distance of 0.8 m x 1.2 m between beds. After that the seedlings were transplanted to the field upon reaching the length of about 2 to 3 in long and with the support of net for the vine to creep on. Data recorded for yield dan related traits fruit weight (g), fruit length (mm), fruit circumference (mm), fruit flesh thickness (mm), fruit number/plant and yield/plant. Analysis of variance was conducted using SAS statistical package.

Results and Discussion

A simple analysis of variance (ANOVA) was carried out on twelve bitter gourd accessions as shown in Table 1. These include the significant differences between all six traits for fruit weight (g), fruit length (mm), fruit circumference (mm), fruit flesh thickness (mm), fruit numbers/plant and yield/plant. Table 2 shows a comparison of means of yield and related traits measured. The accessions showed not much different from one and another for flowering and fruiting time. Among the twelve accessions studied, P11 produced the biggest fruit weight with 838.60 g and PK is the lowest with 29.80 g. The highest fruit length was recorded by P10, P11 and P94 with 359.20 mm, 347.20 mm and 310.60 mm respectively while PK produced the shortest fruit of 83.00 mm. However, P11 showed the biggest fruit size in both widest fruit circumference and thickest fruit flesh of 291.20 mm and 13.37 mm respectively. These include maximum number of fruit produced P101 (25.60), followed by P97 (25.40) and P92 (25.00). However, the highest yield per plant was P92 with 1830 g and the lowest was PK

with only 211 g yield per plant is considered an important trait for any crop improvement. In this study accession P92 give the best in term of highest yield, and produced a large number of fruits. However, P10 has a potential for any future breeding program as it has a largest fruit size, widest fruit length and fruit flesh thickness.

Table 1: Analysis of variance for yield and related traits in twelve accessions of bitter gourd.

Source	df	Fruit weight (g)	Fruit length (mm)	Fruit circumference (mm)	Fruit flesh thickness (mm)	Fruit numbers	Yield/plant (g)
Accession	15	232740.25**	27151.33**	9244.62**	19.63**	151.56**	4364691.64**
Error	44	6377.69	548.78	327.72	4.06	45.50	402656.10
Mean		327.62	235.28	195.43	11.36	17.67	1964.87
CV		24.28	9.96	9.26	17.73	38.18	32.29

*** Significantly different at p = 0.01.*

Table 2: Comparison of means of six traits for yield and related traits in bitter gourd accessions.

Accession	Fruit weight (g)	Fruit length (mm)	Fruit Circumference (mm)	Fruit flesh thickness (mm)	Fruit numbers	Yield/plant (g)
P10	460.80^{bc}	259.20^{a}	229.40^{b}	12.33^{bc}	14.20^{bc}	1422.00^{ab}
P11	838.60^{a}	347.20^{a}	291.20^{a}	13.37^{a}	14.60^{bc}	1313.00^{ab}
P13	86.80d^{e}	141.80^{e}	145.40^{e}	10.14^{cd}	21.80^{ab}	577.00^{def}
P92	452.00^{bc}	260.40^{c}	234.40^{b}	11.93^{bc}	25.00^{a}	1830.00^{a}
P93	550.60^{b}	317.00^{ab}	229.00^{b}	12.42^{bc}	18.20^{abc}	1728.20^{a}
P94	561.20^{b}	310.60^{a}	236.40^{b}	13.37^{b}	13.40^{bc}	681.00^{c-f}
P96	362.60^{c}	330.6^{ab}	204.40^{c}	12.23^{bc}	8.80^{c}	686.00^{c-f}
P97	164.20^{d}	204.40^{d}	172.0^{d}	11.01^{bcd}	25.40^{a}	1108.00^{bc}
P98	169.80^{d}	239.20^{c}	165.80^{de}	9.61^{cde}	17.60^{abc}	747.00^{cde}
P101	171.60^{d}	204.60^{d}	163.40^{de}	11.08^{bcd}	25.60^{a}	1020.00^{bcd}
PK12	83.40d^{e}	125.40^{e}	151.40^{de}	8.73^{de}	13.00^{bc}	366.00^{ef}
PK	29.80^{e}	83.00^{f}	121.20^{f}	7.34^{e}	14.40^{bc}	211.00^{f}

Values within a column with the same letter(s) are not significantly different at p = 0.05 according DMRT.

Conclusions

The information of yield performance and related traits in this study provide basic tool and an earlier that can be applied in bitter gourd breeding and varietal improvement. In addition, the superior traits in bitter gourd collections can be explored to produce superior hybrids through hybridization. This is because hybridization will combine the desirable genes found in two or more different varieties/parents and to produce pure-breeding progeny superior in many characteristics respect to the parental type.

Acknowledgements

The author wishes to thank Mr. Mohd Zamri Kamarudin and Mr. Mohd Isa Darus for their assistance in conducting the trial. The author also wishes to thank MARDI Development Fund (P-RH403-1001) for funding this project.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effect of Nano Fertilizer on Number of Leaves and Chlorophyll Reading for Dwarfed Long Bean (*Vigna sesquipedalis)*

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Introduction

Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering. Nanotechnology is expected to have a significant impact on human life in the 21st century (Sahoo et al., 2007; Kaplan et al., 2017). The impacts and interests of nanotechnology in the future are gaining momentum in many developed and developing countries at present. Based on information obtained from the United States, Asia Pacific and Europe between 1997 and 2005, it is projected to be RM 2.3 trillion of products and two million jobs will be involved with nanotechnology until 2017 and in the world market it will potentially generate up to seven million jobs, especially in agricultural sector (Boehmer-Christiansen, 2012).

Most farmers do not realize that not all single fertilizers used can be fully absorbed by plants (Romig et al., 1995). According to previous studies, synthetic nitrogen in the market is from reactive nitrogen. It does not really stay local and it can also interact with the surrounding resources such as by soil carbon, oxygen and water. It is estimated that only 30-50 percent is absorbed by the crops and the rest is due to waste due to the surrounding resource response. This study was conducted to compare the effects of nano fertilizers and commercial single fertilizers application on the number of leaves produced and chlorophyll content of dwarfed long bean (*Vigna sesquipedalis*)

Materials and Methods

The nano fertilizer (NPK 20:20:20) was obtained from commercial company (Iran). The urea, triple super phosphate and muriate of potash fertilizers were purchased locally. The results of the experiments were analyzed by using statistical software (SPSS ver. 22, 32bit).

Randomized Complete Block Design (RCBD) was used for the present project. A mixture of mineral soil (3:2:1(mineral soil: paddy husk: filtered sand) was used as a plant medium under rain shelter. Five treatments (Table 1) were used with 4 replications. SPAD meter (Konica Minolta) was used to determine relative chlorophyll content. The treatments comprised of single NPK fertilizer 34:56:56 kg/ha, nano fertilizer at rates of 4, 8 and 12 kg/ha and without fertilizer 0 kg/ha (Table 1). All fertilizers were applied directly to the soil (pocket method).

Table 1: Types of fertilizer treatment for dwarfed long bean (*Vigna sesquipedalis*).

Treatment	Type of fertilizer	Rate (kg/ha)	Dose (g/polybag)
1	Single fertilizer:Urea	34:56:56	
	Triple Super Phosphate (TSP)		0.3
	Muriate of Potash (MOP)		0.48
			0.37
			Total: 1.15
2	Nano fertilizer NPK 20:20:20 (basic element)	4	0.2
3	Nano fertilizer NPK 20:20:20	8	0.4
4	Nano fertilizer NPK 20:20:20	12	0.6
5	Without fertilizer	0	0

Results and Discussion

The findings showed a positive and significant improvement on the average chlorophyll reading measured by SPAD for all fertilizer treatments. Figure 1 shows the average decrease of chlorophyll reading in week 1 to week 2 of the study and coherence with the previous findings (Chen et al., 2007). The data showed an average increase of chlorophyll reading of dwarfed long bean from week 3 to week 4 for all treatments in this study where the highest average chlorophyll reading was in the treatment of nano fertilizer compared to mixed fertilizer. The average reading of chlorophyll at 5 and 6 weeks for 3 levels of nano fertilization treatment and treatment without using the fertilizer showed a drop in reading patent while only chlorophyll reading for single fertilizer treatment still showed increased reading in 5 and 6 weeks after transplanting.

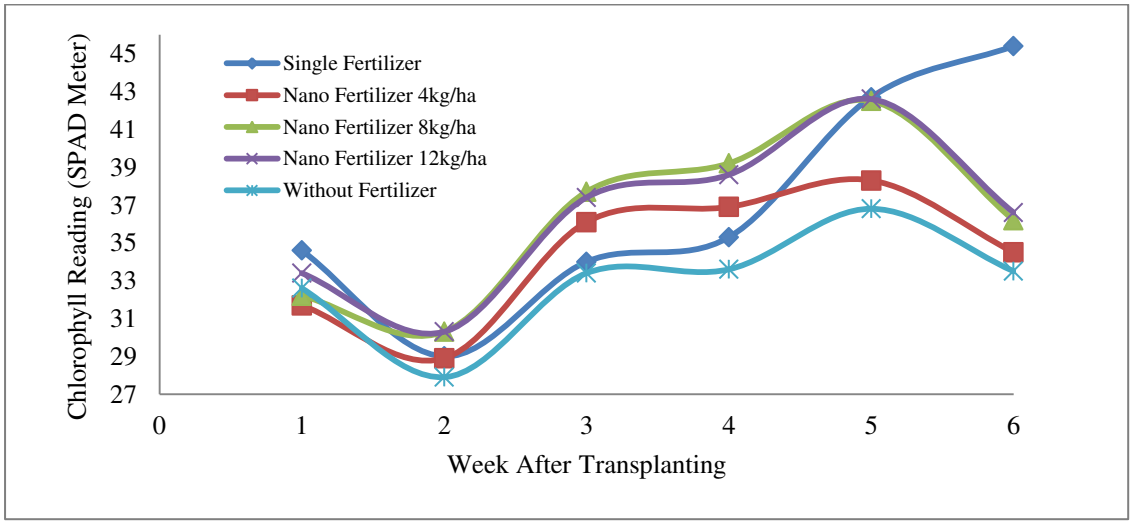

Figure 1: Effect of different types of fertilizer on chlorophyll content of dwarfed long bean.

Based on Table 2, the results showed a very significant difference in leaf chlorophyll reading between treatments from week 3 to week 6 ($p<0.05$). The highest chlorophyll reading in the 3rd and 4th weeks of the study was on T3 treatment (8 kg/ha nano fertilizers) with 37.65 and 39.17 readings while the highest chlorophyll reading was 5 and 6 weeks in T1 treatment (single fertilizer) with a reading of 42.70 and 45.37 (Please refer to Table 2 where all treatments show significance difference, $p<0.05$). Without fertilizer, chlorophyll reading was the lowest for the entire weeks. The data of this study showed that the increase of chlorophyll reading and the number of leaves for the treatment of nano fertilizers after fertilization (week 2-3) were an evidence for the significant improvement of nano fertilizer as compared to a single fertilizer. In this case, nano fertilizers may provide nitrogen to the plant in advance after fertilization is made compared to the conventional single fertilizer. The leaf

chlorophyll reading reflects nitrogen absorption. Nitrogen is an essential element in proteins production for plant growth.

Table 2: ANOVA (One way) for chlorophyll reading at week 3, 4, 5 and 6.

		Sum of Squares	df	Mean Square	F	Sig.
Chloropyll Week3	Between Groups	237.747	4	59.437	6.629	.00012
	Within Groups	672.473	75	8.966		
	Total	910.220	79			
Chloropyll Week4	Between Groups	335.791	4	83.948	10.489	.00000
	Within Groups	600.251	75	8.003		
	Total	936.042	79			
Chloropyll Week5	Between Groups	574.120	4	143.530	9.456	.00000
	Within Groups	1138.414	75	15.179		
	Total	1712.534	79			
Chlopyll Week6	Between Groups	1617.615	4	404.404	23.021	.00000
	Within Groups	1317.522	75	17.567		
	Total	2935.137	79			

The findings also showed a positive increase in actual number of leaves produced for all fertilizer treatments from week 3 to week 6 after transplanting (Figure 2). Based on Table 3, the results showed a significant difference in the actual number of leaf produced starting from week 4, 5 and 6. Plants treated with 12 kg/ha nano fertilizer had the highest number of leaves in week 4 and 5 however at week 6, the highest number of leaves was produced by plants treated with the mixed single fertilizer. This shows that the use of nano fertilizers can accelerate the growth of the number of leaves from the 4th week rather than the use of single fertilizers.

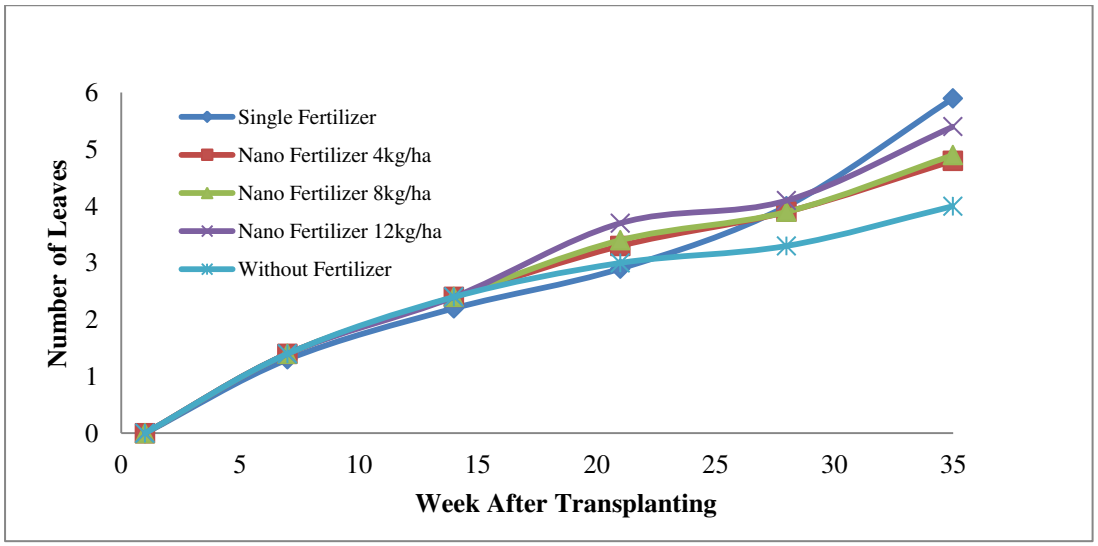

Figure 2: Effect of different types of fertilizer on the number of leaves of dwarfed long bean.

Table 3: ANOVA (Oneway) for the number of leaves at week 3, 4, 5 and 6.

		Sum of Squares	df	Mean Square	F	Sig.
No. of leaves Week3	Between Groups	.675	4	.169	.580	.67787
	Within Groups	21.813	75	.291		
	Total	22.488	79			
No. of leaves Week4	Between Groups	5.925	4	1.481	6.326	.00019
	Within Groups	17.563	75	.234		
	Total	23.488	79			
No. of leaves Week5	Between Groups	6.200	4	1.550	4.709	.00191
	Within Groups	24.688	75	.329		
	Total	30.888	79			
No. of leaves Week5	Between Groups	32.550	4	8.138	4.752	.00180
	Within Groups	128.438	75	1.713		
	Total	160.988	79			

Conclusions

The results showed significant effects on chlorophyll reading and number of leaves by different rates of nano fertilizers in early growth after fertilization. Nano fertilizer at 8 kg/ha was the most efficient in early growth compared to the other nano ferilizer rates and single fertilizers. In conclusion the use of nano fertilizers can provide nutrients on the early growth of plants. Preliminary result showed that the nano fertilizers promote the growth and yield of the experimental plants significantly. This fertilizer probably can be used in a larger scale on the suitable food crop which may increase the yield production.

Acknowledgement

This work was financially supported by MARDI Development Fund.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Screening of Selected Cucumber Mosaic Virus Resistance Chilli Genotypes Obtained from World Vegetable Center for High Yield in Malaysian Condition

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Introduction

Chilli (*Capsicum annuum* L.) is grouped in the genus *Capsicum* belonging to the Solanaceae family. It is closely related with tomato (*Solanum lycopersicum* L.*),* tobacco (*Nicotiana tabacum* L.), petunia (*Petunia* spp. Juss.) and brinjal (*Solanum melongena* L.). Chilli is an annual crop and facultatively self-pollinated with 7-91% incidence of outcrossing in an open field plantation (Bosland, 1993) especially with the presence of pollinators, particularly bees (Berke, 2000). Chilli is an herbaceous plant with densely branched stem. Its height can reach up to 1.5 m and some can be as short as less than 25 cm. The plant produced solitary flower with five to six white petal. It bears berry-type fruits which vary from round to elongate shape. The fruit is usually green at immature stage and turn red at mature stage. In a tropical country like Malaysia, chilli plants are grown as annual crops to obtain quality marketable fruits. To date, there are forty *Capsicum* species recorded. Out of that, only five species are popularly domesticated that is *C. annuum*, *C. frutescense, C. chinense*, *C. baccatum* and *C. pubescence* (The Plant List, 2013).

In most countries, chilli is consumed fresh as well as processed such as for spices, condiments, sauces and pickles. Green chilli contains higher Vitamin C than any other vegetable crops with 242.5 mg per 100 g serving (USDA, 2013). Chilli is also a good source of Vitamin E (Alpha tocopherol), Riboflavin, niacin, folate, iron and magnesium, dietary fibre, Vitamin A, Vitamin K, Vitamin B6, potassium, copper and manganese (USDA, 2013). The use of chilli has widened for other purposes than as food. Chilli can also be used as active ingredients in organic insect repellent (Kazembe and Makusha, 2012) and natural colouring agents (Szabo and Horvath, 2015). The capsaicin in chilli could lower blood pressure (Yang et al., 2010) and also contain antimicrobial properties against bacteria (Chichewicz and Thorpe, 1996; Careaga et al., 2003).

The importance of chilli as vegetables is reflected by the increasing local demand and high import value. At present, annual consumption per capita is 2.0 kg (DOSM, 2015) but the local production only supports about half of its self-sufficiency level (65.6%). The production of chilli is limited due to problems of pests and diseases. Virus disease is considered as the paramount problem in chilli production and among the viruses, Cucumber mosaic virus (CMV) is considered the most serious and giving devastating problems to the chilli cultivation (Mohamad Roff and Ong, 2000). The price of chilli per kilogram is undeniably high compare to other common vegetables in the market which is on the average RM9.00 to RM10.00/kg compared to tomato (RM3.60-RM5.30/kg), brinjal (RM4.65-RM4.85/kg) and cucumber (RM2.30-RM2.50/kg) (FAMA, 2014). To meet the increasing demand, the planting of chilli need to be expanded however, despite that, the industry of chilli plantation in Malaysia is still very much dependent on imported seeds. Limitation of new varieties developed locally minimised the choices for the farmers, thus, forcing them to obtain seeds from other countries. Therefore, this study was conducted with the objective to evaluate introduced chilli genotype with resistance to CMV for high yield and good fruit quality under local environment. The selected genotypes subsequently could be used as parental materials in breeding programs.

Materials and Methods

Seventeen CMV resistant chilli genotypes (Table 1) renamed as, CH1, CH2, CH3, CH5, CH9, CH10, CH11, CH12, CH13, CH14, CH15, CH16, CH17, CH18, CH19, CH20 and CH21 obtained from World Vegetable Center and three local varieties, Kulai, MC11 and MC12 were used in two separate experiments.

Table 1: List of chilli genotypes and their original name, source and pedigree used in this study.

Genotype	Original name	Source	Pedigree
CH1	PP0337-7546	CCA4824	PBC325/PBC308
CH2	PP0438-8543	CCA5239	IR*3/PBC932//Susan's Joy
CH3	PP0637-7515	CCA5837	UF8752/CCA321
CH5	PP0537-7516	CCA5215	Jin's Joy/PBC 481 sel.
CH9	PP0042-31	Mr. Lee No.3 seln	Jin's Joy//Arunalu/IR
CH10	PP0537-7513	CCA5213	PBC 481 sel.//Kulium/HDA248
CH11	PP0337-7554	CCA 4851	VC232/9945-1856
CH12	PP0337-7065	CCA 4799	F1LongChili/F1TM-888
CH13	PP0107-7048	CCA 3468	MI-2/Taiwan83-168-1-1//MI-2
CH14	PP0537-7558	CCA5218	Jin's Joy//Kulai*3/PBC932
CH15	PP0537-7531	CCA5216	Jin's Joy//Arunalu/IR
CH16	PP0537-7503	CCA5213	PBC 481 sel.//Kulium/HDA248
CH17	PP0007-2269	CCA 119-A	Szechwan/HDA249
CH18	PP0637-7529	CCA5835	Tit-Super/CCA321
CH19	PP0637-7519	CCA5829	Jatilaba/CCA321
CH20	PP0637-7505	CCA5850	KR-B/ICPN12#4
CH21	PP0537-7539	CCA5217	Jin's Joy//Kulium/HDA248
MC11	MC11	MARDI	Langkap
MC12	MC12	MARDI	MC4/CH207
Kulai	Kulai	Local variety	-

Chilli seeds were germinated under insect-proof nursery. Peat moss was used as the germination media were soaked in water before being utilized. Chilli seeds were planted in germination tray (98 square cells 50 mm depth). One seed was placed in each cell at 1.5 cm to 2 cm deep from the pre-watered media level. The germination trays then were wrapped using dark plastic for 3-4 days until the seeds germinated. The seedlings were raised in nursery for 30 days before transplanted in the MARDI vegetable research plot which stands of mineral soil type. The plot sizes prepared were 3.5 m x 1.5 m. Fourteen plants were planted in two beds for each genotype. Data recorded from the ten plants in the middle of the plot from each replication. Fertilizer application for chilli in this experiment was based on the recommendations provided by MARDI (2000). Chicken manure was applied as basal dressing at the rate 20 t/ha 3-5 days before transplanting while the compound fertilizer NPK was applied at the rate of 1.5 t/ha. Watering of chilli plant in the field was done manually using a hose pipe twice daily at 8:30 in the morning and 5:30 pm in the afternoon.

The chillies were evaluated for the quantitative characters which include plant height (cm), plant canopy width (cm), yield per hectare (kg/ha), yield per plant (g), fruit number per plant, fruit weight (g), fruit width (mm), fruit length (cm) days to flowering and days to fruiting. The experiment was conducted in a randomized complete block design in three replications.

The performance of the genotypes was analysed using analysis of variance (ANOVA) and mean comparison was conducted using Duncan new multiple range test (DNMRT). The data collected were analysed using SAS software package. ANOVA was used to determine the effects of genotypes and blocks. A Biplot analysis was run to visualize the relationship between genotypes and traits. Data matrix with columns representing the traits and rows representing the genotypes was first standardized,

subsequently decomposed into principle components (PC) through singular value decomposition (SVD) using NTSYS program.

Results and Discussion

Significant variations in the field performance of the chilli genotypes were obtained from this evaluation (Table 2).

Table 2: Mean squares for traits measured on 20 chilli genotypes.

SOV	d.f	Mean Squares									
		FY (kg/ha)	FYP (g)	FNP	FW (g)	FL (cm)	FD (mm)	DTF	DTM	PH (cm)	PCW (cm)
Blocks	2	7521817	10577	346	22.8	3.7	9.8	0.4	15.5	383.3	889.7*
Gen.	19	65791453**	92519**	8154**	190.5**	143.0**	132.2**	29.1**	142.9**	2497.5**	982.2**
Error	38	15092560	21224	998.4	25.1	12.8	13.3	0.9	6.0	310.8	269.8

*SOV = Source of variation; Gen = Genotype; FY =Fruit Yield; FYP = Fruit Yield per Plant; FNP = Fruit Number per Plant; FW = Fruit Weight; FL = Fruit Length; FD = Fruit Diameter; DTF = Days to Flower; DTM = Days to Maturity; PH = Plant Height; PCW = Plant Canopy Width. **, * Significant at $\rho \leq 0.01$ and 0.05, respectively.*

Among the introduced resistant lines, wide variations can be seen in all the traits evaluated (Table 3). For yield trait, the introduced lines showed highly significant variation from 5 t/ ha (CH3) to 22 t/ha (from CH20 and CH21). Most of these introduced resistant genotypes could adapt well and producing comparable yield amount with the local varieties in Malaysian environment. Six introduced chilli genotypes including CH21, CH20, CH5, CH10, CH9 and CH18 produced yields more than 20 t/ha which is considered high yield based on current local varieties yield.

Most of the introduced lines have small to medium fruit size and short plant stature while the local varieties generally have bigger fruit and plant stature. These happen as a result of different breeding purpose for these chillies. The local chillies, MC11, Kulai and MC12 main breeding focus was to get high yield and meet the local chilli preference where bigger fruit will have better grade and better price. The introduced lines used in this evaluation were breed for resistance to viral diseases although some of them were found not resistant in local environment (Ahmad Fadzil et al., 2014). The source of resistance to CMV for pungent chilli usually came from the chilli of small fruit type (Grube et al., 2000; Chaim et al., 2001).

Many introduced lines however could produce high yield despite of small fruit size where the high number of fruits per plants among this genotypes compensate the low average fruit weight (Table 3). Melor (2008) and Jabeen et al. (2009) reported that the main components for the yield in chilli are the fruit weight and fruit number. Other traits such as plant height and number of branches (Zhani et al., 2015) and fruit diameter and fruit wall thickness (Cancaya et al., 2010) also gives impact on chilli yield. Wider plant canopy width also can give advantage in producing more yields due to increased number of secondary and tertiary branches which are the locations for fruit bud formation (Faby, 1997).

The introduced chilli genotypes is this evaluation also showed earliness to flowering and fruit maturity (Table 3). The impact of virus infection will be greater during juvenile period of the infected plant therefore earliness in flowering and maturity can give advantage for the plant to escape disease incident. Genotype CH12 is the best candidate for earliness characteristics, with days to flowering only took 11.3 days and maturity in 69 days after transplanting.

Table 3: Mean values for traits measured on 20 chilli genotypes.

Gen.	Mean									
	FY (kg/ha)	FYP (g)	FNP	FW (g)	FL (cm)	FD (mm)	DTF	DTM	PH (cm)	PCW (cm)
CH1	18580^{bcde}	697^{bcde}	122^{efg}	8.97^{c}	12.0^{cd}	12.8^{de}	17.0^{fgh}	93.0^{a}	41.3^{gh}	49.3^{bc}
CH2	14130^{cde}	530^{cde}	114^{efg}	8.00^{cde}	11.8^{cd}	12.8^{de}	17.0^{fgh}	80.7^{bcde}	47.3^{def}	45.3^{cd}
CH3	5266^{f}	198^{f}	23^{h}	5.89^{hij}	8.4^{g}	14.0^{b}	14.7^{ij}	81.7^{bcd}	38.0^{hi}	35.4^{f}
CH5	21275^{abcd}	798^{abcd}	144^{defg}	8.27^{cde}	11.5^{d}	12.5^{e}	14.3^{j}	80.3^{bcde}	42.9^{g}	43.1^{de}
CH9	20618^{abcde}	773^{abcde}	87^{g}	10.79^{ab}	11.7^{d}	15.0^{a}	24.3^{a}	94.7^{a}	44.3^{fg}	55.9^{a}
CH10	21244^{abcd}	797^{abcd}	322^{a}	3.13^{k}	6.9^{h}	8.6^{h}	19.3^{cde}	73.7^{f}	48.2^{def}	49.7^{bc}
CH11	19967^{abcde}	749^{abcde}	187^{bcd}	7.70^{def}	9.6^{f}	12.4^{ef}	17.3^{fg}	84.0^{b}	51.1^{d}	52.4^{ab}
CH12	15614^{bcde}	586^{bcde}	126^{efg}	6.08^{hij}	10.7^{e}	10.1^{g}	11.3^{k}	69.0^{g}	35.6^{i}	49.1^{bc}
CH13	15114^{bcde}	567^{bcde}	363^{a}	2.41^{k}	6.1^{i}	8.3^{h}	13.7^{j}	77.3^{def}	49.0^{de}	52.9^{ab}
CH14	13774de	517de	103^{fg}	6.50^{ghi}	9.6^{f}	12.2^{ef}	18.3^{def}	83.0^{bc}	45.5^{efg}	46.6^{cd}
CH15	18164^{bcde}	681^{bcde}	170^{bcde}	5.38^{ij}	13.2^{b}	8.7^{h}	17.0^{fgh}	94.3^{a}	41.4^{gh}	45.8^{cd}
CH16	17095^{bcde}	641^{bcde}	207^{bc}	5.06^{j}	8.6^{g}	10.2^{g}	16.3^{ghi}	76.7^{ef}	44.3^{fg}	53.3^{ab}
CH17	13180^{e}	494^{e}	129^{defg}	7.26^{efg}	9.5^{f}	13.6^{bcd}	22.3^{b}	90.3^{a}	44.5^{fg}	40.6^{e}
CH18	20602^{abcde}	773^{abcde}	143^{defg}	6.8^{fgh}	9.9^{ef}	12.8^{de}	20.0^{cd}	78.3^{cde}	49.6^{d}	49.6^{bc}
CH19	19293^{bcde}	723^{bcde}	163^{cdef}	8.46^{cd}	10.4^{e}	13.6^{bcd}	18.3^{def}	80.0^{bcde}	42.9^{g}	45.4^{cd}
CH20	22156^{ab}	831^{ab}	227^{b}	4.95^{j}	12.1^{cd}	8.9^{h}	18.3^{def}	90.7^{a}	55.7^{c}	54.8^{a}
CH21	22581^{ab}	847^{ab}	208^{bc}	8.89^{cd}	11.9^{cd}	11.6^{f}	13.7^{j}	82.0^{bcd}	47.5^{def}	43.7^{de}
MC11	21455^{abc}	805^{abc}	126^{efg}	10.46^{b}	14.1^{a}	13.9^{bc}	18.0^{efg}	81.0^{bcde}	71.5^{a}	55.3^{a}
MC12	26921^{a}	1010^{a}	133^{defg}	11.83^{a}	12.7^{bc}	14.9^{a}	15.3^{hij}	78.0^{def}	57.5^{c}	52.6^{ab}
Kulai	22158^{ab}	831^{ab}	116^{efg}	10.55^{b}	14.1^{a}	13.1^{cde}	20.7^{c}	82.0^{bcd}	64.2^{b}	51.8^{ab}
Mean	18459	692	160.6	7.38	10.74	12.0	17.4	82.5	48.2	48.8
SE	1.00	37.58	16.6	0.36	0.30	0.30	0.7	1.5	1.2	0.9
CV%	21.1	21.05	19.67	28.42	14.01	13.1	5.5	3.0	15.8	16.7

Mean values followed by the same letter in the same column are not significantly different at $\rho \leq 0.05$ based on DMRT.
Gen. = Genotype; FY =Fruit Yield; FYP = Fruit Yield Per Plant; FNP = Fruit Number Per Plant; FW = Fruit Weight; FL = Fruit Length; FD = Fruit Diameter; DTF = Days To Flower; DTM = Days To Maturity; PH = Plant Height; PCW = Plant Canopy Width.

Based on the agronomic traits measured, there are four groups of chilli identified (Figure 1). Group I consist of all the local chilli and also one introduced genotype CH9 that produced significant high yield. Traits including plant height, plant canopy width, fruit weight and fruit length are the most influential factors in this group. The Group II showed all the introduced genotypes that are generally have the strength in terms of earliness in flowering and maturity but medium fruit size and plant stature. This group consists of wide variation in terms of its yield performance; from equally high yield genotypes (>20 mt/ha) to low yield genotype (13 mt/ha). Group III consists of two resistant genotypes with high number of fruit per plant and small fruit type. Group IV consists of CH3 genotype which cannot withstand the local environment thus not performing well.

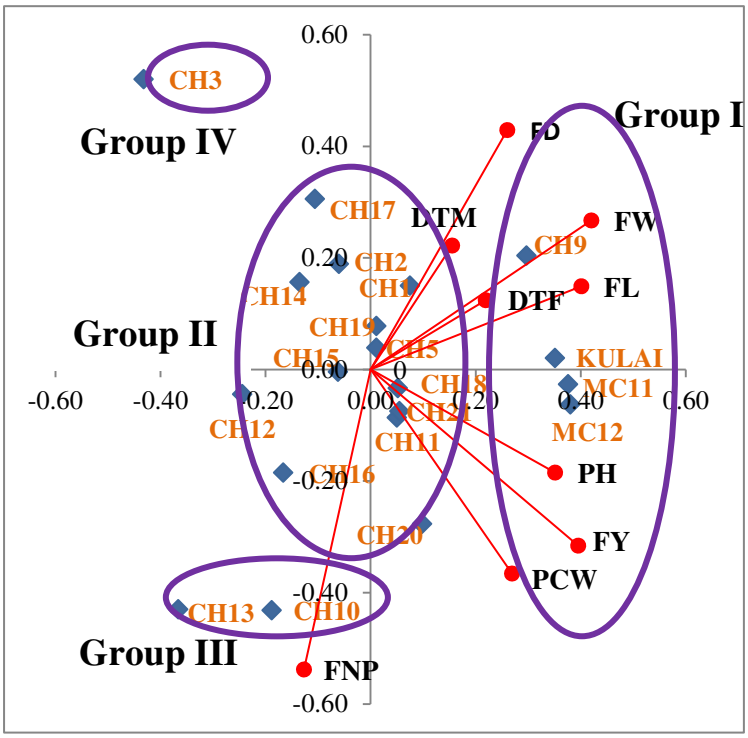

Figure 1: Singular value decomposition biplot showing the relationships among traits measured and chilli genotype.

Conclusions

From this evaluation, it is concluded that variation showed from the introduced resistant chilli genotypes could be explored further for different chilli markets preference and special traits. Most of these introduced chilli genotypes could adapt well and performed good yielding in Malaysian environment. The resistant traits that carried together in most of these genotypes could be useful in future breeding works.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Preliminary Growth Performance of Limau Madu Accessions

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Introduction

Citrus belongs to the subfamily Aurantioideae of the family Rutaceae that comprises about 158 genera and 1900 species. It believed to have originated from the region within Northeast India, South China, Indonesia and Peninsular Malaysia (Hajivand et al., 2009). It is and extremely an important crop with the total world production of at estimated 95.1 million metric tons (USDA, 2017). Citrus products play an important role in human diets as citrus fruits are high in vitamin C content and contain other important nutrients including folate and potassium. Fresh form of citrus is good sources of dietary fiber (Thomas, 2009). In Malaysia, citrus is also known as "limau" and grown in commercial orchards, backyard orchards and small plantation in various parts of the country; providing a livelihood to many growers who supply mainly to the domestic market. Citrus species commonly cultivated are *Citrus maxima* (Burm.) Merr. (Limau Bali), *Citrus reticulata* Blanco (Limau Madu, synonym *Citrus suhuiensis* Hort. Ex Tanaka), *Citrus hystrix* DC. (Limau Purut), *Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle (Limau Jari) and *Citrus aurantifolia* (Christm.) Swingle (Limau Nipis) (Elcy et al., 2012). Japan usually follows the botanical names of the taxonomy by referred to Tanaka system while elsewhere it is more popular to classify the genus citrus into Swingle system from Florida.

In Malaysia, mainly in Terengganu more than 2000 hectares are planted with *Citrus suhuiensis*. This species is also planted in Thailand, Indonesia and Vietnam, where its fruit is known as Somkeae Wan, Jeruk Siam and Duong respectively. Two cultivars of *C. suhuiensis* Hort. Ex Tanaka, limau madu and limau langkat, are commercially important citrus fruit crops (loose-peel mandarins) that differ in size, shape, aroma and in susceptibility to Trestiza virus and Phytophthora collar rot diseases (Makeen et al., 2007). The fruit, one of the finest mandarin oranges in the country, is oblate, globose and smooth and produces juice with a weak aroma. Basically, the fruit of this species is spherical in shape with shiny green or greenish yellow peel and its skin can be easily peeled. The fruit typically contains 12-15 seeds per fruit and plant generally propagated with Cleopatra root stock (Suri et al., 2016). Besides, a local variety of limau madu is widely cultivated in the states of Pahang, Kedah, Johor, Terengganu and Sarawak. Limau manis makes up most of the production, while limau madu becoming increasingly more popular although pummelo and limau kesturi are also grown in certain areas. Limau madu consists of 9-15 easily separated segments which are pale orange in colour; the flesh is juicy and sweet (Elcy et al., 2012). Fruit are cheaper than imported oranges andcan be eaten raw, as squeezed fruit juice or used for falvoring.

Even though limau madu is considered as a cross pollination crop (Makeen et al., 2007), it is expected to show minimal genetic variability as the plants are vegetatively propagated by the nurseries to ensure maintenance of elite genotypes with desirable traits (Singh and Rajam, 2009). For conservation purposes limau madu collections was established by seeds which are polyembryonic and have notable genetic diversity, particularly of the pummelo and some of the related genera (Hajivand et al., 2009). The aim of this work was to evaluate the growth performance of limau madu accessions in breeders' collection in term of trunk girth and tree size. Potential accessions that are associated with agronomic and fruit quality trait such as yield, external and internal fruit attributes can be identified based on the

superior genotype obtained and selected. By studying the plant development within limau madu populations, this would greatly assist the breeders in management the germplasm that is available for cultivar improvement programs.

Materials and Methods

Eleven accessions namely Thailand, Setiu, Ponkan, Kuala Kangsar, Pokok Sena, Dungun, Ulu Tiram, Maran, Lanchang, Kahang and Jeliwere collected from farmers' collection all over the Peninsular of Malaysia in November 2012, as seeds. They were sown and grew for a year in the nursery and then transplanted at MARDI Kluang plot in December 2013 in 9 x 9 m tree spacing. A total of 132 plants were planted in a randomized complete block design (RCBD) with four blocks and three replicates. Experiment was conducted on mineral soil with the study area has a hill and slope topography.

Girth of the trunk was measured at a height of 15 cm from ground level using a measuring tape and expressed in centimeters (cm). As a measure of tree size, the height was first determined and then the tree spread was taken in two directions at right angles (North-South and East-West) and expressed in centimeters (cm). For plant height of the tree, data took from the base of the trunk at ground level to the top of the canopy where the leaves emerge out from the tip of the stem and also expressed in centimeters (cm). Tree size (S) was then calculated according to the following formula (Oppenheimer, 1960).

$$S = 1/3 (d + h)$$

Where; d = mean diameter of tree canopy
h = tree height

Data were subject to analysis of variance and mean values were compared using Duncan's Multiple Range Test at the 0.05 level.

Results and Discussion

According to analysis of variance results (Table 1), both parameters of trunk girth and tree size showed no significant differences among accessions, replication and interaction between accessions in block. However, significant differences between block (land surface of planted area) are detected in tree size but not in trunk girth. All trees that planted in slope area obtained good development and more vigorous compared to highly land area. Results showed that topography influenced the growth of limau madu plants most probably attributable to its water and nitrogen efficiencies. The plots with flat slope contained significantly more nutrient contents and had consistently higher soil moisture than steep slope and hilly plots (Robert, 2016). Result on mean of both plant characteristics and coefficient of variation (CV) are also shown in Table 1. It showed that there is small variation among the plant characteristics studied, where coefficient of variation data gives less than 20%. The finding of this study showed that most of the trunk girth and tree size sampled were very stable among limau madu accessions in Kluang.

Accession Thailand (45.91 cm) and Setiu (45.42 cm) gave fastest increase in trunk girth development (Table 2) followed by Ponkan (44.50 cm), Kuala Kangsar (44.33 cm) and Pokok Sena (43.10 cm). Meanwhile, Jeli exhibited the slowest trunk growth with average mean of 38.53 cm followed by Kahang (39.00 cm) as compared to other accessions. For tree size parameter, Thailand resulted the highest among accessions at mean 235.30 cm and the slowest size development was observed in Ponkan with average of 195.15 cm. Westwood and Roberts, (1970) reported that trunk measurements can be used to estimate the yield efficiency as fruit weight per cm^2 trunk cross-section of any orchard tree without major pruning. Tree size gives an estimate of branch and leaf development of a tree. The determination of tree size in the different cultivars would, therefore supply information on the

development of the potential bearing surface of each cultivar (Abutiate, 1988). Tree size was influenced by many factors (quantitative traits) and can be manipulated to achieve the desired shape and size.

Field observation showed that most of the accessions were good in growth performance. Bitters, (2012) also mentioned that seedling trees of most varieties are vigorous upright growers, extremely thorny, and are late in coming into bearing. However, the plant growth of Ponkan retarded even after four years planting in the field. Based on Figure 1, the tree remained quite slow in comparison with other accessions. Branch development was also sparse. Lanchang and Jeli also exhibited low performance after Ponkan. This retarded development might be due to accession genetically itself. Normally, tree size will show a very close relationship with girth measurements. As in a case of girth development, tree size was greater in Thailand (45.91 cm, 235.30 cm) followed closely by Pokok Sena (43.10 cm, 233.68 cm) and Kuala Kangsar (44.33 cm, 231.92 cm). In contrast, Ponkan (44.50 cm, 195.15 cm) and Lanchang (39.55 cm, 210.89 cm), the results showed that the tree size was less developed with girth improvement.

Table 1: Mean squares from ANOVA for trunk girth and tree size of eleven limau madu accessions.

Sources	df	Plant characteristics	
		Trunk girth (cm)	Tree size (cm)
Rep	2	29.40	385.93
Block	3	30.51	5252.60*
Acc	10	81.69	1769.69
Block*Acc	30	103.00	1110.11
Error	82	48.80	617.20
Total	127		
Mean		42.16	223.78
CV		16.57	11.10

** Significantly different at p<0.05.*

Table 2: Mean of trunk girth and tree size of eleven limau madu accessions.

Accessions	Plant characteristics	
	Trunk girth (cm)	Tree size (cm)
Thailand	45.91^{a}	235.30^{a}
Setiu	45.42^{ab}	230.22^{ab}
Ponkan	44.50^{abc}	195.15^{c}
Kuala Kangsar	44.33^{abc}	231.92^{ab}
Pokok Sena	43.10^{abc}	233.68^{ab}
Dungun	41.82^{abc}	230.85^{ab}
Ulu Tiram	41.50^{abc}	224.26^{ab}
Maran	40.25^{abc}	228.33^{ab}
Lanchang	39.55^{abc}	210.89^{bc}
Kahang	39.00^{bc}	228.33^{ab}
Jeli	38.53^{c}	213.97^{abc}

Mean values with the same letter(s) are not significantly different at p<0.05.

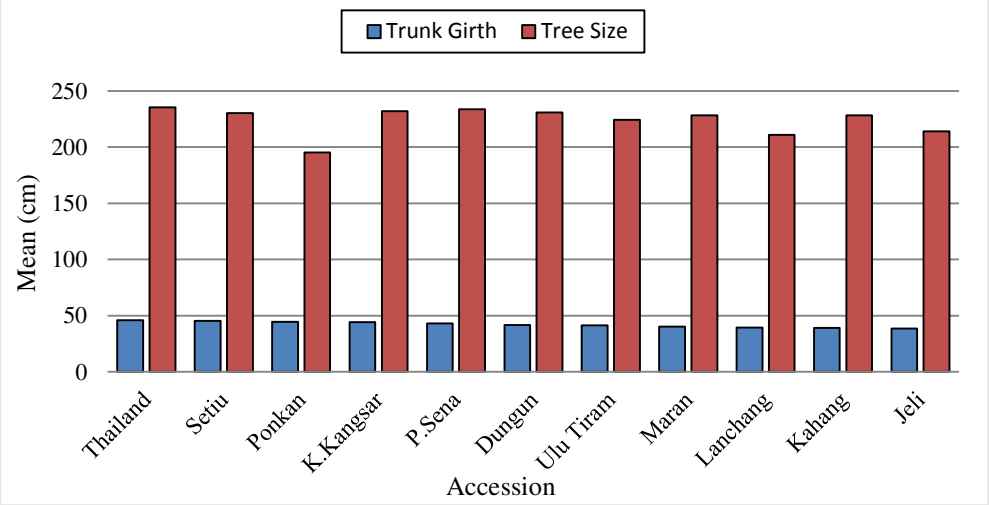

Figure 1: Growth performance of eleven limau madu accessions based on trunk girth and tree size.

Conclusions

Overall from the evaluation, accession Thailand and Pokok Sena showed good performance in plant growth compare to accession Ponkan and Lanchang. In conclusion, although slope area influenced nutrient contents that contributed to general growth of limau madu accessions, however there are no affected interaction between accessions and land surface to the well growth at Kluang plot especially for both promising accessions. Further study is required to evaluate these limau madu accessions in term of fruit quality to confirm its potential and good genotype should be selected to improve this cultivar for future breeding programme.

Acknowledgements

The author is grateful to all staff involved for their assisting in limau madu field management and handling plants over for assessment. The assistance of practical student and the entire staff of Horticulture Unit at MARDI Kluang are grateful acknowledged. Finally, the author wishes to express her thanks to the Director of Horticulture Research Centre and Deputy Director of fruits programme for the facilities made available for the research and permission to present and publish this paper. This work was supported by MARDI Development Fund.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Viorica: A Promising Rootstock in Producing Highly Tolerance Grafted Papaya against Papaya Dieback Disease

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Introduction

Papaya (*Carica papaya*) is a highly nutritional tasty fruit. The plant is widely cultivated in the tropical and sub-tropical region. China is the largest importer for Malaysian papaya, followed by Hong Kong and Singapore. The Malaysian export value for papaya in 2016 is USD 515,234 (third highest) after durian (USD 6,693,648) and watermelon (USD 5,067,998) reported by Global Trade Information System, (Anon, 2017). Papaya dieback disease (PDD) outbreak caused by the notorious pathogen, *Erwinia mallotivora* was first recorded in 2003 and still remain as the main constraint in papaya industry in Malaysia (Noriha et al., 2011). The common symptoms of PDD include greasy, water-soaked lesions and spots on petioles, leaves, trunks and fruits (Wan et al., 2017).

Three intensive phases have been implemented for PDD research strategy in MARDI i.e. increasing the gene-pool to obtain resistant gene, glass house and hotspot screening, and finally followed by the proper breeding program (Sarip, 2016a). The development of new varieties through conventional and molecular breeding becomes more challenging due to the lack of resistant accessions until Viorica was discovered in 2012 (Rogayah et al., 2016). Viorica was found to be resistant and has been filed for IP: PVP 2013/09/0014 (Sarip, 2013; Sarip, 2016b). It was developed through selection and purified through composite controlled pollination to increase heterosis and purity (Sarip et al., 2013). The plant is vigorous with purple petiole and peduncle. Therefore, the discovery of Viorica offers new light in papaya industry.

Various breeding strategies including development of F_1 and double-crossed hybrids have been conducted in MARDI to develop high quality papaya varieties with resistance against PDD (Sarip et al., 2016). However, there is a possibility to produce elite grafted papaya seedling using Viorica as a resistant rootstock. The idea of using resistant rootstock is due to the metabolite translocation and interaction between scion and rootsctock as proposed by Aloni et al. (2010). A study of grafted grapevine onto crown-gall resistant rootstock conducted by Sule and Burr in 1998 show bigger crown-gall form on grapevine on susceptible rootstock rather than resistant rootstock. In watermelon (Yetisir et al., 2003) and tomato (Sarip and Moore, 1994), the fusarium wilt resistant rootstock was used to influence disease resistance, fruit yield and quality. The same conclusion also made by Nisini et al. (2002) in their muskmelon study. However, there is no research has been done on papaya.

With our hypothesis, the metabolites or proteins produced in the Viorica rootstock could be translocated to the susceptible elite scions, Eksotika and Sekaki to induce resistant against PDD, grafting experiment was conducted to study the effect of resistant rootstock in reducing the susceptibility of scion.

Materials and Methods

Selection of rootstocks and scions

Selection of rootstocks based on the resistant level from glass house and hot spot screening (Sarip et al., 2012). Viorica was the first disclosure to the public in 2012, known as Purple Lady and MARDI Purple. Viorica has been observed to possess the characteristics of moderate fruit quality and high tolerance against PDD (Sarip, 2012; Sarip and Siang, 2013). Based on the excellent agronomic traits, two commercial varieties namely Eksotika and Sekaki were selected as the elite scions (Sarip and Noor Faimah, 2012; Simoh and Sarip, 2015).

Grafting procedure and seedling preparation

Six treatments (grafting combinations) - Eksotika onto Viorica, Sekaki onto Viorica, Viorica onto Eksotika, Sekaki onto Sekaki, Eksotika onto Eksotika and Viorica onto Viorica were used in this study. Hundred healthy papaya seedlings were chosen for each treatment. The middle part of the seedling that would be grafted was sterilised with 50% ethanol using sterilised cotton. The scions were cut in 'V' shape using a grafting knife. The rootstocks with a similar diameter size of scions were cut in vertical order. The scions were inserted into the rootstocks. The grafted part was secured with parafilm. All of the grafted seedlings were covered and arrested under transparent plastic for 14 days. After 3 weeks, 20 uniform and healthy seedlings from each treatment were randomly planted at the hotspot MARDI Serdang. Randomized Complete Block Design (RCBD) was adopted.

Disease severity and score

The disease severity level of papaya plants was observed and recorded. The disease severity was divided into five levels i.e. free from PDD symptom (score 0), symptom appear only on petiole (score 1), symptom appear on petiole and leaf (score 2), symptom appear on petiole, leaf, fruit and stem (score 3), and symptom appear on all part of the plants or died as score 4 (Table 1). Average disease score was calculated.

Table 1: Disease severity level and score for papaya plant.

	Severity level				
Score	Disease free	Petiole	Leaf	Fruit/Stem	Shoot/Died
0	√				
1		√			
2		√	√		
3		√	√	√	
4		√	√	√	√

(A) (B) (C)

Figure 1: Disease severity level and score (A) Score 2, (B) Score 3 and (C) Score 4.

Results and Discussion

After 12 months, all plants generated from elite scions grafted onto Viorica still survive with none or mild symptom of PDD. However, all of the controlled susceptible plants were severely infected or died. The Average Disease Score (ADS) of resistant control, Viorica onto Viorica contribute 0.55 with 9 and 11 plants scored 0 and 1 respectively. However, the ADS of susceptible control, Eksotika onto Eksotika contribute 3.90 with 2 and 18 plants scored 3 and 4 respectively (Table 2). The maximum ADS possible value to be obtained is 4 when all 20 plants having score 4.

Table 2: Average disease score for grafted plant after 12 months at hotspot.

Plant combination		Number of plants					Disease score (Average)
Scion	Rootstock	Score 0	Score 1	Score 2	Score 3	Score 4	
Eksotika	Viorica	5	12	3	0	0	0.90
Sekaki	Viorica	8	10	2	0	0	0.70
Viorica	Eksotika	6	11	3	0	0	0.95
Sekaki	Sekaki	0	0	1	11	8	3.25
Eksotika	Eksotika	0	0	0	2	18	3.90
Viorica	Viorica	9	11	0	0	0	0.55

The ADS for 6 plant combinations indicates that the Eksotika onto Viorica (0.90) and Sekaki onto Viorica (0.70) have lower ADS compared to the Eksotika onto Eksotika (3.90) and Sekaki onto Sekaki (3.25). But the combination Viorica onto Viorica has lower ADS compared to Eksotika onto Viorica and Sekaki onto Viorica. Viorica onto Eksotika (0.95) has lower ADS compared to the susceptible control Eksotika onto Eksotika. The 12 months old papaya plants with Viorica as a rootstock still survived and produced good yield (Figure 2).

(A) (B) (C)

Figure 2: Papaya plants at the age of 12 months (A) Eksotika grafted onto Viorica, (B) Sekaki grafted onto Viorica and (C) Viorica grafted onto Eksotika.

Further research

The research will be repeated in a larger scale with RCBD experimental design to validate the effect of resistance rootstock in reducing the susceptibility of elite scions, to identify the metabolites or proteins and their role in the mechanism of resistance, and to study the influence of Viorica on yield and fruit quality of elite scions.

Conclusions

Sufficient grafting method was established and more than 90% success rate was obtained. Viorica, a resistant rootstock to PDD possible to be used in reducing the susceptibility of elite scions.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

A Preliminary Data on Melon Root Affected by Developed Nano Fertilizer

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Introduction

In order to increase crop production, fertilizer has been used to deliver nutrients efficiently to the plant. Reports indicate 30-50% of agriculture product is a result of fertilizer usage either organic or chemical (Liu and Zhao, 2013; Xiaoyu et al., 2013). Some of the fertilizers used are not absorbed by the plant which can cause leaching that leads to environmental problems such as eutrophication phenomena (Liu and Zhao, 2013). A study on development of nano fertilizer was done in order to synchronize the release of fertilizer-N and -P with their uptake by crops, preventing undesirable nutrient losses to soil, water and air via direct internalization by crops and avoiding the interaction of nutrients with soil, microorganisms, water and air (Naderi, 2013).

The degree of nutrient mobility and the availability of nutrients to a crop planted within the soil are dependent on a variety of factors. These factors also influence the productivity of applied fertilizer such as soil moisture content, chemical activity of soil components, nutrients balance of applied fertilizer and responsiveness of particular crop to fertilizer (Gellings and Parmenter, 2004).

The nitrogen, phosphorus and potassium (NPK), which are required in large amounts for plants, are not adequately available in natural soils to support the sustained growth of plants. Therefore, these macronutrients (NPK) are needed to be applied externally through fertilizers. A nano strategy, involving a smart carrier fertilizer based on modification of fertilizer using hydroxyapatite (HA) nanoparticles was studied. The HA ($Ca_{10}(PO_4)_6(OH)_2$) nanoparticles are rated as one of the prominent candidates in agricultural applications, which can provide phosphorus nutrient. In this study, melon was chosen because this plant has become one of the main premium fruit plantations in Malaysia which contributes to major income for exportation. The root branching of melon using our newly developed nano fertilizer was determined.

Materials and Methods

Experimental site

The experiment was located at transgenic glass house, Biotechnology and Nanotechnology Research Centre, MARDI Serdang, Selangor, with a relative humidity ranged 65-80% at night and 40-60% during the day and the temperature of 27-40^{o}C. The favourable variety by farmers and its ability to fight diseases, are among the criteria of the chosen variety. The seeds were germinated overnight before transplanting into the peat block.

Reagents

All the reagents used were analytical purity. The HA nanoparticles were synthesized using aqueous solution as mention in Azima et al. 2016. Adsorption of nutrients and HA can include covalent, electrostatic, Van der Waals and hydrogen bonds.

Treatments

This root comparison studies focussed on the root branching between the second and the third link of root in six different treatments. These six treatments were 50%, 100% and 150% of nutrient requirements with HA, HA, Agroblend (control) and conventional fertilizer (control). Seeds were sown in seedlings tray for overnight before transplanting them into peat blocks. The size of peat blocks used is 7.62 cm x 6.35 cm x 6.35 cm and was observed twice daily. Fertilizers with modified hydroxyapatite were applied in peats block composition according to six treatments in completely randomized design (Figure 1). All six treatments have four replicates with each treatment has eight replicates. Weeds were controlled manually. Watering was done manually twice a day. These root branching studies were done using root analyser at Horticulture Research Centre MARDI after 14 days using one plant from each replicates. This second and third link branching studies is very important in order to know the efficiency of this fertilizer for nutrient adsorption. This method is a destructive method. The rest of the melon plants were transplanted into polybag for further studies.

Figure 1: Cultivation of melon in peat blocks for HA treatment; a) melon seedling in peat blocks at day 3 for HA treatment; b) melon transplant at day 14 for HA treatment and (c) peat block transplanted plant into polybag for further studies.

Results and Discussion

From all the six treatments, HA alone is expected to give a significant result because of a high amount of phosphorus in its composition. The plant phosphate status systemically influences the expression of many genes and the root development (Desnos, 2008).

The results show that HA alone portrays the third highest branching with 3587 number of branching for the third link compared to other treatment (Figure 2). This may be due to the highest amount of phosphorus that enhanced the ability of branching. Meanwhile, 50% of nutrient requirement with HA shows highest data which is 1930 and 4976 for second and third branching, respectively, which portray sufficient amount of nutrient for root branching. This particular data is very important to determine the significant finding for this study.

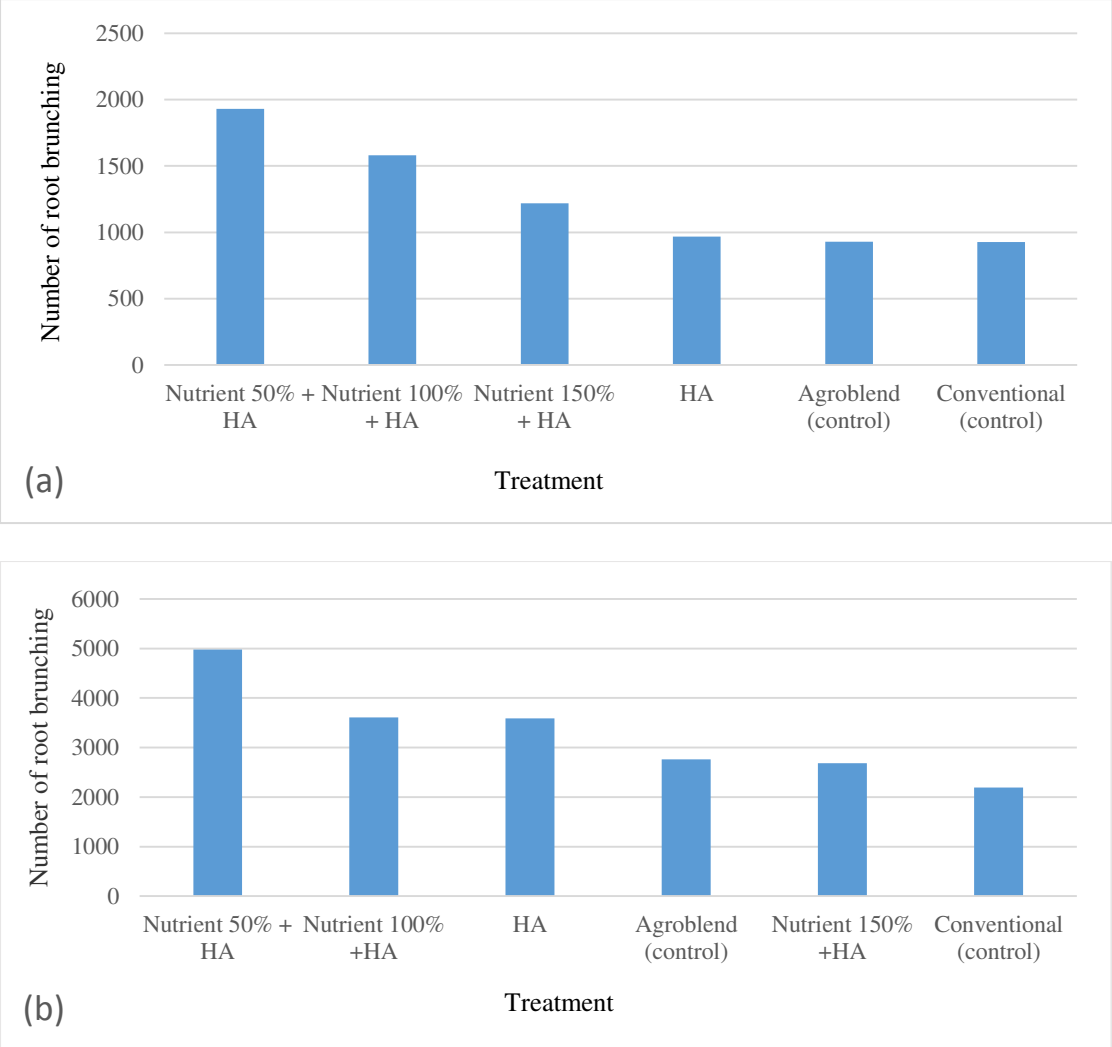

Figure 2: Root branching data for (a) second and (b) third link for six treatments; 50%, 100% and 150% of nutrient requirements + HA, HA, Agroblend (control) and conventional fertilizer (control).

The results show that 50% of nutrient requirement with HA has the highest number of root link for both second and third link branching compared to the conventional fertilizer which is also the lowest for both second and third link branching. This shows that this particular treatment at 50% nutrient requirement is sufficient for early development of melon root. Figure 2 has also shown that HA alone

is the third highest at third link, meaning that HA also gives a big contribution for root development because of its composition which is high in phosphorus in comparison to conventional which does not have any HA treatments.

Root development is strongly affected by nutritional value and its effectiveness to absorb nutrients. Therefore, plants may response to a spatially restricted nutrient availability by root architecture system displaying enhanced branching root development into nutrient rich patches. This response is nutrient specific and observed only if overall nutrient is limited (Desnos 2008). Studies on root branching especially on second and third link are also important to indicate the fertilizer efficiency towards nutrient transporting through root development.

Conclusions

From the data obtained, 50% nutrient requirement with HA treatment shows the highest branching for second and third link root branching, amounting to 2000-5000 branching. The HA alone shows a very good branching due to phosphorus release which is an important nutrient to branch. As a preliminary data, this study shows a potential outcome of this newly developed nano fertilizer.

Acknowledgements

The author would like to thank all parties that involve in this study especially MARDI's officers and staff for their guidance and facilities provided. These studies were funded Mega Project from MARDI Development Fund entitled Development of Nano fertilizer for Melons (P-RB405B-1001).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Bio-Soil Amendment on the Growth of Okra (*Abelmoschus esculentus* L. Moench)

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Introduction

Agriculture is a soil-based industry that extracts nutrients from the soil (Kwadwo and Christian, 2015). Soil fertility is an important form of renewable natural capital (Sanchez et al., 1997). Depletion is one of the major concerning problems for declining per capita food production in worldwide. Although chemical fertilizers release their nutrient rapidly into the soil for productivity, their effects may have brought a lot of concerns to sustainable of crop production (Crew and Peoples, 2004). Organic soil amendment has been shown to have beneficial effect on soil nutrients, soil physical, soil biological and crop viability (Kang et al., 1981). It is a nematode management option and numerous aspects of research and will practically applied in commercial agriculture for the amelioration of pest problem (Akhtar and Malik, 2000). Soil amendment is materials added to the soil to improve it physical properties and the goal is to provide a better environment for plant roots (Davis and Wilson, 2002). Application of bio-soil amendment as a plant probiotic is becoming accepted practice among farmers to maximizing yield crop production instead minimizing the use of chemical fertilizer. Incorporation of organic amendment into the soil, will enhance soil nutrient status and reduced the incidence of pest (Adilakhsmi et al., 2008). Due this effect, the decreasing of pest infestation may increase the healthy plants and give a potential of high yield production.

Okra (*Abelmoschus esculentus* L. Moench) or ladies finger which is also known as Bendi belongs to family of Malvaceae. Okra has an economic importance because of its nutritional value that has improved food security (FAO, 2006). For production of okra, soil fertility is the main thing that needs to be emphasized for sustaining plant growth. Low production of okra will affect its utilization (Nwangwu, 2016). Effective and efficient approaches to slowing nutrients removal and returning nutrient to the soil will be required in order to maintain and increase crop productivity and sustain agriculture for long term (Grunh et al., 2000). Considering the cost of excessive use of chemical fertilizers, this study was conducted to compare the effects of different level concentration of bio-soil amendment on the growth characteristics of okra.

Materials and Methods

Site description

The experiment was carried out at the Research Plot of the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan, under rain shelter sizing (24.40 m x 6.10 m). The mean minimum and maximum monthly temperature were 28°C and 32°C, respectively.

Treatments/experimental design

The experimental design used was randomized complete block design (RCBD) with six treatments replicated five times each. The treatments were: T1: 0 ppm as control, T2: 50 ppm, T3: 100 ppm, T4: 200 ppm, T5: 400 ppm and T6: 800 ppm of bio-soil amendment. The dilution factors were prepared

based on the supplier's recommendation i.e. one sachet of 20 g is diluted into 100 litres of water. The solutions were applied directly to leaves and soil surface. Treatment was carried out during vegetative growth at 20-25 days after sowing (DAS) on cloudy days with high air humidity.

Agronomic practices

Okra seeds of cultivar 551 Hybrid named Green Torpedo were grown in plastic pots with loamy sandy soil as growth medium. Three seeds were sown per pot and later seedlings were thinned to one plant per stand ten days after germination. Bio-soil amendment as a microbial supplement i.e Artisan Plant Probiotic "Zyme" distributed by AsiaBio's partner company of Artisan has been selected for this experiment. This product is a 100% home grown microbial soil supplement and it has been proven to reduce chemical fertilizer dosage. The content is mainly diatomite powder as carrier and organic materials (soy powder and bran meal) for the plant growth enzyme. For the application, the powder was diluted into 25 litre of water. The dilution factors were prepared based on the supplier's recommendation i.e. one sachet of 20 g is diluted into 100 litre of water. The solution was applied directly onto leaves and soil surface during vegetative growth at 20-25 DAS in the early morning. 2-grams NPK (12:12:17:2) was applied to each pot at two weeks after planting.

Parameters measurement

For the shoot and root length, all the tagged plants were removed from the soil and cleared thoroughly. The plants were cut on the collar (the dividing line between root and stem). Shoot and root were measured using a meter ruler. The shoot length was measured from the surface of the medium to apical tip of the plant and the root length was determined by trace the roots on paper, measure each of the tracings, and calculate root length from the tracings. Plant height was measured with a meter ruler from the first nodes of the plants to the tip of the terminal buds and the average measurements were recorded for each treatment. Mean pod fresh weight (g) of okra was determined by weighing pods individually using a digital analytical balance (± 0.01 g). It was taken from tagged plants and the average weight was recorded for each treatment. The pod length (cm) was measured as the distance from the pod cap scar at the base to the tip end of the pods. The length of three fruits was taken and measured using a meter ruler and the average was recorded for each treatment.

Statistical analysis

The data of all the parameters studied were subjected to one-way analysis of variance (ANOVA) using the XLSTAT 2016. Means were separated using the Least Significant Difference (LSD) at 5% level of probability.

Results and Discussion

Table 1: The growth of okra as affected by bio-soil amendment application.

Treatment	Root length (cm)	Shoot length (cm)	Plant height (cm)	Pod length (cm)	Pod weight (g)
T6	85.53[a]	38.32[a]	48.70[a]	11.73[a]	13.76[a]
T5	75.58 [b]	38.27[ab]	43.60[b]	11.65[a]	11.99[b]
T4	68.60[bc]	37.92[abc]	36.70[c]	10.25[b]	10.84[bc]
T3	64.77[cd]	37.00[abc]	35.20[d]	9.87[b]	10.53[bc]
T2	55.15[de]	36.12[bc]	34.70[e]	9.85[b]	10.46[c]
T1	51.50[e]	35.80[c]	32.00[f]	9.77[b]	10.35[c]
Pr > F	< 0.0001	NS	< 0.0001	0.001	< 0.0001
CV	20.38	4.88	15.32	17.60	20.64

Means having similar letter (s) are statistically not significant and those having different letter (s) differ significantly by LSD at 0.05 levels of probability. T1-control (water application) + NPK, T2-50 ppm bio-soil amendment + NPK, T3-100 ppm bio-soil amendment + NPK, T4-200 ppm bio-soil amendment + NPK, T5-400 ppm bio-soil amendment + NPK, T6-800 ppm bio-soil amendment +NPK. CV- Coefficient variation. NS- Not Significant.

The results indicated that the growth of okra responded significantly to the bio-soil amendment. All the parameter studied increased four weeks after planting in the following order: T6>T5>T4>T3>T2>T1. At full maturity, at 60 DAS, the plants applied with 800 ppm of bio-soil amendment (T6) had produced significantly tallest plants (48.70 cm) than other treated plants. Whereas, the shortest plant height was found from the non-treated plant. The results indicated that the application of bio-soil amendment significantly increased plant height due to nutrient available to the plants and improve soil fertility. Likewise, root length of okra in this study was also significantly enhanced due to an increase concentration of bio-soil amendment and it increased by 40% compared to the control. Similar results were reported by Wright et al. (1995), who observed that maximum root growth and rooting depth of barley crop were higher in treatments, which received organic amendment during cultivation. However, there was no significant difference in shoot length as influence by the application of bio-soil amendment. Effect of bio-soil amendment application on the yield parameters of okra plants showed that the fresh pod length and weight increased with increasing bio-soil amendment. The longest pod was found from T6 which was statistically similar to those in T5. Pod length is a tone character for economic yield which depends upon various factors such as genetic makeup of the cultivars and their responses to prevailing environmental conditions (Khan et al., 2013). Naik and Srinivas, (1992) discovered that fruit length in okra was significantly improved by the application of N and P. Maximum weight of individual pod was found in T6 while minimum in T1. Adebayo et al. (2013) found that the compost application had been found to have a significant effect on dry matter accumulation and fruit yield of okra. Vegetative growth and crop yield (fruit yield and fruit weight, number of leaves, plant height and stem girth) of okra have been found to increase by the application of organic based nitrogen fertilizer sources (Okello-Baw, 2014).

Conclusions

These experimental results revealed that the growth of okra could be modified by the application of different concentration level of bio-soil amendment as the following order; T6>T5>T4>T3>T2>T1 (control). Bio-soil amendment can be used to provide nutrition to okra and attain yields attribute such as pod weight and pod length. In conclusion, growing okra in a pot with the application of bio-soil amendment in 800 ppm resulted in the optimum growth.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Photosynthetic Response of Pepper to Application of Beneficial Microorganisms on Sandy Textured Soil

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Introduction

Since ancient times, *Piper nigrum*, also known as the "King of Spice" has always been one of the most widely traded agricultural products around the world. Pepper is used as an important component of many recipes and to flavor foods. Though the price tends to fluctuates in recent years, pepper remains one of the most sought-after commodities in the world. Even up till now, demand for pepper continues to exceed the market supply, both locally and globally. This is true with the world-renowned Sarawak creamy white pepper which is still a more preferred pepper brand especially in Japan. Because of this, Sarawak pepper can still fetch a higher price in the international market.

Introducing beneficial microorganisms in pepper planting through the Natural Farming approach will be part of an effort by the Malaysian Pepper Board that supports green development. This is in line with the National Commodity Policy heading towards an environment-friendly industry. Currently, some pepper farms in Sarawak can be found in areas with marginal soil conditions such as sandy textured soil. This particular soil type usually contributed to lower yield of pepper. As the Natural Farming approach is closely related to soil fertility, it is with hope that introducing several liquid fertilizers containing beneficial microorganisms to sandy textured soil type can help to improve the soil's chemical and physical properties.

Nevertheless, enhancing food security through this approach in Malaysia is still a not well-established option due to lack of technical documentation and knowledge in the required subject. The desired effects for applying beneficial or effective microorganism fertilizers to soils can vary, at least initially. Therefore, this study was conducted to compare selected properties of soil as well as certain *P. nigrum* photosynthetic responses after application with different Natural Farming liquid fertilizers.

Materials and Methods

Experimental plot

The study was conducted at Kampung Serayan Hilir, Lundu, Sematan, Sarawak, Malaysia with an area coordinate of 1.722322, 109.769786. The study plot was approximately 0.01 hectare. The soil series at the study site was Miri Series of the Miri Family which is a sandy, siliceous, isohyperthermic, strongly cemented Typic Haplorthods. Due to their sandy textures as well as very poor moisture and fertility status, soils of the Miri Series are not suitable for agriculture and are best left under their natural vegetation (Teng, 2004). The crop involved in this study was *Piper nigrum*. Duration of the study was from July 2015 to June 2016.

Experimental design and treatments

The experiment used a randomized complete block design (RCBD) with 5 treatments replicated 5 times giving a total of 25 plants. Treatments were: (i) F0 – control, (ii) F1 – Indigenous Microorganisms (IMO), (iii) F2 – Fermented Plant Juice (FPJ), (iv) F3 – Fermented Fruit Juice (FFJ),

and (v) F4 – Lactic Acid Bacteria Serum (LABS). All liquid fertilizers containing beneficial and effective microorganisms were applied to the pepper vines and soil every once a month.

Selected soil properties determination

To further understand the fertility status of the soil, samples were collected at a depth of 0-25 cm and analyzed for its soil organic matter (SOM), pH, total nitrogen (N), exchangeable phosphorus (P), exchangeable potassium (K) and cation exchange capacity (CEC) using the method by Tan (1995). The soil bulk density (BD) was determined using a soil sample ring kit (Model C, Eijkelkamp, Holland).

Piper nigrum gas exchange measurement

Gas exchange measurement was determined according to the method by DiCristina and Germino (2006), carried out on young fully expanded leaves with the same orientation and the same layer in the crown (middle bottom). Measurements of net photosynthesis on an area basis (*A*) (µmol CO_2 $m^{-2}s^{-1}$), leaf stomatal conductance (*gs*) (mol H_2O $m^{-2}s^{-1}$) and transpiration rate (*E*) (mmol H_2O $m^{-2}s^{-1}$) of fifteen (15) different leaves per treatment were monitored using a LICOR LI-6400 XT (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set to PAR at 1500 µmol $m^{-2}s^{-1}$which was presumed to be the intensity where photosynthetic rates for black pepper would be maximal (Mathai, 1983; Vijayakumar et al., 1984). The CO_2 flow into the chamber was maintained at a concentration of 400 µmol mol^{-1}. The humidity flow into the chamber was fixed at 500 µmol s^{-1}. Measurement was done on gas exchange parameters at between 1100 to 1200 h.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) with the SPSS software (version 15). The Tukey's Honest Significance Difference (HSD) Test, at $\alpha = 0.05$ level of significance was done to compare the means and to determine whether there were any differences in selected soil properties and *P. nigrum* gas exchange characteristics.

Results and Discussion

Soil properties

The selected sandy textured soil properties after application of different beneficial and effective microorganism liquid fertilizers are presented in Table 1. The result indicates that soil SOM in IMO, FPJ, FFJ and LABS were 42%, 51%, 42% and 38% higher respectively than that of control (Table 1). This result is in agreement with a study by Javaid and Bajwa (2010) which reported that due to the ability of effective microorganisms (EM) to form humus from decomposed plant material, SOM increased significantly in the treated soils.

Result also showed a significant ($p<0.05$) difference between soil pH under beneficial microorganism treatments and that of the control (Table 1). The soils applied with beneficial microbes revealed higher mean pH (in both H_2O and KCl) levels when compared to that of the control. In a report by Zuraihah et al. (2012) stated that this could be due to the decomposition of organic matter which helps to increase soil acidity.

Table 1 showed that total N for treatments F1, F2, F3, and F4 increased when compared to that of the control. Park and DuPonte (2008) were of the opinion that beneficial microorganism activities might accelerate decomposition of organic matter which led to additional N content in the soil. In addition, a

similar study done by Higa (1987) found that farms treated with beneficial microorganism will increase the nitrogen cycling function of the soil.

Result in Table 1 also showed that soil treated with Fermented Plant Juice (FPJ) and Fermented Fruit Juice (FFJ) demonstrated a profound increased in available P and exchangeable K. Soil available P in treatment FPJ and FFJ were 111.45 mg/kg and 99.75 kg respectively while soil available K were 0.53 and 0.55 cmol(+)/kg respectively. The findings concurred with a study by Chan (2008) which reported that the build-up and accumulation of nutrients P and K in the soil is due to the breakdown of organic matter from the plant residues from the environment as well as in the FPJ and FFJ concoction itself. Both FPJ and FFJ liquid fertilizers are made of plant parts that consist of high P and K factor including leaves, stems, roots and fruits.

As an indicator for level of soil fertility, study on soil CEC was conducted. The soil samples obtained from treatments IMO, FFJ, FPJ and LABS showed significantly ($p<0.05$) greater CEC values than that of the control (Table 1). This could be due to the accumulation of organic matter observed in the surface soil layer that might have contributed to the increase in negative charge, and consequently, in the CEC (Ngome et al., 2011).

The soil bulk density under different Natural Farming (NF) treatments was presented in Table 1. The insignificant difference among the bulk densities of F0 through F4 suggested that NF liquid fertilizers application did not affect hardness of the soil within the time frame of this study. Bulk densities were ranged from 1.11 to 1.27 g/cm^3 and were typical of this soil series. In a report by Cai et al. (2002) mentioned that bulk density of mineral soil that ranges from 1.1 to 1.5 g/cm^3 in surface horizons are generally ideal for plant growth.

Piper nigrum gas exchange characteristics

Comparison of gas exchange characteristics between the control and *P. nigrum* applied with beneficial microorganism treatment are shown in Table 2. The results revealed that the photosynthetic rate in pepper applied with FPJ and FFJ were significantly ($p<0.05$) higher compared to that of IMO, LABS and the control (Table 2). This result could be attributed to improvement in the soil fertility which led to higher availability of soil N, P and K for pepper uptake which are important for the development of major photosynthesis drivers including leaf chlorophyll content and Rubisco enzymatic activity (Wang et al., 2012). Multiple studies have also been done describing the increment in plant photosynthetic rate and productivity associated with high levels of N availability (Eck, 1984; Jacobs and Pearson, 1991).

Result for *P. nigrum* leaf stomatal conductance showed that treatment FPJ and FFJ recorded significantly ($p<0.05$) higher value when compared to treatment IMO, LABS and control (Table 2). A similar study by Longstreth and Nobel (1980) reported that stomatal conductance increased profoundly with higher concentration of soil nutrients. They also added that the increase in stomatal conductance with increasing NO_3^-, PO_4^{2-}, and K^+ levels reflected a greater CO_2 mesophyll conductance, which was due to an increased in the development of the plant leaf cell dimension.

Table 2 also shows that the *P. nigrum* transpiration rate in treatment applied with FPJ and FFJ depicted a significantly ($p<0.05$) greater transpiration value than the other treatments. The result concurred with a study by Shimshi (2007) who mentioned that in the condition of sufficient soil moisture, the transpiration rates were regulated by the degree of stomatal opening. With that being mentioned, the stomata of N, P, K-supplied plants tend to open widely than those of N, P, K-deficient plants.

Table 1: Effect of different beneficial microorganism liquid fertilizers on soil organic matter (SOM), pH, total nitrogen (N), available phosphorus (P), exchangeable potassium (K), cation exchange capacity (CEC) and soil bulk density (BD).

Treatment	Soil organic matter (SOM)	pH in H_2O	pH in K_2O	Total N (%)	Available P (mg/kg)	Exchangeable K (cmol(+)/kg)	CEC (cmol(+)/kg)	Bulk density (g/cm^3)
F0	2.06 ± 0.79^a	4.07 ± 0.37^b	3.81 ± 0.63^b	0.04 ± 0.42^b	58.45 ± 0.86^b	0.30 ± 0.28^b	8.95 ± 1.46^b	1.11 ± 0.34^a
F1	2.93 ± 0.67^b	5.14 ± 0.71^a	4.52 ± 0.68^a	0.11 ± 0.17^a	60.6 ± 1.35^b	0.35 ± 0.72^b	11.50 ± 0.87^a	1.25 ± 0.48^a
F2	3.10 ± 0.41^b	5.06 ± 0.45^a	4.52 ± 0.33^a	0.10 ± 0.89^a	111.45 ± 0.94^a	0.53 ± 0.54^a	11.43 ± 1.16^a	1.22 ± 0.26^a
F3	2.93 ± 0.38^b	5.20 ± 0.31^a	4.46 ± 0.63^a	0.09 ± 0.31^a	99.75 ± 1.56^a	0.55 ± 0.68^a	12.05 ± 0.68^a	1.27 ± 0.43^a
F4	2.85 ± 0.65^b	5.18 ± 0.31^a	4.21 ± 0.79^a	0.09 ± 0.76^a	65.50 ± 0.78^b	0.30 ± 0.28^b	11.67 ± 1.57^a	1.13 ± 0.66^a

Means with same letter superscript within columns are not statistically different using Tukey's at P>0.05 probability level. Treatments are F0 – control, F1 – Indigenous Microorganisms (IMO), F2 – Fermented Plant Juice (FPJ), F3 – Fermented Fruit Juice (FFJ) and F4 – Lactic Acid Bacteria Serum (LABS) (mean ± S.D., n = 15).

Table 2: Effect of different beneficial microorganism liquid fertilizers on *Piper nigrum*'s gas exchange characteristics.

Treatment	Photosynthesis, *A* (μmol CO_2 $m^{-2}s^{-1}$)	Stomatal conductance, *gs* (mol H_2O $m^{-2}s^{-1}$)	Transpiration, *E* (mmol H_2O $m^{-2}s^{-1}$)
F0	4.14 ± 1.56^{c}	0.05 ± 0.98^{b}	1.47 ± 0.91^{b}
F1	4.24 ± 1.44^{c}	0.06 ± 1.54^{b}	1.38 ± 1.28^{c}
F2	6.83 ± 1.85^{a}	0.11 ± 1.68^{a}	2.74 ± 1.75^{a}
F3	7.07 ± 1.19^{a}	0.10 ± 1.39^{a}	2.68 ± 1.09^{a}
F4	5.00 ± 1.21^{b}	0.05 ± 0.88^{b}	1.53 ± 1.44^{b}

Means with same letter superscript within columns are not statistically different using Tukey's at P>0.05 probability level. Treatments are F0 – control, F1 – Indigenous Microorganisms (IMO), F2 – Fermented Plant Juice (FPJ), F3 – Fermented Fruit Juice (FFJ) and F4 – Lactic Acid Bacteria Serum (LABS) (mean $\pm$ S.D., n = 15).

Conclusions

This study has shown that application of different treatment of beneficial microorganism liquid fertilizers responded better in terms of its selected soil properties as well as the gas exchange characteristics of black pepper. Application of IMO, FPJ, FFJ and LABS improved the selected soil properties significantly by showing higher SOM, pH, total N, available P, available K and CEC whereas bulk density of the soil recorded insignificant difference among treatments. It was also found that pepper photosynthetic, stomatal conductance and transpiration rate grown under exposure to treatment FPJ and FFJ were significantly higher than IMO, LABS and control. Through the action of certain beneficial microorganism, the fertility of the sandy textured soil can be improved considerably and therefore led to higher availability of soil nutrients for pepper uptake which positively affect its gas exchange characteristics.

Acknowledgement

The authors are grateful to Malaysian Pepper Board for providing the financial support for the project.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Growth Performance of Eight Years Old *Aquilaria malaccensis*: Some Management Considerations

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Introduction

Sustainable forest plantation management and good silvicultural practices for *Aquilaria* or karas tree species play an integral role in ensuring continuous supply of wood and other non-wood products. Agarwood or gaharu is an important non-timber forest product (NTFP), promising many exciting economic value added products, a highly fetched commodity, provides sustainable livelihood to many local dwellers and derived from all species of *Aquilaria/Gyrinops* (Lok, 2014). With good management practices, these *Aquilaria* tree species can be successfully domesticated, cultivated and considered a promising choice species for both rehabilitation and reforestation projects. Until recently, it has generated much interests/demands in both local and international markets (cultivation, research programs, trading partners and other land investment schemes) due to its rare unique aromatic products, pharmaceutical and cosmetic values, ornamental, religious ceremony products and other traditional uses. To prevent the unsustainable harvesting of *Aquilaria* genetic resources, preservation, all species have been listed in Appendix II of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) since 1995 and in the Red List of Threatened Plants published by International Union of Convention of Nature (IUCN, 2014). Red List classifies these species as vulnerable base on the reduction of at least 20% over three generations caused by levels of exploitation and declined in population (Barden et al. 2000; Hilton-Taylor, 2002). There are more than 15 known *Aquilaria* species in tropical Asia; mostly naturally found in South and Southeast Asia, almost half of the total species are found and cultivated in Malaysia. It is a medium sized tree with light coloured soft wood but with no distinction in colour between sapwood and heartwood (Desch, 1954). Among them, *Aquilaria malaccensis* is a very popular, well distributed, domesticated and highly sought after agarwood probably due to its rich strong spicy aroma smell among the species. Efforts must be taken to enforce any indiscrimate felling in the wild before it become critically endangered. However, domesticated trees must be induced after achieving maturity age and it is believed that these can be done by notching, fungal infection, insect infestation or using effective inducement techniques via biological and organic means (Ng et al., 1997). Present inducement means maybe sceptical, that more intensive and comprehensive research and development for effective inducer programs for quality and large quantity of resins. To date there are over 24000 ha. reported to be cultivated with several *Aquilaria*, aiming for production of agarwood, oil and other high value added products (Zahari, 2013; Lok, 2017).

The expected rotation cycle is expected to be between 4 to 8 years old, depending on the species, growth performance, site suitability, proper management techniques and use of effective inducement applications (Lok, 2010). Hence, this paper shares the early and mature growth performance of cultivated *A. malaccensis* and discusses some of the management practices aiming for early optimum growth and inoculation treatments.

Materials and Methods

Site location

The plots in FRIM are located at about 3° 14' N and 101 38 E with the mean daily temperature ranges from 27 to 30°C and an annual rainfall between 2000 to 2900 mm. They are established in F44 on May, 1997 and F53 on April 2009, at the lower slope of the lower ridge of Bukit Hari, Selangor, Malaysia. Aspect is southerly and with an altitude of 200-220 m above sea-level. At both sites, the soil is of heavy clay loam granitic origin, reddish brown in colour with an average pH of 4.5. In F44, the trees were initially interplanted with *Azadirachta excelsa* at a planting distance of 3 x 3 m to study possible domestication in the open and growth performance with a fast growing tree while F53 was using monoculture cropping with a large planting distance of 4 x 3 while smallest spacing was 2 x 2 m. The trees were all planted with young potted seedlings obtained from the private nurseries and wilding collected at FRIM's ground. However, trees of other species were collected from other individual plots that were available at different locations. Each sample plot consists of at least 100 to 350 trees, measured randomly. Parameters measured were stand age, average diameter and height, mean annual increment (MAI). Other maintenance routines carried out were pruning, fertilizer applications and weeds control.

Table 1: Comparison on age, species and growth of *Aquilaria malaccensis* and other *Aquilaria* species.

Species	Age (year)	MAI	Mean diameter (cm)	MAI	Mean height (m)
A.malaccensis (FRIM)	5	2.8	14.0	2.0	9.8
A.malaccensis (FRIM)	7	3.2	22.1	2.1	14.5
A.malaccensis (FRIM)	8	2.9	23.0	2.0	15.5
A.malaccensis (FRIM)	13	1.4	18.3	1.1	13.8
A.malaccensis (FRIM)	19	2.4	45.8	1.6	30.8
A. hybrids (Perak)	2	1.6	3.2	1.2	2.4
	3	3.8	11.4	1.8	5.4
A. sinensis (Selangor)	3	2.1	6.0	1.2	3.6
A. crassna (Vietnam)	4	1.6	6.4	1.1	4.7
A. crassna (Cambodia)	6	2.1	12.6	1.3	7.8

Results and Discussion

There are about four species of *Aquilaria* commonly cultivated in Malaysia. These include *A. malaccensis*, *A. hybrids*, *A. crassna*, *A. sinensis* (Table 1). Some other species have also been cultivated but this was not recorded here. However, all species are an important source of agarwood. *Aquilaria* is known to be best raised and easily propagated by seedlings (Ahmad Zuhaidi et al., 2015; Lok and Ahmad Zuhaidi, 2016). Earlier results obtained showed that there is lack of silvicultural understanding of the species resulting in high mortality rates (Lok, 1996). However, since then several attempts have been successfully carried out using good management practices and knowledge acquired, for instant, trials of planting spacing give better understanding to tree growth and other environmental conditions required. Early age results for *A. malaccensis* showed that the growth rates were promising, reaching mean annual diameter and height growth of 2.9 cm and 2.0 m at age 8. The achievable average diameter and height was 23 cm and 15.5 m, respectively (Table 1). Comparatively, using smaller planting distance of 2.5 x 2.5 m and at age 5, the achievable mean diameter and height was 14 cm and 9.8 m (Table 1). This showed that at about age 4 or 5, the cultivated trees could have achieved diameter size of ≥10 cm, a recommended size for inoculation considerations.

Silviculture and management considerations

Some of the recommended considerations for successful cultivation include:

1. Uniform planting stocks with good genotype-seedlings and tissue culture. Seedlings must be hardy, easily adaptable to local conditions.
2. Develop fast growing clones or hybrids with optimum growth rates.
3. Proper land preparation and treatments-during pre and post planting.
4. Trials on optimum planting distance and provide recommendations.
5. Pruning and inducement techniques-to promote optimum growth and effective inducement techniques.
6. Other silvicultural treatments and technical know-how: tending and weeding, fertilizer treatments and pest and diseases.

Conclusions

A promising forest tree species for cultivation: among others-its fast growth rates, adaptable to diverse growing conditions, prized commodity, short cutting cycle and low establishment costs. Planting distance may be a main factor to promote early growth but other management considerations are necessary to achieve desirable sizes for inducements. Recommended maturity age: 4-5 years is possible with the expected growth rates attained with good silvicultural practices and suitable environmental conditions. Do required cost-effective inducers, quality planting stocks, more supportive related research programs and strong legislative mechanisms.

Acknowledgements

The authors wish to express their gratitude to FRIM and MSPP for the financial support. Our appreciation also to associated planters, individuals and private landowners for the site visit permissions, data collections and knowledge shared during the course of the project.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

The Influence of Nitrogen Application on Early Vegetative Growth of *Ficus carica* L. var. Ipoh Blue Giant (IBG)

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Introduction

Ficus carica L. or its common name is fig, belong to genus *Ficus* which been classified under Eusyce section of mulberries family, Moraceae (Andersen and Crocker, 1994; Flaishma et al., 2007). It was believed that the oldest fruits being cultivated by man and this species is originated from the Old World Tropics, the Asia Minor and the Mediterranean region (Morton, 1987; Andersen and Crocker, 1994). Fig planting in Malaysia is still new. At first, fig was reported to be brought to Malaysia by unknown individual for hobby purposes (Kosmo, 2015).

Nitrogen (N) is one of the most important nutrients that are required by plants that play a major role in plant growth and development. It is also essential for constituent of protein and chlorophyll (Sahadevan, 1987). Besides that, N is also a constituent of many compounds such as amino acid, enzymes, and nucleic acids. Deficiency of this nutrient can cause reduction in foliage and roots expansion, loss in photosynthesis capability and disturbance of all metabolic function required in plant (Marschner, 1995). Lacking of information on fertilization for this species during early vegetative growth under Malaysia climate warrants further investigation. Therefore, the objective of this study is to determine the optimum N rate on the early vegetative growth of *F. carica* L. cutting.

Materials and Methods

Plant material and treatment

The experiment was conducted at Ladang 15, Faculty of Agriculture, Universiti Putra Malaysia, Serdang (2° 59' 01" N, 101° 44' 00" E). This experiment was conducted under 50% shade netting. Environment factor are considered uniform throughout the experiment. Hardwood cutting of *F. carica* L. variety Ipoh Blue Giant (IBG) with 1.0-2.0 cm diameter and 15 cm long were prepared and planted in a container filled with moist sand and the polybag were covered with a black plastic bag for two weeks. At week 5, cuttings were then transferred into 16 × 16 in polybag sized filled with mixed soil (3:2:1 - top soil, sand and organic matter).

Six rates of N (0, 10, 20, 30, 40 and 50 g N/plant/year) were applied for every interval 4 weeks. Without any application of N (0 g/plant/year) was served as a control. This treatment was applied when cutting reach 6 week and end after 12 weeks. Beside N, potassium (K) and phosphorus (P) was also given to the fig cutting at constant rate of 40 g for each element/plant/year. K and P was applied together with N. N was given in the form of urea, P in Christmas Island Rock Phosphate (CIRP) and K in Muriate of Potash (MOP).

Measurement of growth and physiology

Shoot length (SL) and shoot diameter (SD) were taken every 2 weeks starting from the day of treatment to the end of the experiment. For SL and SD, average of two same shoot was recorded. Reading for SL and SD was expressed in centimeter (cm) and millimeter (mm) respectively. Total chlorophyll content (TCC) was measured every 4 weeks using SPAD Meter. Total leaf area (LA), total

root length (RL), average root diameter (RAD), total root surface area (RSA) and root volume (RV) was measured at the end of the experiment. TLA was measured using LI-3100 Leaf Area Meter, while RL, RAD, RSA and RV was scanned using EPSON Expression 1680 root scanner and then analysed using WinRHIZO 2007d. LA, RL, RAD, RSA and RV was expressed in centimeter square (cm^2) cm, mm, cm^2 and centimeter cube (cm^3) respectively.

Statistical analysis

All the treatment was arranged in Randomized Complete Block Design (RCBD) using factorial treatment arrangement with 4 replicates. The data were analysed using analysis of variance (ANOVA) and the difference among the means was separated using Least Significant Difference (LSD) at $P<0.05$ with SAS version 9.3 (SAS, 2011).

Results and Discussion

Growth

Application of N fertilizer to *F. carica* cutting affected the early vegetative growth significantly. The result obtained in Figure 1 and 2 showed that there were significant differences in shoot length (SL) and shoot diameter (SD) toward different N fertilizer rates. The result shows application of N at 30 g N/plant/year increased the SL and SD significantly compared to other treatment.

The highest SL and SD were observed at 30 g N/plant/year with the value at the end of experiment is 68.68 cm and 9.69 mm, respectively. Both values were significantly higher than other treatments. This then followed by 20 g N/plant/year and 40 g N/plant/year. The lowest SD and SL value was observed in control plant (0 g N/plant/year) which is at 42.40 cm and 6.97 mm.

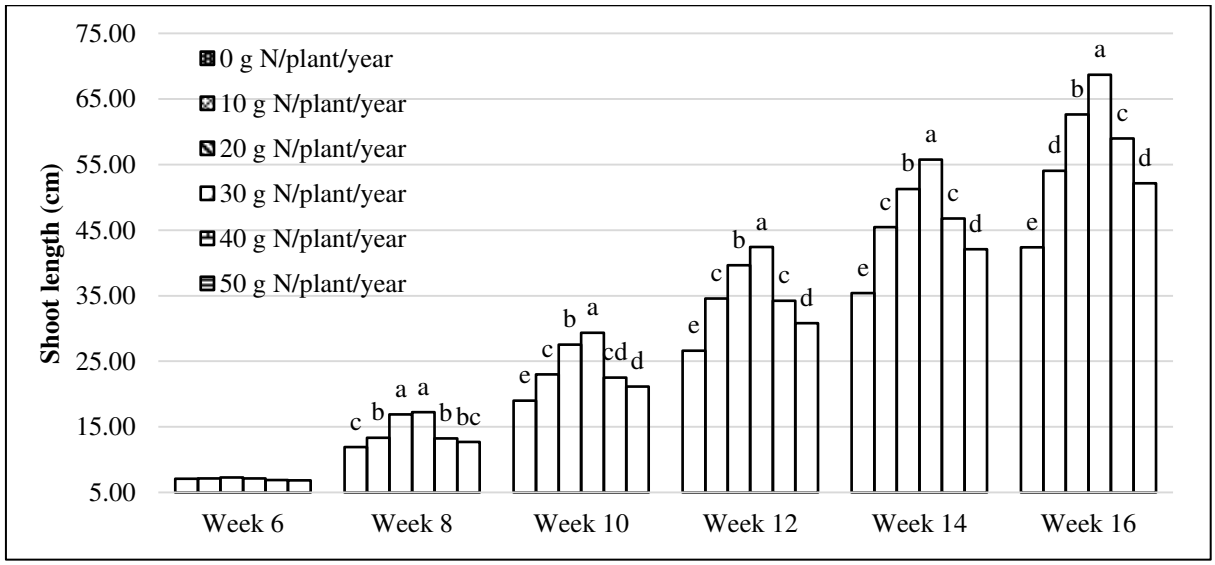

Figure 1: Shoot length (SL) of *F. carica* L. as influenced by different application of N fertilizer. Mean with the same letter(s) are not significantly different at $P>0.05$.

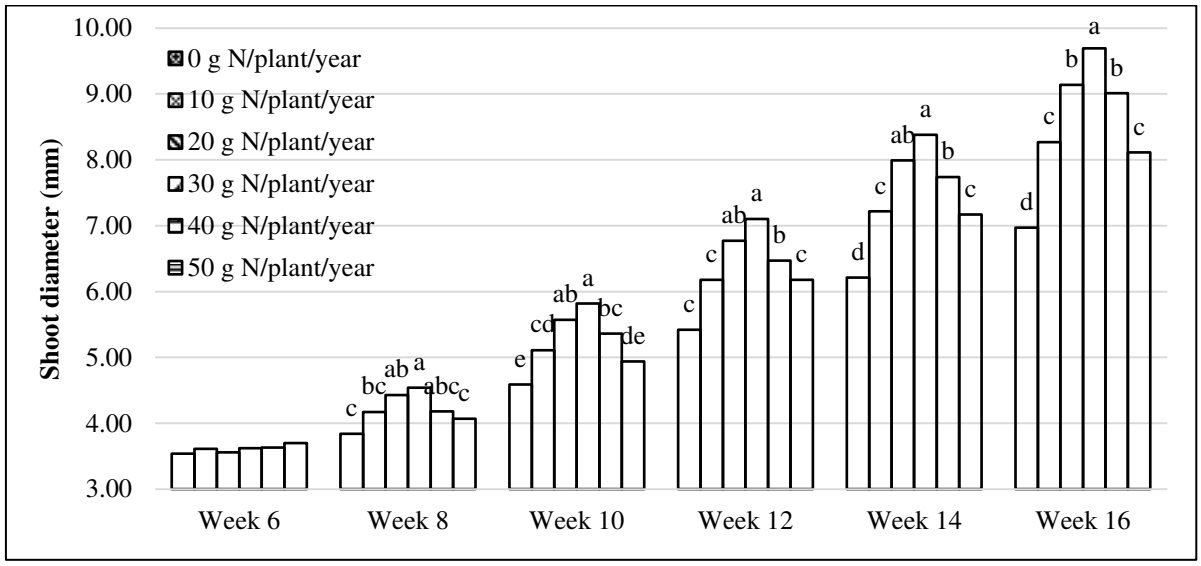

Figure 2: Total shoot diameter increments (TSDI) of *F. carica* L. as influenced by different application of N fertilizer. Mean with the same letter(s) are not significantly different at P>0.05.

For root length (RL), root surface area (RSA), root volume (RV) and root average diameter (RD), application of different N fertilizer rate have affect these parameters significantly (Table 1). The highest RL, RSA and RV were observed at 30 g N/plant/year followed by 20 g N/plant/year and 40 g N/plant/year. The lowest RL, RSA and RV were recorder at 0 g N/plant/year. The lowest RAD was recorded at 30 g N/plant/year followed by 20 g N/plant/year and 40 g N/plant/year. The highest RAD was recorded at 0 N g/plant/year with RAD of 0.9126 mm.

Table 1: The root length (RL), root surface area (RSA), root volume (RV), root average diameter (RAD) of *F. carica* L. influenced by different application of N fertilizer at the end of experiment. Mean with the same letter(s) are not significant at P>0.05.

N rate (g/plant/year)	RL (cm)	RSA (cm^2)	RV (cm^3)	RAD (cm)
0	3624.9^{e}	942.26^{e}	17.41^{e}	0.9126^{a}
10	5206.5^{c}	1226.38^{cd}	26.33^{c}	0.5887^{c}
20	6010.4^{b}	1537.06^{ab}	29.69^{b}	0.5420^{de}
30	6703.8^{a}	1576.80^{a}	32.26^{a}	0.5306^{e}
40	5531.3^{c}	1371.32^{bc}	28.78^{b}	0.5630^{d}
50	4403.0^{d}	1161.30^{d}	21.74^{d}	0.6382^{b}
$LSD_{P<0.05}$	452.91***	191.09***	2.24***	0.0232***

**** The LSD is at P<0.001.*

N rate significantly affected the total leaf area (TLA) (Figure 3). The highest TLA was recorded at 30 g N/plant/year which is 4186.2 cm^2. This followed by 20 g N/plant/year and 40 g N/plant/year with TLA of 3856.6 cm^2 and 3683.0 cm^2. The lowest TLA was recorded at 0 g N/plant/year with TLA of 1769.9 cm^2.

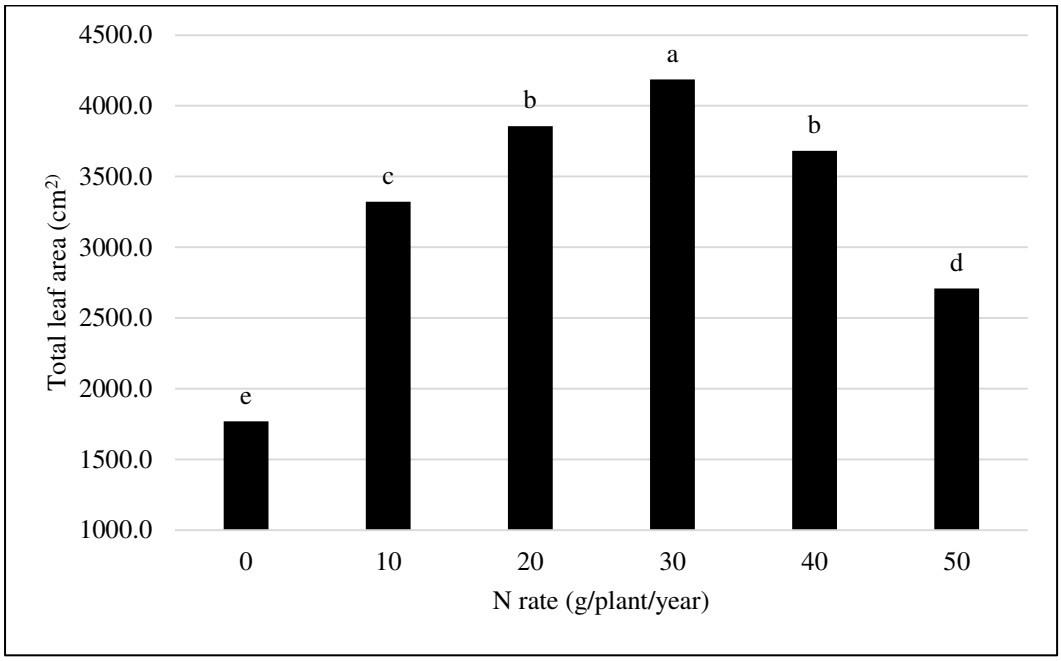

Figure 3: Leaf area of *F. carica* L. as influenced by different application of N fertilizer. Mean with the same letter(s) are not significantly different at P>0.05.

All these equivalent to study done by Sevil et al. (2007) which stated that N fertilizer is an essential element that has important function on plant growth, yield and quality. Shortage of N fertilizer application can cause reduction in plant cell growth include shoot, leaves and root as N plays major role in cell division (Hignett, 1982; Moghazy et al., 2014).

However, when the N fertilizer application exceed 30 g/plant/year all the growth parameters value decreases significantly and if exceed 50 g N/plant/year, it even has lower parameters value than 10 and 20 g N/plant/year except for RAD. As stated by Scagel et al. (2011), excess N fertilizer application can cause the toxicity to plant which cause the imbalance of others nutrient uptake and it also can increase the water stress which later can decrease the growth.

Chlorophyll content

It is known that N and chlorophyll content is closely linked (Bojović and Marković, 2009). N fertilization application has a significant affect to the total chlorophyll content (TCC) on *F. carica* cutting leaves. Application of N fertilizer at 30 g N/plant/year contained significantly highest TCC compared to other treatments for all three reading (Figure 4). This is due to N is a major component of chlorophyll (Sahadevan, 1987). This result was equivalent to study done by Hokmalipour and Darbandi (2011) and Keshavan et al. (2011) which stated that with increase in N rate, it will increase the chlorophyll content in leaves.

However, when N is given exceed 40 g/plant/year it reduced the TCC. This due to when N is excess, it can increase water stress to the plant (Scagel et al., 2011). According to Sanchez et al. (1983), water stress can affect the chlorophyll content, stomatal conductance and photosynthesis. As water stress increase, it reduced the chlorophyll content although the N content in leaves was not affected.

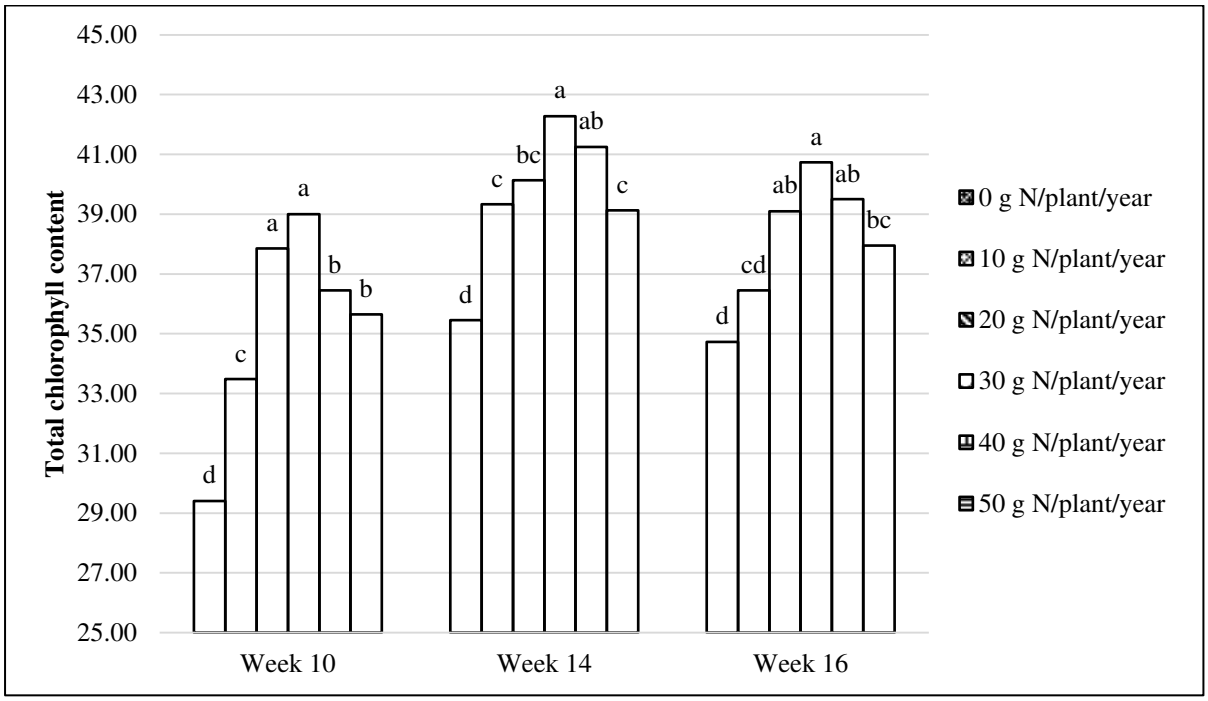

Figure 4: The total chlorophyll content (TCC) in the *F. carica* L. leaves (SPAD meter) as influenced by different application of N fertilizer. Means with same letter(s) are not significantly different at (P>0.05).

Conclusions

Through this study, the optimum early growth and leaves TCC was achieved from application of 30 g N/plant/year. From the results, it clearly showed that N was one of the main factors that can influence the early growth of fig. As N rate increased it will increased the early growth and leaves TCC of fig. However, as N given increased beyond 30 g N/plant/year it reduced the early growth and TCC significantly.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effect of Planting Distance on Growth and Yield of Eggplant (*Solanum melongena* L.)

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Introduction

Eggplant or brinjal is a famous edible fruit in Asian countries. This edible fruit belongs to the family Solanaceae, one of the largest families in vegetables. It was reported that the origin of eggplant was from India due to the diversity of eggplants in the country (Kalloo, 1988). Other report says that eggplant originates from the African country Lester and Hasan, (1991). The fruit is rich in essential vitamins and minerals. It contains 89.0 g water, 1.4 g protein, 1.0 g fat, 8.0 g carbohydrate, 1.5 g celloluse, 130 mg calcium, 105 mg vitamin c and 1.6 mg Iron. In particular, eggplant is a good source of calcium, phosphorus and iron salts for bone and blood cell formation in the body, as well as a reasonable source of vitamin A (Carotene), Vitamin B-complex and vitamin C, all essential for good health (Romain,2001).

Globally, vegetable production has grown intensively especially on a per capita basis, which has increased 60% over the last 20 years. This trend is particularly strong in developing countries. According to The Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) (2014), total world eggplant production is 50.2 million tonnes where China and India are the major growing countries. Malaysian total production of eggplant is comparative low at 46,557 metric tons of 2,640 ha planted area (Anon, 2016). The Department of Statistics Malaysia (2015) reported that, Malaysian self-sufficient ratio (SSR) of eggplant is 101%, there are increase in import dependency ratio (IDR) of 12% in 2014 compared to the previous year. As a developing country, Malaysia needs continuous research intensively on vegetable crops including eggplant.

In spite of the economic and nutritional value of the crop, this plant as well as other vegetable plants also susceptible to pests and diseases problem. The production is hampered by the fruit and shoot borer i.e. *Leucinodes orbonalis*. It is regarded as the most damaging insect pest of the crop (Raju et al., 2007; Rashid et al., 2008; Onekutu, 2011). The eggplant is also subjected to the attack of many diseases affecting roots, leaves, stems and fruits. The severity in any particular disease may depend on the environment, eggplant variety, soil and other factors.

The eggplant can be grown practically on all soils types from light sandy to heavy clay. The soil should be fertiled and well-drained. The growth of eggplant is well develop in the soil pH at optimal level at 5.5 to 6.0. The eggplant is moderately tolerant to acidic soil. Several cultivars are grown successfully under high pH level with a rich application of farm yard manure or green manuring practiced prior to transplanting.

Materials and Methods

The field experiment was set up with three replications at the Malaysia Agricultural Research and Development Institute (MARDI), Serdang Selangor. The plot area was maintained organically on a mineral soil type. Three varieties were used in the experiment i.e. Terung MTE2, Terung Bulat and Terung Panjang. All seeds were sown in the glasshouse and transferred to the field at 2 weeks after

germination. At 4 days before planting, the planting holes were applied with 200 g of biochar-based organic fertilizer. Experimental design was Completely Randomised Design (CRD) with three replications. Each replication consists of 10 plants per variety. The treatments were different planting space between plant in a row which were 0.6, 0.8 and 1.0 m. Standard culture practices and drip irrigation were applied. Data for plant height at flowering, diameter and length of fruit, fruit weight and fruit yield were recorded. Analysis of variance was used to distinguish the plant means (SAS software).

Results and Discussion

The eggplant can be grown practically on all soils types from light sandy to heavy clay with well-drained. The soil texture in the experimental plot was sandy clayloam with the pH 6.12. The percentage of N content is low at 0.20% but the total carbon content in the soil is moderate at 2.45%. The cation exchange rate (CEC) is 12.37 cmol/kg i.e. within optimum rate (12-25 cmol/kg).

Result from this study showed there was no significant difference on the growth and fruit yield between different planting distances of the treatment. However, higher fruit yield was obtained for Terung Bulat and Terung MTE2 that was harvested from the planting distance of 0.6 m i.e. at 14.08 kg and 14.5 kg respectively. While, Terung Panjang gave maximum yield of 9.9 kg at planting distance of 1.0 m. These two types of eggplant may have different basic needs of growth. Terung Panjang (long fruit shape type) maybe need wider planting space to grow for better yield compared to Terung Bulat and Terung MTE2 (round fruit shape type). In this study, biochar-based organic fertilizer was used instead of compose or manures in the organic farming plot system. The biochar-based organic fertilizer was applied to achieve better crop production through higher nutrient supply for more efficient crop uptake under organic farming system. Previous research reports that, varieties of crops such as maize and corn showed significant differences in their yield response to increased plant density (Fanadzo et al., 2010; Roekel and Coulter, 2011; Mohseni et al., 2013; Pepó and Sárvári, 2013). Meanwhile, Giesbrecht (1969) found that the row spacing did not affect the yield.

Planting distance determine the plant densities and thereby, plant density could affects the production. Besides yield, the plant height was measured for growth data. It was measured at flowering as the plant growth has completely matured. Result also showed that all the varieties (Terung Bulat, Terung MTE2 and Terung Panjang) at the closer planting space (0.6 m) produced taller plants as compared to the plant in wider spacing (0.8 and 1.0 m). This is in agreement with previous findings that an increase in plant density increased plant height (Tetio-Kaho and Gardner, 1988; Maboko and Du Plooy, 2009). The increased in plant height can be associated with competition for active photosynthetic radiation. Plant tend to be taller due to competition for sunlight. This study showed that close and wider space of planting does not give significant effect to the growth and fruit yield. Therefore, from this study plant spacing of 0.6 m is sufficient for eggplant cultivation to get the maximum density and production. Planting at a distance of 0.6 m between plants can provides 15,000 plant density compared to 9,000 plants for 1 m spacing in 1 hectare. Hence higher densities will result in higher production. Nevertheless, study effect for planting distances of less than 0.6 m are yet unknown.

Table 1: Effect of plant distance between plant and cultivar on growth and yield of eggplant. Treatment A (0.6 m), Treatment B (0.8 m), Treatment C (1.0 m).

	Yield (kg)	Plant Height (cm)	Fruit Weight (g)	Fruit Diameter (cm)	Fruit Length (cm)
Terung MTE2					
• Treatment A	14.5	48.6	142.9	22.8	8.9
• Treatment B	9.8	43.7	134.0	22.8	9.0
• Treatment C	12.1	37.6	140.4	22.5	8.6
Terung Bulat					
• Treatment A	14.1	52.1	160.0	22.0	8.5
• Treatment B	8.1	51.4	130.4	20.7	10.8
• Treatment C	11.1	48.1	148.0	23.0	9.5
Terung Panjang					
• Treatment A	9.9	58.1	72.0	9.2	18.0
• Treatment B	3.5	56.8	86.9	9.7	19.1
• Treatment C	8.0	53.8	97.8	9.7	14.6

Conclusions

The results indicate that there is no significant difference on the growth and fruit yield between different planting distances of the treatment in this study. Therefore, in this study the best plant distance for Terung MTE2, Terung Bulat and Terung Panjang is 0.6 m between plants. It should be noted that the results presented here are only data collected for one season and does not take into account the problem of pests and diseases occurred. There is a need to study further the effect of closer plant spacing and other factors such as pests and diseases as well.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effect of Nano Fertilizer on Early Growth, Height and Stem Diameter of Dwarfed Long Bean (*Vigna sesquipedalis)*

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Introduction

The growth of the plant depends on the type of fertilizer and the suitability of fertilization time. Treated trees cannot accept the fertilizer provided that the fertilizer used does not meet its requirements. Fertilization is actually intended to restore nutrient content in soil that has been absorbed by the plant through its physiological processes where nutrient deficiency can cause the growth of a plant to be stunted, easily broken and leaves become yellow. Fertilizers are defined as substances for nourishing plants containing substances such as nitrogen, phosphorus and potassium either naturally or artificially, and can be added by mixing trace elements such as Fe^{2+} and Mg^{2+} according to the desired combination (Aziah, Mohd Fazli Tajuid, 2016).

Plants require some nutritional elements to grow well. Plants can derive the above elements from the three main sources i.e. from air, water and soil while carbon, hydrogen and oxygen are obtained by trees from water and air. Other elements are all obtained from the soil either from fertilizers or organics from the soil. For improved yield and good growth, fertilization is an important thing in agriculture. In general, fertilizers are categorized into two type's namely organic fertilizer and inorganic fertilizer. Organic fertilizers are usually derived from crop and animal waste either in liquid or solid form while inorganic fertilizers are a chemical mixture in liquid or solid form with certain metrics processed from the plant. Inorganic fertilizers can also be produced for only one element such as urea fertilizer for nitrogen (N), *Muriate of Potash* (MOP) for potassium (K) and *Triple Super Phosphate* (TSP) for phosphate (Anonymous, 2011).

The study and information of the effects of nano fertilizers is still low at this country and is difficult to obtain as a reference. Therefore, this study was carried out to verify the effect of using nano fertilizers to prove the advantages of using nano fertilizers over the growing of dwarfed long bean (*Vigna sesquipedalis*). On this awareness, the study of nanoscale especially in agricultural sector should be carried out by relevant agency. According to the nano fertilizers theory, the size of a very small nano-molecular weight <100 nm can help plant roots continue to absorb nutrients such as nitrogen, phosphorus and potassium in plants more efficiently and does not require too much quantity as used in ordinary commercial fertilizers. The rate of recommendation on the use of these nano fertilizers is minimal, and low cost. The results of this study will determine the effectiveness of nano fertilization technology in agricultural applications.

Materials and Methods

The design of the study is randomized complete block design (RCBD). Each treatment contains 10 trees and 4 replicates for each treatment. The independent variables in this study were the fertilizer types. The constant variables in this study are the same crop media. Growths of trees such as tree height and stem diameter were quantitatively measured.

Types of treatment were as follows:
Treatment 1 (T1): Single Fertilizer rate 34:56:56 (kg)/ha (control)
Treatment 2 (T2): Nano Fertilizer 20:20:20– rate 4 kg/ha

Treatment 3 (T3): Nano Fertilizer 20:20:20– rate 8 kg/ha
Treatment 4 (T4): Nano Fertilizer 20:20:20– rate 12 kg/ha
Treatment 5 (T5): Without fertilizer

Results and Discussion

The findings of the study showed positive and significant growth to average height reading of trees according to the type of fertilizer used. The significance differences from week 1 to week 5 are shown in Figure 1.

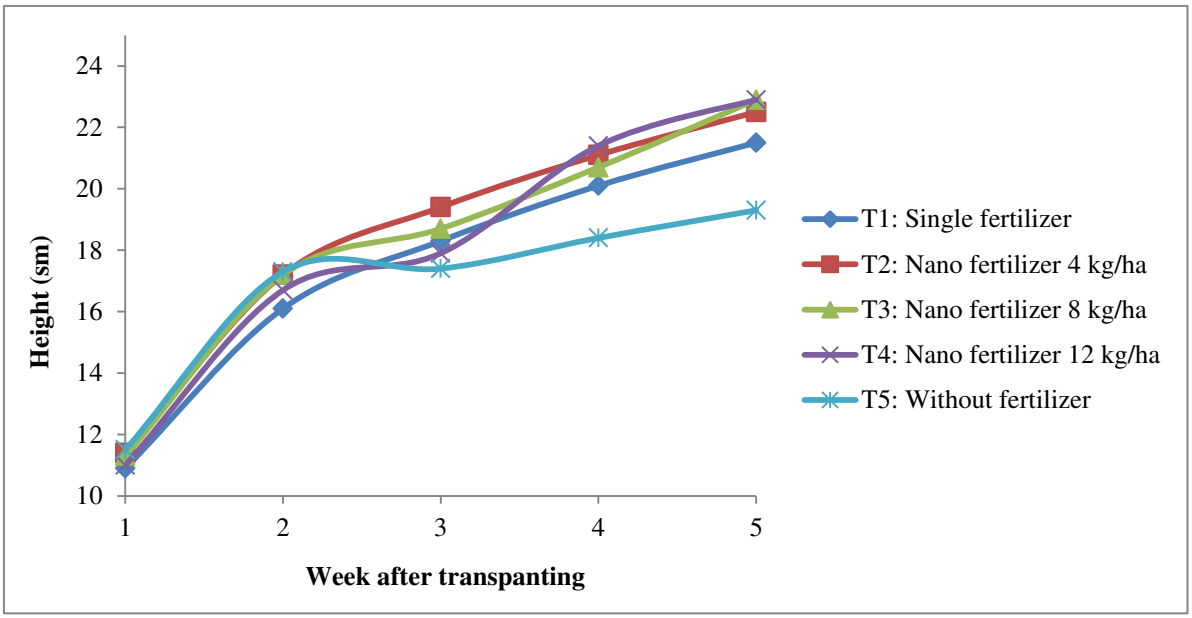

Figure 1: Plot showing the comparison for the height of dwarfed long bean treated with single fertilizer and nano fertilizers.

Table 1: ANOVA (One way) data of height according all treatment at week 3 to week 5.

		ANOVA (Oneway)				
		Sum of Squares	df	Mean Square	F	Sig.
Height week 3	Between Groups	25.956	4	6.489	2.348	.062
	Within Groups	207.266	75	2.764		
	Total	233.222	79			
Height week 4	Between Groups	58.368	4	14.592	3.088	.021
	Within Groups	354.391	75	4.725		
	Total	412.759	79			
Height week 5	Between Groups	74.469	4	18.617	2.808	.031
	Within Groups	497.203	75	6.629		
	Total	571.672	79			

Based on the Table 1, All treatment showed the significant reading $P<0.05$ from week 4 to week 5 proving that there was a significant difference between the fertilizer treatments used on the effect of the height of the dwarfed long bean. Based on the Figure 1, the most significant increase in elevation was the treatment of 8 kg/ha nano fertilizers compared to other treatments.

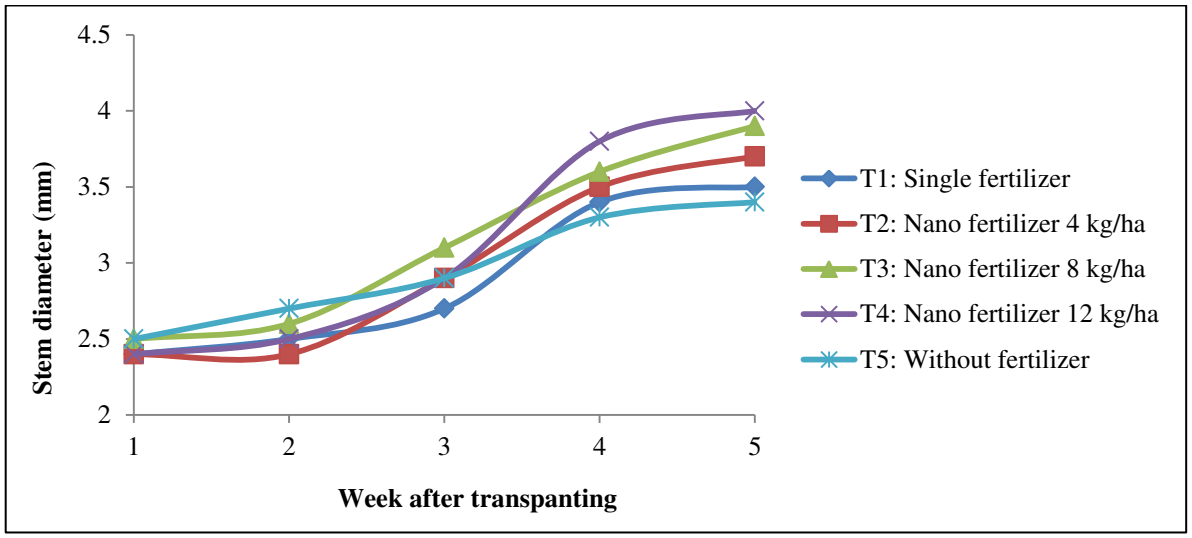

Figure 2: Plot showing the comparison for the stem diameter of dwarfed long bean treated with single fertilizer and nano fertilizers.

The findings of the study showed positive and significant growth to the mean reading of the stem diameter of the dwarfed long bean for all the fertilizer treatments used. Figure 2 above shows an increase in the average readings of stem diameter from week 1 to week 5 for all fertilizer treatments used in this study.

Table 2: ANOVA (One way) data of stem diameter according all treatment at week 3 to week 5.

		ANOVA (Oneway)				
		Sum of Squares	df	Mean Square	F	Sig.
Stem Diameter week 3	Between Groups	1.176	4	.294	3.939	.0058
	Within Groups	5.596	75	.075		8759
	Total	6.772	79			
Stem Diameter week 4	Between Groups	1.997	4	.499	7.019	.0000
	Within Groups	5.335	75	.071		7438
	Total	7.332	79			
Stem Diameter week 5	Between Groups	4.036	4	1.009	10.474	.0000
	Within Groups	7.226	75	.096		0086
	Total	11.262	79			

The observed data shows dwarfed long bean treated with nano fertilizer increase its height compared to the untreated and single fertilizer plants. The effect of nano fertilizer improved the optimized capacity of nutrients received by the dwarfed long bean by the process of slow release system performed by the binder carrying the micronutrients in the nano fertilizer system. The micronutrients received by the plants through the slow release process system by the nano fertilizer enhanced the growth of plant height and stem diameter.

Conclusions

Preliminary result showed that the nano fertilizers promote the growth on height and stem diameter of the experimental plants significantly. This fertilizer probably can be used in a larger scale on the suitable food crop which may increase the food production of the crop that used this fertilizer.

Acknowledgement

This work was financially supported by MARDI Development Fund (P121003001210001).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Growth Performance of *Ruellia brittoniana* as Affected by Daminozide Concentrations

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Introduction

Ruellia brittoniana is one of the species that shows very strong growth tendency and has less flower. *Ruellia sp.* is fast growing flowering ornamental plant. Most growers prune the plant mechanically on regular basis to reduce growth, promote side-shoot branching and initiate flowering. This technique is labor intensive but not cost effective. To reduce labor requirement, chemical pruning using suitable growth retardant at appropriate dosage could be an effective alternative. Growth regulators are commonly being used to improved establishment characteristics as well as aesthetically attractive flowering plants. Thus, plant growth retardants are applied to agronomic and horticultural crops to reduce unwanted longitudinal shoot growth without lowering plant productivity. Most growth retardants act by inhibiting gibberellin biosynthesis (Rademacher, 2000). Growth retardants have an inhibiting effect on cell division and enlargement of cell in plants. Therefore, they are widely used for height control in floriculture (Pasian, 1999). However, plant response to (PGRs) may differ even within the same species and depends on the retardant type, dose and application method, frequency of application, age of plant, nutritional status and environmental conditions (Basra, 2000; Barett and Bartuska, 2010; Salachna and Zawadzinska 2013; Sprzaczka and Laskowska, 2013; Ahmad et al., 2015). Having looked at these scenarios, the objective of this study was formulated as to evaluate the effects of different concentrations of daminozide on vegetative growth and the flowering of *R. brittoniana.*

Materials and Methods

Plant materials and growth condition

This experiment was conducted in the Agrotechnology Unit, Faculty of Agriculture, Universiti Putra Malaysia. The plant species, *R. brittoniana* were obtained from local nursery at Sungai Buluh, Selangor and the seedlings were planted in polyethylene bags with cocopeat. The plants were fertilized a month after planting with an organic fertilizer NPK (8.0N: 8.0P_2O_5: 8.0K_2O: 3MgO) about 6 g/plant every two weeks during the two months of experimental period. The plants were grown under 50% shade on a on a 1.2 meter bench and the average temperatures during the study were between 25°C (night) and 33°C (day).

Daminozide treatments

The plant's cuttings were obtained after two months of the establishments using hardwood cuttings. The plants were pruned regularly to get uniform cuttings. Each plant was pruned to about the same height at approximate of 12 to 15 cm length with 2 to 3 nodes. The young leaves were fully emerged after 15 days following the trimming. The plants were treated with a daminozide at five concentrations (0, 1000, 2000, 3000, 4000 mg/L, 50 mL for each plant) through foliar spray. The plants were sprayed with a fine spray using hand sprayer. The control plants were treated with tap water.

Data collection

Plant height was measured before and after daminozide treatment. The increase in plant height was measured twice a month for two consecutive months. Measurement of plant height was taken from the point of cutting to the higher shoot tip using a measuring tape. Internode length was determined twice a month by using measuring ruler. Leaf areas were measured and recorded as total leaf area per plant using automatic leaf area meter (MODEL LI-300, LI-COR) while the leaf number was manually counted based on fully expanded leaves. The whole plants were then separated into leaves, stems and roots and the dry weight of each part was determined after drying for 72 hours at 75°C in the oven.

Experimental design and data analysis

The treatments were arranged in a randomized complete block design (RCBD) with four blocks, five treatments and each plot comprised of eight plants. Data generated were subjected to analysis of variance (ANOVA) utilizing SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ levels.

Results and Discussion

Vegetative growth

All vegetative parameters taken were significantly affected by daminozide concentrations ($P<0.01$) (Table 1). Increasing in daminozide concentrations significantly reduces the plant height at every week intervals (Figure 1). The effect of reduced in plant height is attributed with decrease internode length which caused by daminozide action in interposes stride of gibberellin biosynthetic pathway. This would be resulted in retardation of elongation and cell division (Mabvongwe, 2014). A decrease of 68% in internode length was recorded in the plants treated with 4000 mg/L of daminozide compared to the control plants. The reduction in internode length consequently led to overall suppressive impact of daminozide on the total vegetative growth of plants. The daminozide treated plant has smaller leaf area as compared to the leaves of the control plants. Leaf area of plant was significantly reduced by 66% as the concentration of daminozide increased from 0 to 4000 mg/L. The decreasing in leaf area is due to the decreasing in number of leaves. The higher concentration of daminozide concentrations exhibited deformations of leaves (Wanderley et al., 2014). The number of flowers was observed to be the highest in the control plants compared to treated plants. Higher daminozide concentration has reduced the numbers of flowers of the plants. Flowering delay after daminozide application could be due to a blockage of gibberellin biosynthesis or from a wholly or partly restriction in action of one or more active endogenous gibberellins, which may be flowering promoters.

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Table 1: Effect of daminozide concentration on plant height, internode length, leaf area, number of flowers and number of leaves of *R. brittoniana*.

Daminozide concentrations (mg/L)	Plant height (cm)	Internode length (cm)	Leaf area/ plant (cm^2)	No. of flowers/ plant	No. of leaves/ plant
0	53.25^{a}	6.98^{a}	977.55^{a}	8.00^{a}	127.25^{a}
1000	27.25^{b}	3.77^{b}	615.28^{b}	4.00^{b}	96.00^{b}
2000	22.50^{c}	3.09^{c}	534.48^{bc}	2.00^{c}	89.25^{bc}
3000	18.00^{d}	2.49^{d}	427.07^{cd}	1.00^{d}	80.00^{c}
4000	14.00^{e}	2.24^{d}	331.23^{d}	0.25^{d}	65.25^{d}
F- test	**	**	**	**	**

***Significant at 1% probability level, *Significant at 5% probability level.*
Means in each column with the different letters within each factor indicate significant differences at P≤0.05% according to DMRT.

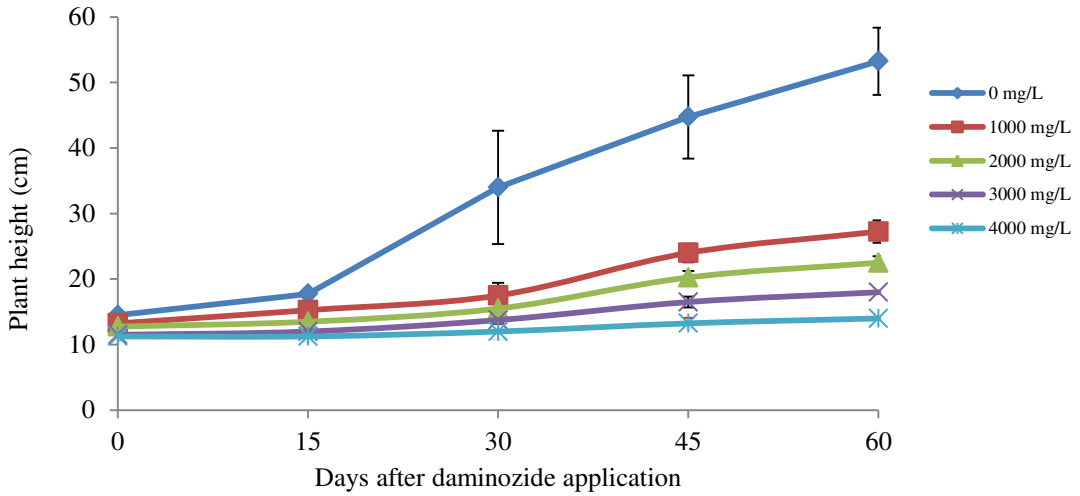

Figure 1: Effects of daminozide concentrations on plant height of *R. brittoniana* during 60 days of propagation. Vertical bars represent standard error of means.

Figure 2: *R. brittoniana* plant heights as affected by different daminozide concentrations i.e. 0, 1000, 2000, 3000 and 4000 mg/L.

Relative chlorophyll content

Daminozide treated leaves of *R. brittoniana* plants have higher chlorophyll contents than the control plants and were significantly effected (P<0.01) by daminozide concentrations (Figure 3). Increasing the daminozide concentrations significantly increased the leaf chlorophyll content. Among the treated plant, daminozide at 1000 mg/L was found to have highest chlorophyll content by 9.0% compared to control leaf (0 mg/L). This can be seen clearly through the light yellowish leaves when higher concentration applied, while other leaves that had been treated with 1000 mg/L appeared to be dark green compared to the non-treated plants. The increase in chlorophyll content will lead to the rise in the number and size of chloroplasts (Chaney, 2005a,b). However, there was no significant difference in chlorophyll content among leaves of plant treated with 2000, 3000 and 4000 mg/L.

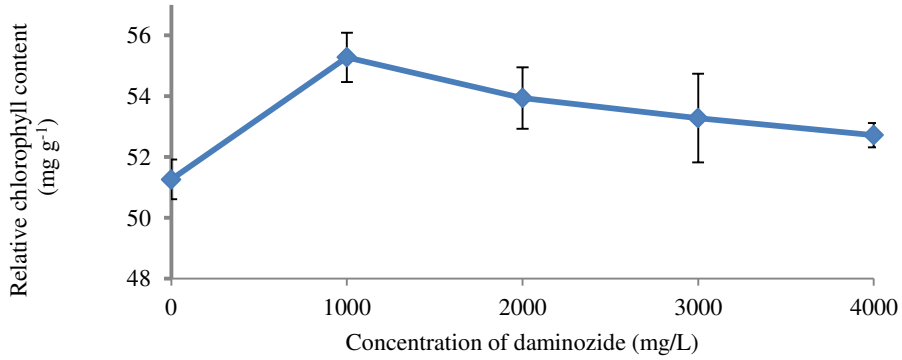

Figure 3: Effects of daminozide concentrations on relative chlorophyll content of *R. brittoniana*. Means in each graph with the different letters indicate significant differences at P≤0.05 according to DMRT.

Reduction of plant dry matter

Results obtained in this study showed that leaf, stem and root dry weight were greatly reduced by daminozide treatments (Figure 4) which in line with the results reported by Hashemabadi et al. (2012). Different level of daminozide concentrations was significantly affected the leaf, stem and root dry weight of plants (P<0.01). Decreasing in leaf dry weight from 8.79 to 3.63 g/plant as the concentration of daminozide increasing from 0 to 4000 mg/L, which was 59% lower compared to untreated plant. Increasing daminozide concentrations significantly reduced the stem dry weight when treated with 4000 mg/L daminozide by 83% compared to control (0 mg/L). The effect of daminozide concentration was significantly reduced the root dry weight by 55% when the concentrations of daminozide were increased from 0 to 4000 mg/L. The reduction of leaf, stem and root dry weight by daminozide application may be related to the reduced of plant height, internode length, canopy width and leaf area.

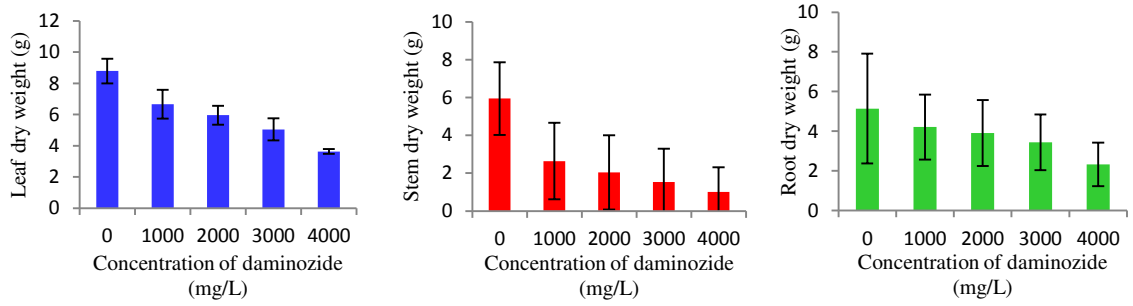

Figure 4: Effects of daminozide concentrations on dry weigh of *R. brittoniana plant parts*. Means in each graph with the different letters indicate significant differences at P≤0.05% according to DMRT.

Conclusions

The rate of reduction depends on the level of daminozide concentrations. In this study, the optimum rate of daminozide concentration was at 1000 mg/L, where it reduced the plant height, internode length, leaf area, and the dry weight of leaves, stem and root with less decreased in number of flowers. Generally, daminozide was found to increase the chlorophyll content of the plant leaves.

Acknowledgements

This research work was supported by Malaysian Agricultural Research and Development Institute (MARDI) and Universiti Putra Malaysia (UPM).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Assessing Salinity Tolerance Level of Four *Cucurbitaceae* Species using Growth and Mineral Ion Content (Na^+ and Cl^-) as Indicators

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Introduction

Cucurbitaceae comprises species consumed as food worldwide that thrives well in tropical, subtropical, arid deserts and temperate regions (Rai et al., 2008). In arid and semi-arid regions, salinity problem is particularly profound due to hot and dry weather combined with scarce water resources that further causing elevation of salinity concentrations (Yadav et al., 2011). In Malaysia, intensive agricultural methods such as high application of fertilizer and manure contribute to increasing soil salinity level. Major *Cucurbitaceae* production sites such a Cameron Highlands was reported to have EC level increased from the normal level to as high as 6.4 dS/m due to intensive rain shelter farming system that did not provide natural rainfall leaching (Wong et al., 2002). With approximately 130 genera and 800 species (Jeffrey, 2005), genetic diversity within *Cucurbitaceae* family is tremendous, and the response against salinity stress may differ greatly among the species in the family. Selecting salinity-tolerant species among genotypes is considered to be an effective method for salinity adaptation (Najqfian et al., 2008). While studies of *Cucurbitaceae* species in response to salinity stress has been done extensively in other regions, information on salinity responses in local Malaysia *Cucurbitaceae* species is still lacking. In view of that, growth parameters as well as mineral concentration; Na and Cl of four *Cucurbitaceae* species: cucumber (*Cucumis sativa*), bitter gourd (*Momordica charantia*), bottle gourd (*Ligenaria siceraria*) and squash (*Cucurbita moschata*) grown under NaCl salinity at vegetative stage were investigated. The objectives of this experiment was to determine the tolerance level of four *Cucurbitaceae* species studied by using responses of growth and mineral ion (Na and Cl) concentration of the species to different levels of NaCl salinity concentrations.

Materials and methods

Plant materials and growth condition

The plant materials used in this study were cucumber (*Cucumis sativa*), squash (*Cucurbita moschata*), bitter gourd (*Momordica charantia*) and bottle gourd (*Lagenaria siceraria*). Cucumber seeds were provided by MARDI (variety MT 1) while the rest were bought from a local seed supplier. Seeds were sown in plug trays filled with peat at University's Agriculture Park nursery, Universiti Putra Malaysia. The 4-days old germinated seedlings were then transplanted into 1.5 litre pot. The growing media used were cocopeat with 6 g/L N:P:K - 15:15:15 compound fertilizer, 2 g/L ground magnesium limestone and 1 g/L mixture of micronutrients. The plants were grown under 50% shade on a 1.2 meter bench and prior to salt treatment, approximately 300 mL of tap water were given to each plant every day.

Salinity treatments

When the first true leaf had fully expanded, four different levels of NaCl concentrations; 0, 25, 50 and 75 mM were given by manually drench the media approximately 300 mL of the solutions once per day for the first 10 days and twice per day for the following days as the plants grew. EC of the media was determined using the pour-through method (Cavins et al., 2000), which were 2.35, 4.12, 6.40 and 8.73

dS m^{-1} for 0, 25, 50 and 75 mM NaCl, respectively. The EC of solutions were checked by the EC meter (5061 Pen SHSX).

Vegetative growth

At 17 days after the first NaCl application, plants were sampled at random from each treatment for determination of plant height, stem diameter and leaf number. Measurement of plant height was taken from the surface of the soil in the polyethylene bag to the highest shoot tip by using a measuring tape. Stem diameter was measured at the lowest part of stem using Electronic Digital Caliper (Model SCM DIGV-6) while the leaf number was manually counted based on fully expanded leaves. The whole plants were then separated into leaves, stems and roots and the dry weight of each part was determined after 72 hours at 75°C in a drying oven. Plant dry matter was expressed as total dry weight of leaf, stem and root. Leaf areas were measured and recorded as total leaf area per plant using automatic leaf area meter (MODEL LI-300, LI-COR).

Mineral content

At 20 days of treatment, the top fully expanded leaf, stem and roots samples were harvested and washed with deionized water prior to drying at 70°C for 72 h. Briefly 0.25 g of the dry sample was transferred to a 100 mL digestion flask and 5 ml concentrated H_2SO_4 was added. The flasks were then heated for 7 minutes at 450°C and 10 mL of 50% H_2O_2 was added to complete the process. The flasks were then removed from the digestion plate, cooled to room temperature and then made up to 100 mL with distilled water. Concentration of sodium (Na) was quantified using an atomic absorption spectrophotometer (Perkin Elmer, Model 3110, USA) while Chloride (Cl^-) was extracted from the dried samples using silver ion titration method (Richards, 1954).

Experimental design and data analysis

The treatments comprising 4 salinity levels and 4 *Cucurbitaceae* species were arranged in a RCBD with three replications; 6 plants per replication. The data obtained was analyzed using ANOVA in the SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatment means were compared using Tukey's Honest Significant Difference (HSD) at $P \leq 0.05\%$.

Results and discussion

Vegetative growth

All vegetative parameters taken were significantly affected by the main effects of Cucurbit species and salinity ($P<0.01$). Significant interactions between the main effects were recorded only for leaf area and plant height ($P<0.05$) while the rest parameters showed no significant interactions (Table 1). In all of the species studied, leaf number and stem diameter started to decrease significantly at 25 mM NaCl. This is associated with the first phase of osmotic stress that resulted in reduction of vegetative parameters. Munns and Tester (2008) stated that osmotic stress phase starts immediately after the salt concentration around the roots increases to a threshold level, resulting in significant reduction in shoot growth. At this phase, leaf expansion is reduced, new leaves emerge more slowly, and lateral buds develop more slowly or remain quiescent, resulting in fewer branches or lateral shoots formation.

Significant interactions in leaf area and plant height revealed that reduction degree on these parameters varied between the species. Compared to control, 25 mM NaCl significantly reduced the leaf area in bottle gourd by 48.14% whereas in bitter gourd, significant reduction by 43.72% was recorded at 75 mM. In the rest of the species, leaf area was significantly reduced at 50 mM. Percentage of leaf area reduction as salinity increased from 0 to 75 mM was highest in cucumber, 68.54% and lowest in bitter

gourd, 43.72%. Plant height was significantly reduced by salinity in all species except in bitter gourd. From 0 to 75 mM increase of salinity, reduction of plant height was the highest in bottle gourd and lowest in bitter gourd with reduction percentage of 79.33 and 24.88%, respectively. Significant interactions between NaCl and *Cucurbitaceae* species in plant height and leaf area indicated that reduction degree as salinity increased was dependable on species. Ghoulam et al. (2002) reported that the degree to which growth is reduced by salinity differs greatly among species and to a lesser extent among varieties within a species.

Table 1: Main and interaction effects of four Cucurbit species and four NaCl salinity concentrations on leaf number, stem diameter, plant height, leaf, stem and root dry weight.

Factor		Leaf number	Leaf area (cm²)	Stem diameter (mm)	Plant height (cm)
Species	Squash	7.08b	751.41a	6.88b	38.56c
	Cucumber	6.85b	594.48b	6.63b	49.95b
	Bottle gourd	5.85c	871.58a	10.17a	40.86bc
	Bitter gourd	13.83a	776.00a	3.68c	105.89a
NaCl (mM)	0	10.27a	1174.93a	7.78a	85.60a
	25	8.42b	748.41b	6.82b	61.39b
	50	7.78bc	577.68c	6.71b	47.96c
	75	7.15c	492.55c	6.06c	40.31c
Species		**	**	**	**
NaCl		**	**	**	**
Species*NaCl		ns	*	ns	*

***Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD.*

Reduction of plant dry matter

As salinity level increased to 25 mM, reduction in plant dry matter in bottle gourd was the highest with reduction percentage of 46.27%. Cucumber and squash shown relatively lower reduction percentage compared to bottle gourd but there was no significant difference among the three species. Reduction in plant dry matter of bitter gourd was the lowest, 19.31% and was significantly lower than reduction of plant dry matter in bottle gourd. However, this reduction shown no significant difference compared to cucumber and squash (Figure 1A). When salinity increased from 0 to 50 mM, bitter gourd remained as species with the lowest reduction of plant dry matter (37.96%), even though no significant difference was recorded between reduction in this species compared to squash (48.37%) and bottle gourd (46.30%). Cucumber recorded the highest reduction in plant dry matter (59.43%), with significant difference compared to reduction of plant dry matter in bitter gourd (Figure 1B). The same pattern of reduction between the species at 0 – 50 mM was also recorded in 0 – 75 mM salinity increment. Bitter gourd remained as species with the lowest reduction in plant dry matter (44.40%), followed by squash (55.24%) and bottle gourd (58.70%), with no significant difference recorded between the species. Reduction of plant dry matter was the highest in cucumber (67.84%) and was significantly difference compared to bitter gourd (Figure 1C).

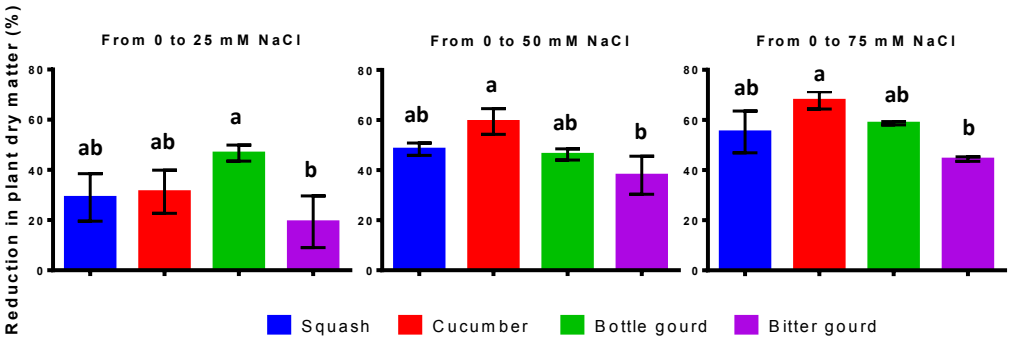

Figure 1: Reduction in plant dry matter from 0-25 mM (A), 0-50 mM (B) and 0-75 mM (C) salinity Means in each graph with the different letters indicate significant differences at P≤0.05% level according to Tukey's HSD.

Na and Cl concentrations

As salinity levels increase from 0 to 75 mM, Na^+ concentrations in roots (root Na) in bitter gourd had the highest increase; 5.71 times, while increment in squash, cucumber and bottle gourd were only 2.89, 2.59 and 2.57 respectively compared to control. Expectedly, Na^+ concentrations in leaf (leaf Na) in bitter gourd had the lowest increase (8.00 times) compared to squash, cucumber and bottle gourd which increase by 19.00, 11.50 and 9.50 times, respectively (Table 2).

Table 2: Main and interaction effects of four Cucurbit species and four NaCl salinity concentrations on Na and Cl contents of leaf, stem and root.

		Na(%)			Cl(%)		
Factor		Leaf	Stem	Root	Leaf	Stem	Root
Species	Squash	0.10c	0.29bc	0.46a	3.70a	5.25b	2.21ab
	Cucumber	0.26b	0.47a	0.35b	3.23a	6.70a	3.39a
	Bottle gourd	0.09c	0.41b	0.44a	2.58b	6.66a	2.68ab
	Bitter gourd	0.33a	0.38c	0.20c	1.82c	2.94c	2.07b
NaCl (mM)	0	0.03d	0.11d	0.15b	1.21c	2.36d	1.31c
	25	0.17c	0.45c	0.41a	2.72b	4.67c	2.37bc
	50	0.25b	0.53b	0.41a	3.17b	6.05b	2.98ab
	75	0.33a	0.56a	0.47a	4.22a	8.78a	3.69a
Species		**	**	**	**	**	*
NaCl		**	**	**	**	**	**
Species*NaCl		**	**	*	ns	*	ns

***Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD.*

High root Na along with low leaf Na in bitter gourd suggests that it has the ability to store relatively high Na ions in its root thus preventing excessive transport to shoot. This strategy of combating salinity stress is described as compartmentation of ions at organ level (Davenport et al., 2005). Leaf blade is the main site of Na toxicity in most plants since after Na ions have been deposited in the transpiration system, they accumulates in the leaf rather than the roots (Munns, 2002). Preventing Na^+ ions from reaching the leaf blades is critical in avoiding Na ion toxicity since plants in general transpire 50 times more water than they retain in leaves (Munns et al., 2006). Otherwise, Na^+ ions will accumulate in older non-expanding leaves and necrosis symptom will first appear at tip and margin of the leaf and extends towards the center of the leaf as Na^+ concentration increases. This symptom is apparent in squash since the leaf Na in this species is the highest among the species studied (Figure 2).

Apart from toxicity in Na ion, toxicity in Cl^- ion is also deleterious in salt-stressed plants. Regulation of Cl^- ions transport and Cl^- ions exclusion from shoots is correlated with salt tolerance in many species in various genus such as *Legumes, Glycine, Citrus* and *Vitis* (Travakkoli et al., 2010). In this study, bitter gourd has the lowest leaf Cl concentrations compared to other species. For root Cl, squash and bottle gourd were ranked lowest together with bitter gourd, with bitter gourd having relatively lower concentration, 2.07% among them. Together with low leaf Na, bitter gourd also had significantly lowest leaf Cl compared to other species. The ability of bitter gourd to maintain low Na^+ and Cl^- ions in its leaf compared to other species does not rely solely on compartmentation of Na ions at organ level. It was also observed in this study that during NaCl treatment, bitter gourd had a very prominent guttation activity in the morning which lasted up to 9.30 am (Figure 3). This phenomenon was not observed in other species. Shabala et al. (2010) stated that Na^+ ions excretion through hydathodes is one of the mechanisms to reduce salinity stress in plants. Hydathodes exude guttation fluid, which contains many organic and inorganic substances. Since it is suspected that bitter gourd guttation would contain high amount of salt, it is expected for the droplets to take longer to evaporate than droplets that are relatively lower in salt concentration.

Figure 2: Marginal leaf necrosis caused by NaCl salinity in older leaf of squash at 14 days of NaCl treatment.

Figure 3: Guttation observed on the leaves of bitter gourd.

Level of salinity tolerance between the species

In order to classify salt tolerance in species, the most suitable response to measure is growth or yield (Carillo et al., 2011). In addition, several indicators of salt tolerant plants can also be considered such as leaf ion status, particularly Na and Cl ions that could avoid ion toxicities (Shannon and Grieve, 1999). In view of that, among the species studied, bitter gourd is classified as least salt-sensitive since the reduction of plant dry matter was the least and this species has the ability to avoid toxicity of Na^+ and Cl^- ions in its leaf. Cucumber is classified as most salt-sensitive species compared to other species studied since the reduction of plant dry matter is the highest and increase in salinity levels results in ion toxicity particularly in its leaf. Between squash and bottle gourd, bottle gourd is less salt-sensitive since reduction in plant dry matter and concentrations of Na and Cl ions in the leaves are lower compared to squash. The level of salt-tolerant between the species from least sensitive to most sensitive is bitter gourd>bottle gourd>squash>cucumber.

Conclusions

1. Based on reduction in plant dry matter and accumulation of toxic ions in leaf, among all species studied, cucumber was classified as most salt-sensitive while bitter gourd was classified as least salt-sensitive species.

2. Salinity level of 50 mM was found to be detrimental to all vegetative parameters which will lead to ionic stress.

Acknowledgements

This research work was supported by Malaysian Agricultural Research and Development Institute (MARDI) and Universiti Putra Malaysia (UPM). The authors thank Dr. Nazmin Yaapar (UPM) for reading the manuscript.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Preparation and Maintenance of Lotus Planting Stock in the Aquatic Nursery for Mass Planting

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Introduction

Lotus (*Nelumbo nucifera*) is an aquatic plant which has aesthetic and medicinal values. In terms of socio-economic, lotus helps to generate income for the local community through ecotourism activities. In addition, lotus improves oxygen content for better aquatic habitat. Dongru et al. 2001 consider lotus as an emergent plant that helps improving water quality in a hypertrophic shallow lake. This in turn will also increase the biodiversity of the aquatic life in the lake.

Lotus is an icon for Tasik Chini (N 3° 25' 57'', E 102° 55' 29''), Pahang, Malaysia. It is the main ecotourism attraction as Man and Biosphere Reserve in Malaysia. Unluckily, annual flooding diminished the lotus population. Hence, preparation and maintenance of lotus planting stock is crucial. Thus, preparation of lotus planting stock is an initial conservation activity that can generate income in future. This paper discusses the preparation of lotus planting stock for mass planting which needs proper maintenance procedures. Previous study reported that this aquatic plant was grown well in muddy soils with water depth less than one meter (Mohd Ghazali et al., 2016).

Materials and Methods

An aquatic nursery size 10 x 10 x 1 m was constructed at Tasik Chini, Pahang. The lotus seedlings were taken from the lake (Figure 1). A bare-rooted plant was uprooted and put into a plastic pot. A stolon with at least three nodes was transplanted into a pot (25 cm in diameter), containing media mixture. The rooting network was preserved and grown in the mixture of soil, organic material and mud (1:1:1) for prolific rooting. The rooting pots were then submerged at the edge of the lake for a month for refreshing the newly transferred planting materials. They were placed in water less than 1 m depth. In order to enable new shoots to grow, planting materials were transferred into larger pots and put into the aquatic nursery for three months. The second stage potting was supplied with 50 g slow release fertilizer (NPK 8:8:8) to ensure healthy growth of the seedlings. Intensive maintenance such as fresh water supply and removing unwanted materials in the aquatic nursery was carried out continuously (Table 1).

Table 1: Maintenance schedule.

Activity	Month					
	1	2	3	4	5	6
Top-up water and residual cleaning	x	x	x	x	x	x
Repotting to larger container			x			
Applying slow release fertilizer	x		x		x	

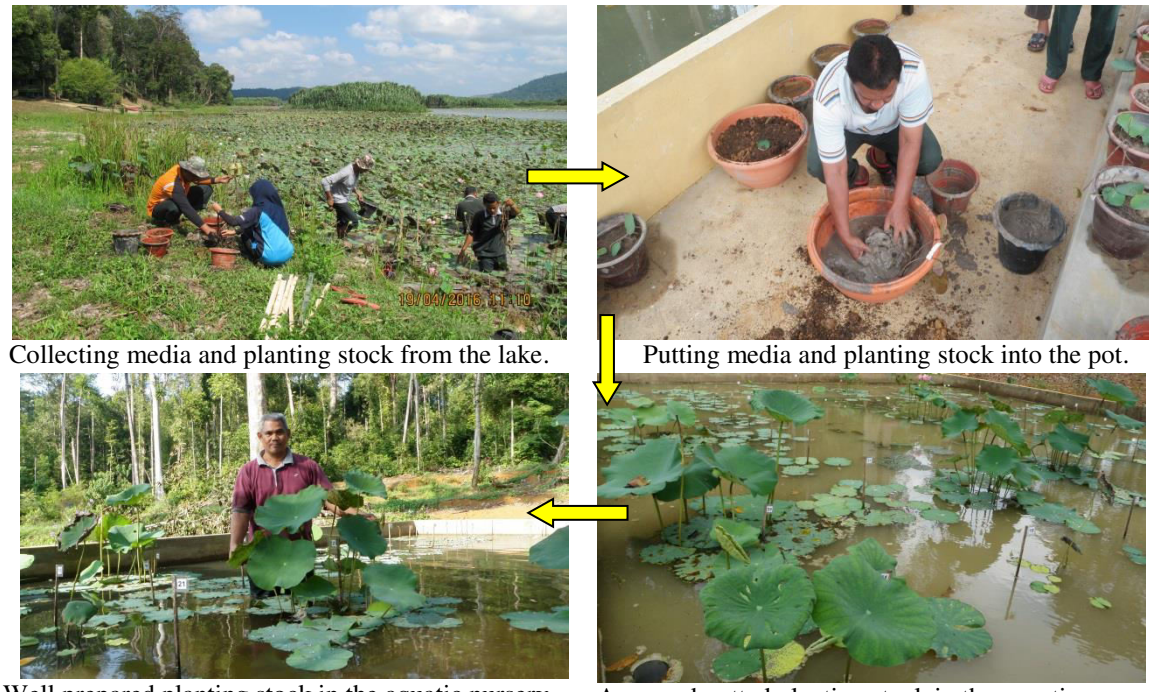

Figure 1: Preparation of media and planting stock from lake into the aquatic nursery.

Observation found that unwanted plants such as algae and *Hydrilla* sp. were established in the aquatic nursery. These plant communities dominated the aquatic nursery within a short period. Monthly, cleaning and weeding was carried out to ensure healthy growth of the lotus seedlings. Figure 2 shows the aquatic nursery conditions after two months without and after cleaning.

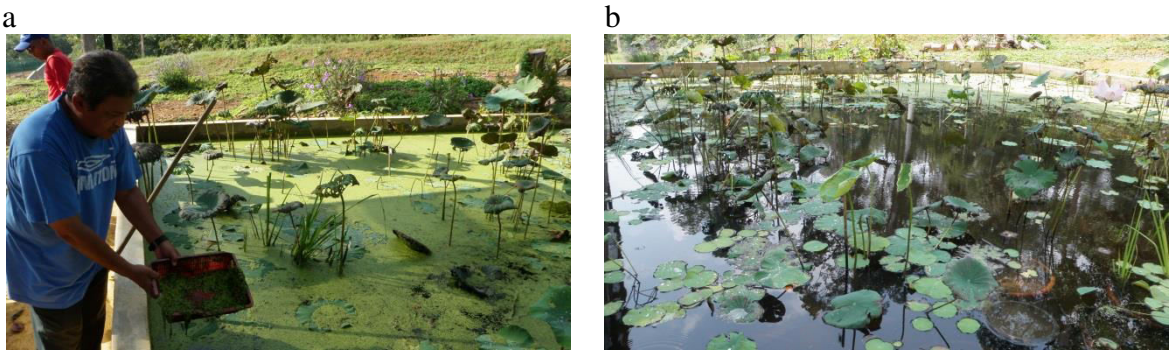

Figure 2: Cleaning and weeding the aquatic nursery. Aquatic nursery pond: (a) before cleaning (b) after cleaning.

Results and Discussion

Preparation of the planting materials required two stages i.e., potting and repotting. Observation showed that appropriate preparation of planting stock need intensive maintenance. The water level should be maintained at appropriate depth less than one meter (maximum depth of the aquatic nursery). Continuous water flowing from tap water and rain water will give dilution effect to reduce water toxicity accumulation due to various living activities in the aquatic nursery. Accumulation of organic materials and dilution of the fertilizer will recondition the water suitable for various aquatic plants. Competition for growing space and nutrients will suppress the lotus health. Hence, continuous

maintenance for the aquatic nursery is crucial. Without proper maintenance various setbacks might occur. Stagnant water enhanced algae population, dominating the surface of the water (Figure 2a). Weeds and algae are the common plants need to be cleaned monthly. Removing dead leaves also need to be done continuously. The well managed and prepared planting stocks can be produced with up to half a meter leaves in diameter two months after repotting to bigger pots in the aquatic nursery.

Proper maintenance of lotus in the aquatic nursery was crucial in order to produce healthy planting stock. Slow release fertilizer at a rate of 50 g should be supplied in the pot to provide ample nutrients supply to the lotus seedlings. Normally, slow release fertilizer can supply nutrient in the pot for six months before giving supplement for second fertilizer cycle but little amount given every 2 months produced well-formed leaves (Figure 1). The nutrient depleted plant showed sign of yellowing leaves and less number of standing leaves. Majority of the leaves will stay on the surface water with fewer flowers showed up. Normally, full blooming flower is the main attraction for lotus community. The flowers also supply seeds for succession of the lotus community. This study showed that potted lotus planting stocks which have at least five well-formed leaves above the water surface, make faster colonization after transferred to the lake.

Conclusions

Preparation and maintenance of lotus planting stock in the aquatic nursery for mass planting is a manageable activity. However, ample knowledge and appropriate technique is needed in order to succeed. This study suggested a sound experiment procedure on stock preparation and maintenance for lotus mass planting. The stakeholders should practice the method to get more experience for successful and better results.

Acknowledgements

We acknowledge the financial support from the Ministry of Natural Resources and Environment for lotus conservation in Tasik Chini which was conducted by FRIM.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Chapter 2

Plant Production

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Stress Tolerance of Cacao Trees (*Theobroma cacao* L.) Subjected to Smart Water Gel®

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Introduction

Cacao seedlings grow in the nursery under optimal conditions with sufficient supply of water and nutrients can be easily subjected to abiotic stress in the field during transplanting which causes major losses to cacao production. The trees planted under natural environment are exposed to different abiotic stresses and it is evident that abiotic stress has a significant impact on plants. Abiotic stress can be defined as environment conditions which reduce growth and yield below optimum levels (Skirycz and Inze, 2010). Among the abiotic stress, drought and heat stresses for cacao are the two critical threats to newly planted trees (Mittler, 2006; Suzuki et al., 2014). Drought stress as a consequence of planting trees during dry period with insufficient rainfall and deficient soil moisture might severely restrict crop growth while high atmospheric temperature might cause heat stress to the plants suffering from water deficit (Seki et al., 2007; Vadez et al., 2012). Water deficit inhibits plant growth by reducing water uptake into the plant cells thus alters the rheological properties of the cell wall. Consequently, inhibition of growth by water deficit occurs prior to inhibition of photosynthesis or respiration (Cramer et al., 1994; Hummel et al., 2010).

Smart Water Gel® or known as hydrogel in powder form is usually applied in tree planting for better water holding capacity. Hydrogel is created by the hydrophilic groups which present in a polymeric network upon the hydration with aqueous solution. Thus, the function of applying gel during planting is to absorb significant amount of water and retain the water in the soil surrounding the roots (Rosiak and Yoshii, 1999).

Currently, it is urged to find a solution to increase the survival rate of trees during planting especially during dry season in order to ensure normal growth after planting which contributes to high productivity of cacao. The aim of this experiment was to (i) study the growth performance and physiological response of cacao trees planted under natural environment in the field; (2) compare the effects of smart water gel and temporary shade to the newly planted cacao trees. The study will help the cocoa farmers to uncover the importance of shade and soil moisture after planting to ensure high survival rate and normal growth of cacao under instable climates.

Materials and Methods

Plant material and experimental site

The cacao seedlings were grown under optimal conditions in the nursery. A total of 340 hybrid cacao seedlings were transplanted to the experimental plot at Cocoa Research and Development Centre of Malaysian Cocoa Board, Bagan Datuk, Perak on 26 July 2016. 120 hybrid cacao seedlings were transplanted to the experimental site at six months after sowing. Trees were transplanted in July 2016 where there was low rainfall (124.8 mm^2) even during the subsequent months in August (42.1 mm^2) and September (89.1 mm^2). The average temperature from July - September 2016 was 28.9°C and the average daily maximum temperature was 35.2°C in April. The weather was considered hot and dry with low rainfall during the planting period.

Preparation of gel, tree planting and experimental design

The gel content is prepared by weighing 10 g of powder gel and mixed with 1 litre of deionised water. The mixture was stirred until it formed jelly-like aqueous material. A hole was dug before planting and 150 g of Christmas Island Rock Phosphate (CIRP) was applied to the hole. Next, 2 litres of gel material were applied to each hole and seedling was planted into the hole and covered with soil to fill up the hole. Temporary shades were set up by using coconut fronds to cover areas surrounding the cacao trees. Watering was done once a week for a month. A randomized complete block design was used and the experimental units were replicated four times.

Determination of survival rate

The survival rate of plants was recorded by counting the trees which survived after planting. Survival percent was recorded as number of trees survived x 100 / total number of planted trees.

Determination of growth traits

Growth parameters such as plant height (cm), stem girth (mm) and number of leaves were collected at 2 weeks intervals. Plant height was measured at 5 cm from the soil surface to the shoot tip of the plant using a measuring tape while stem girth was measured using a digital calliper. The number of leaves was determined by counting the leaves attached to the plants.

Determination of chlorophyll content and leaf area

Chlorophyll content was measured using a chlorophyll meter (CCM-200, Opti-Sciences, USA) which measured the optical absorbance in two different wavebands of 653 nm for chlorophyll and 931 nm for near infra-red. Leaf area was measured using a portable leaf area meter (CI-202, CID Bio-Science, USA).

Statistical analysis

Data were subjected to analysis of variance and the means were evaluated by using Least Significant Difference (LSD) test at $p<0.05$. Analyses were carried out using SAS statistical software.

Results and Discussion

Effects of gel and shade on the survival rate and growth of cacao trees

During the first month after planting, cacao plants applied with gel and covered with shade achieved 100% survival while control plants without gel and shade only gained 74% survival (Figure 1). Control cacao plants which were fully dependent on the natural field conditions, with no application of gel and shade, had the lowest survival rate (35%) with more than half of the transplanted trees died by three months after planting. Control cacao plants experienced stunted growth with heavy leaf loss and scorching. This might be due to the roots of control plants which failed to absorb sufficient water to satisfy the transpiration from the leaves and hence, they began to dehydrate. Trees with gel and shade obtained more than 95% survival even after three months of planting. During hot and dry period, transpiration can be one of the main factors that contribute to the loss of moisture in the upper soil zone and indirectly affects the vegetation and productivity of the cacao trees in the field.

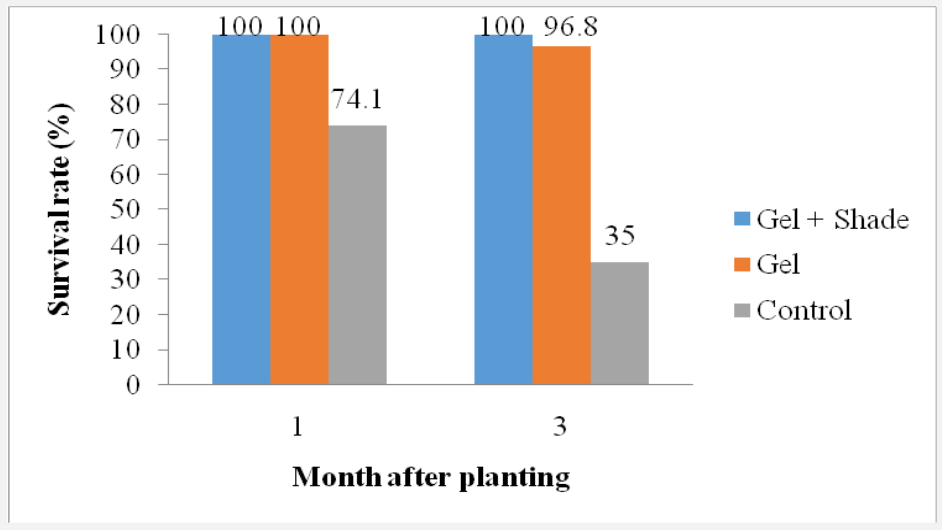

Figure 1: Survival rate of cacao trees subjected to treatments.

For growth measurements on height (Figure 2) and girth (Figure 3), cocoa planted under temporary shade and gel performed significantly better than cocoa planted with only gel ($P<0.05$) as plants matured. Cocoa planted with gel seemed to have stopped growing and this might be due to the reason that the heat generated from the atmosphere caused increase in evapotranspiration especially during long dry period and it was unlikely that the root absorption of water from the gel be sufficient to offset the moisture loss to the atmosphere. From the result, even though the girth for cocoa planted with gel also did not show significant increment after planting, the plants still survived and were eligible for grafting. Due to the insufficient number of trees for data collection, control plants were not encountered in the treatment comparison.

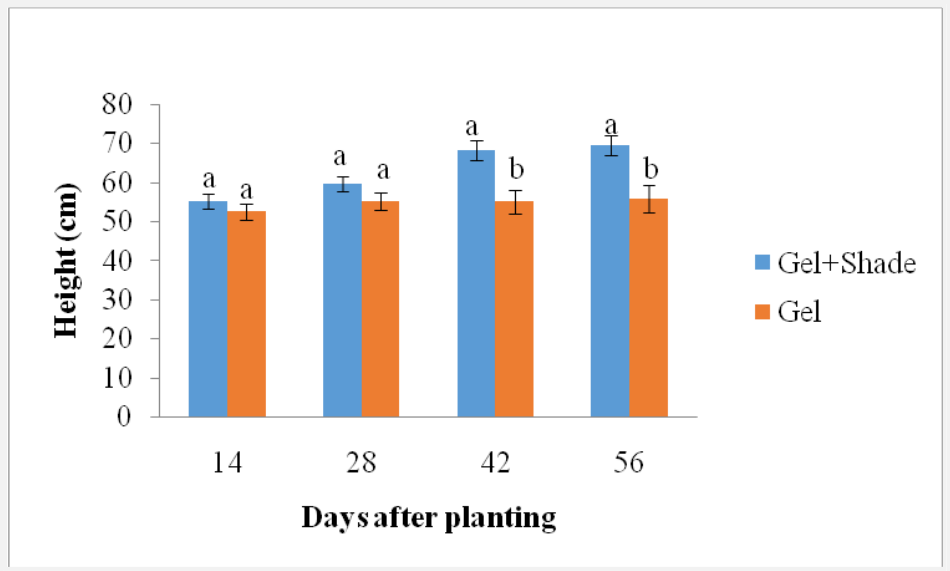

Figure 2: Effects of shade and gel on the height of cacao trees.

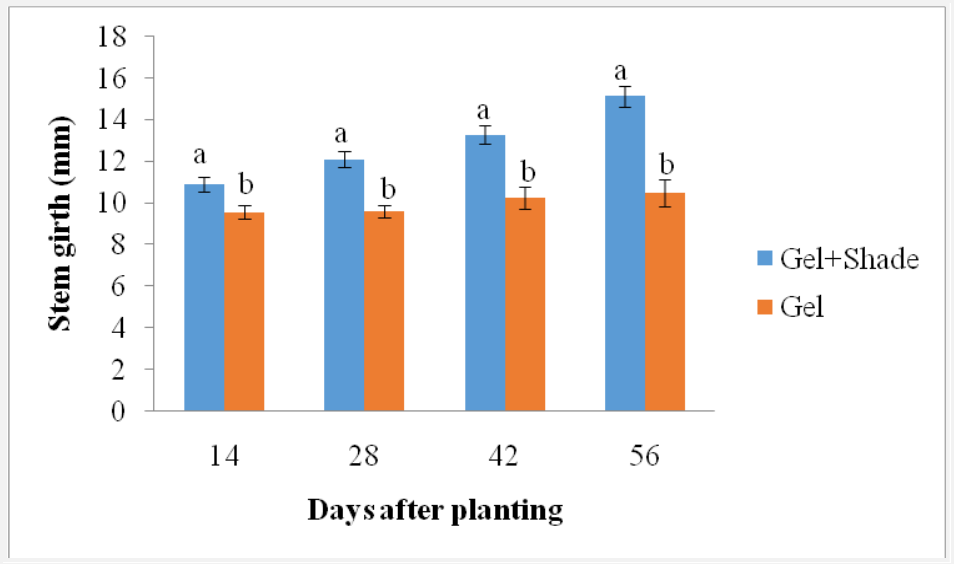

Figure 3: Effects of shade and gel on the stem girth of cacao trees.

The leaf count showed a significant difference ($P<0.05$) between cocoa planted with additional shade, even both treatments were with gel. Cocoa without shade lost more leaves during the dry period after planting (Figure 4). However, the cocoa plants flushed during 56 days after planting. Loss of leaves might be attributed to the effect of heat stress due to excessive temperature during development and during day time; leaf temperatures are often higher than that of surrounding air and when solar radiation peaks, the soil water reserves are depleted. Thus, leaves dry up fast and drop.

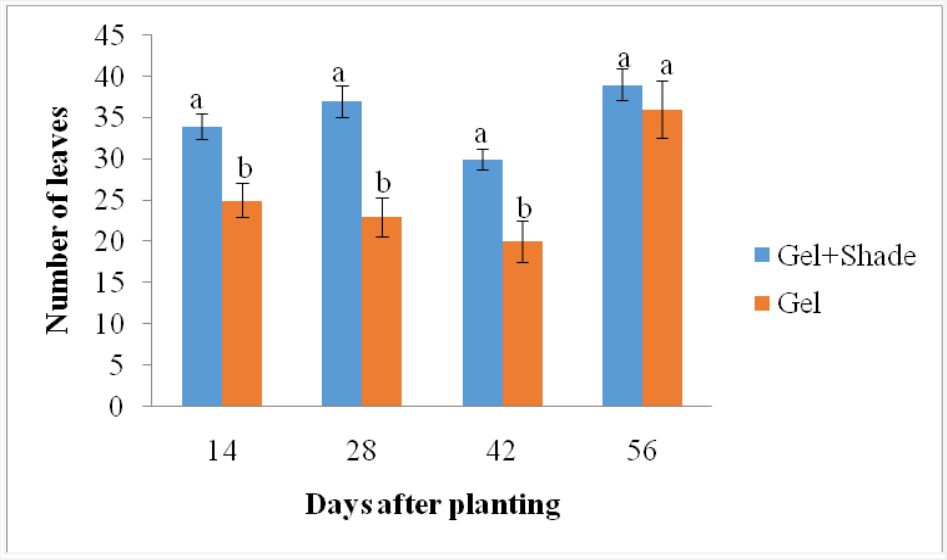

Figure 4: Effects of shade and gel on the number of leaves of cacao trees.

Both chlorophyll (Figure 5) and leaf area measurements (Figure 6) showed that cocoa planted with shade performed significantly much better than cocoa without shade ($P<0.05$). These results coincided with the physical growth measurements. Measurement of the chlorophyll content can be used as an indicator to understand the photochemical efficiency of cacao trees in PSII and thereby detect the damage of stress in PSII (Baker and Rosenqvist, 2004). Thus, cacao trees with gel and shade performed efficiently in photochemical reaction under field condition. This explains that the gel is

capable to enrich soil moisture while the shade manages to reduce the temperature of the trees thus reduces the loss of moisture through transpiration.

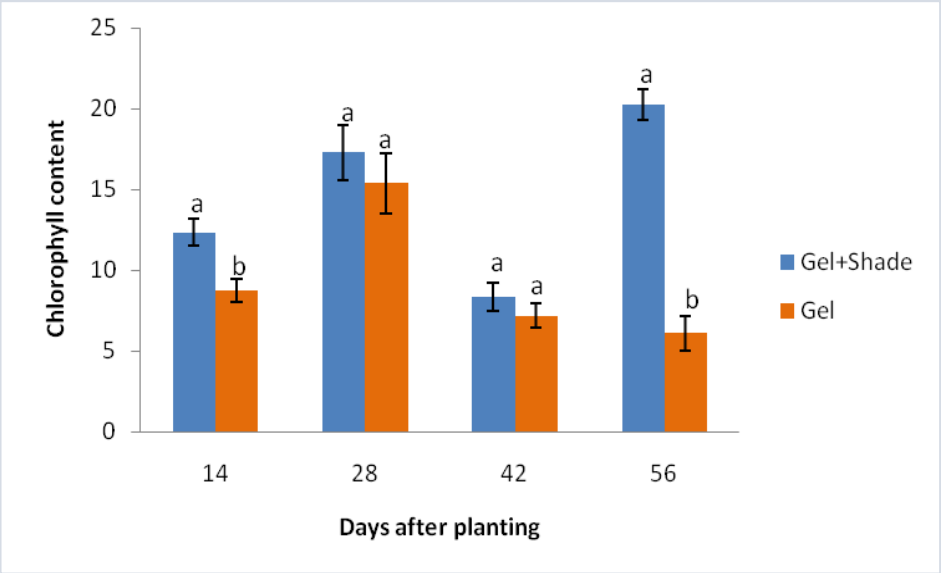

Figure 5: Effects of shade and gel on the chlorophyll content of cacao trees.

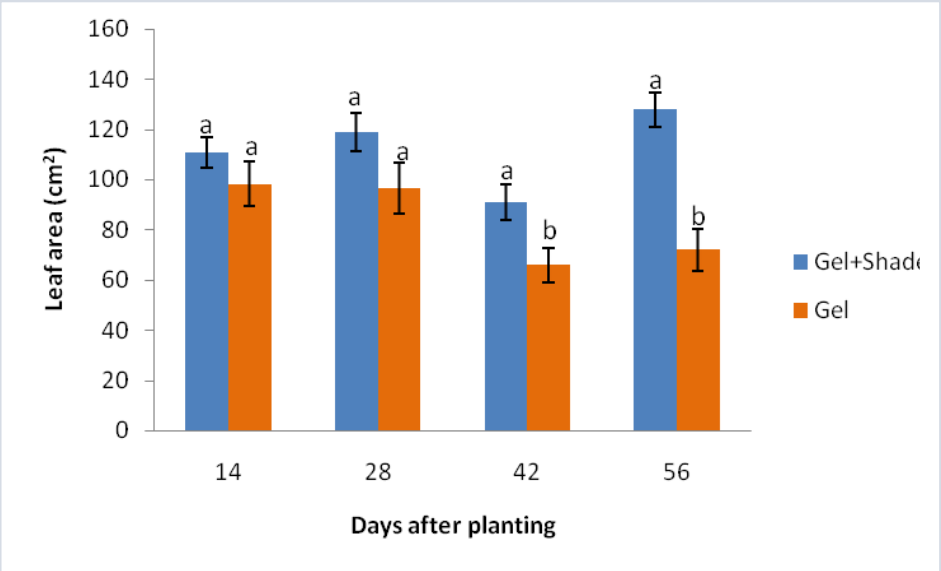

Figure 6: Effects of shade and gel on the leaf area of cacao trees.

Conclusions

Application of Smart Water Gel® enables the cocoa seedlings to be planted and survive during dry period. This preliminary field trial showed that cocoa grew better under temporary shade and gel. Cocoa planted with gel has achieved more than 95% survival rate by three months after planting and the plants survived for grafting in the field. Application of gel and shade had significantly increased the efficiency of cacao plants to survive under hot and dry period in terms of growth and physiological responses. Cacao plants with shade and gel had higher chlorophyll content and larger leaf size.

Acknowledgements

The study was financially supported under 11th Malaysian Plan, Economical Planning Unit from the Prime Minister's Department: Development of Cocoa Planting Management Towards Climate Change (P20001001116007). We thank Ministry of Plantation Industries and Commodities (MPIC) for her long-term support for our research. Special thanks go to the Director General of Malaysian Cocoa Board and the Director of Cocoa Upstream Technology, Malaysian Cocoa Board for their kind approval in publishing this research. We also thank Dr. Rozita Osman as the Head of Section for Crop Management Technology, Malaysian Cocoa Board, for her time and effort in reviewing the paper.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Different Soilless Media and Planting Density on the Flower and Rhizome Yield of *Curcuma alismatifolia*

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Introduction

Curcuma alismatifolia Gagnep. (Zingiberaceae) commonly known as Siam Tulip or Lotus Ginger is an ornamental ginger originated from the tropical and subtropical areas of northern Thailand and Cambodia (Apavatjrut et al., 1999). Its colourful lotus shape inflorescent comprising pink upper bracts and green lower bracts have long-lasting vase-life which can last for more than two weeks (Bunya-atichart et al., 2004). *Curcuma alismatifolia* has potential to be introduced as Malaysian low-lands cut flower and potted plant.

However, growing *C. alismatifolia* in soil is easily attack by bacterial (*Ralstonia solanacearum*) disease (Ruamrungsri and Apavatjrut, 2003). Production of *C. alismatifolia* is moving towards soilless culture in order to prevent the soil-borne bacterial disease. Planting media which provide good aeration and able to store moisture and nutrient are preferable for curcuma cultivation (Kuehny et al., 2005). In addition, the materials should be readily available locally. Cocopeat and burned rice husk are commonly used as growing media for horticultural crops in Malaysia. These materials are suitable to be used as planting media for cut flower production (Taweesak et al., 2014). Planting density for plants with underground storage are depend on the size of storage organ, plant parts that are going to be harvested and the growing behaviour of the plant. High density planting induce competition of plants for space, light, water and nutrient while wider planting distance obtain lower yield per unit area (Chang, 1996; Hossain et al., 2005). A study has been conducted to investigate suitable soilless media with optimum planting density for curcuma flower and rhizome production in Malaysia.

Materials and Methods

The experiment was conducted in a rain-shelter in Ladang 2, UPM. Black polyethylene plastic trough size 1 m x 1 m x 0.2 m was constructed under the rain-shelter. Black nylon netting was constructed 2 m above the ground to provide 70% shade to the growing area. Rhizomes of *C. alismatifolia* were planted in three different mixtures of soilless media with three planting densities namely cocopeat: sand (2:1 by volume) (C), burned rice husk: sand (2:1 by volume) (B) and cocopeat: burned rice husk: sand (1:1:1 by volume) (M) at 9 plants/m^2, 16 plants/m^2 and 49 plants/m^2. Treatments were arranged in RCBD with 3 × 3 factorial and four replications. Data on total number of flowers and flower quality was collected throughout the flowering season. Two inflorescences with 3-5 opened true flowers were collected from each treatment and the diameter of the inflorescences was measured using a calliper. Dormant rhizomes were collected in the end of the growing season. Daughter rhizomes with diameter measured at least 1.5 cm and contained at least 3 storage roots were considered as marketable rhizomes. Data collected was analyzed with generalized linear model (GLM) using Statistical Analysis System (SAS) version 9.1 (SAS Institute Inc. Cary, NC, USA) and means were separated by Duncan's Multiple Range Test with significant level at $P<0.05$. Data for number of flowers and daughter rhizomes were subjected to arcsine transform before analyzed.

Results and Discussion

C. alismatifolia grown in soilless media M produced the largest flower (8.09 cm) and highest mean number of flowers/plant (2.85) (Table 1). The highest planting density (49 plants/m^2) produced the highest number of flowers/m^2 (62), tallest plant (102.71 cm) with longest flower stalk (67.92 cm) but had lower mean number of flowers/plant (1.28) and small flower size (7.00 cm) (Table 1). Low planting density (9 plants/m^2) produced the largest flower (8.25 cm) and highest mean number of flowers/plant (3.30) (Table 1). High planting density produced high number of flowers/m^2 but low flower quality and fewer flowers/plant while low planting density obtain higher quality flower but low yield per unit of production area (Chang, 1996).

Total rhizomes yield was not affected by different soilless media. However plants grown in media M produced highest number of marketable rhizomes/m^2 (54.58), highest number of marketable rhizomes/clump (2.95) and high number of storage roots/rhizome (8.92) (Table 2). Planting density at 49 plants/m^2 produced highest number of rhizomes/m^2 (124.17), highest number of marketable rhizomes/m^2 (64) and highest number of unmarketable rhizomes/m^2 (60.17) (Table 2). Total rhizome yield was not increased with reduction of planting density but higher number of low quality or unmarketable rhizomes were produced under high planting density (Hossain et al., 2005). The highest planting density (49 plants/m^2) produced lowest number of marketable rhizomes/plant (1.31), in contrast highest numbers of marketable rhizomes/plant were obtained from planting density at 16 plants/m^2 (2.95) and 9 plants/m^2 (3.12) (Table 2). High planting density reduced the new rhizomes formation and resulting low rhizome production/plant (Chang, 1996).

Cocopeat has high water holding capacity and is slightly acidic while burned rice husk is slightly alkaline (Taweesak et al., 2014). A balance mixture of cocopeat and burned rice husk are suitable to be used as soilless growing media for *C. alismatifolia* cut flower and rhizome production where more flowers and rhizomes were obtained when growing in this media. High planting density induces competition of plants and produced more low quality rhizomes and flowers (Hossain et al., 2005).

Table 1: Effects of types of planting media and planting density on plant height (cm), number of inflorescences/m^2, number of inflorescences/clump, flower stalk length (cm) and inflorescences size (cm) of *Curcuma alismatifolia.*

Treatment	Plant height (cm)	Number of inflorescences/m^2	Number of inflorescences/clump	Flower stalk length (cm)	Inflorescences size (cm)
Planting media					
Coco peat: sand (2:1)	91.53±2.62[a]	44.67±5.03[a]	2.36±0.21[b]	61.51±1.65[c]	7.58±0.25[b]
Burnt rice husk: sand (2:1)	95.62 ±3.03[a]	41.92±4.37[a]	2.29±0.20[b]	65.56 ±1.23[a]	7.39 ±0.17[b]
Coco peat: burnt rice husk: sand (1:1:1)	95.71±2.00[a]	52.25±3.86[a]	2.85±0.20[a]	64.10 ±1.29[b]	8.09 ±0.27[a]
F value	2.98	3.67	5.29*	5.79*	6.36*
Planting density					
9 plants/m^2	85.70±2.36[c]	29.67±4.29[c]	3.30±0.27[a]	60.61±0.81[b]	8.25±0.17[a]
16 plants/m^2	94.44±3.01[b]	46.75 ±1.73[b]	2.92±0.19[b]	62.65±1.69[b]	7.81±0.29[b]
49 plants/m^2	102.71±2.28[a]	62.42±7.24[a]	1.28±0.15[c]	67.92±1.67[a]	7.00±0.23[c]
F value	37.89*	34.63*	84.51*	19.62*	19.60*
Planting media × Planting density	0.70	0.28	0.50	0.43	0.86
CV (%)	5.08	10.64	9.05	4.63	6.46

*Data of number of inflorescences/m^2 and number of inflorescences/clump were square root transformed before analyze. F values followed by * are significant at P<0.05. Mean values followed by the same letter within a column are not significantly different at P≤0.05 as determined by Duncan's multiple range test.*

Table 2: Effects of types of planting media and planting density on number of rhizomes/m^2, number of marketable rhizomes/m^2, number of unmarketable rhizomes/m^2, number of marketable rhizomes/clump and number of storage roots/rhizome of *Curcuma alismatifolia.*

Treatment	Number of rhizomes/m^2	Number of marketable rhizomes/m^2	Number of unmarketable rhizomes/m^2	Number of marketable rhizomes/clump	Number of storage roots/rhizome
Planting media					
Coco peat: sand (2:1)	76.25±9.17[a]	42.33±4.96[b]	33.92±4.93[a]	2.12±0.23[b]	8.33±0.44[ab]
Burnt rice husk: sand (2:1)	81.83±12.76[a]	42.33±6.03[b]	35.90±8.20[a]	2.31±0.25[b]	7.58±0.54[b]
Coco peat: burnt rice husk: sand (1:1:1)	99.33±15.04[a]	54.59 ±5.5[a]	44.75±9.85[a]	2.95± 0.26[a]	8.92±0.47[a]
F value	2.80	4.06*	1.75	6.57*	5.09*
Planting density					
9 plants/m^2	49.92±5.78[c]	28.08±2.00[c]	21.83±4.30[c]	3.12±0.22[a]	8.71±0.62[a]
16 plants/m^2	83.33±9.41[b]	47.17±5.35[b]	36.17±5.20[b]	2.95±0.33[a]	8.08±0.45[a]
49 plants/m^2	124.17±21.78[a]	64.00±9.14[a]	60.17±13.49[a]	1.31±0.18[b]	8.04±0.38[a]
F value	24.69*	25.32*	20.24*	45.72*	1.49
Planting media × Planting density	0.55	0.55	0.56	1.07	1.93
CV (%)	4.63	13.75	19.37	11.62	6.31

*Data was square root transformed before analyze. F values followed by * are significant at P<0.05. Mean values followed by the same letter within a column are not significantly different at P≤0.05 as determined by Duncan's multiple range test.*

Conclusions

Soilless media of cocopeat: burned rice husk: sand (1:1:1 by volume) with planting density of 16 plants/m^2 is suggested for better flower and rhizome yield of *C. alismatifolia*.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Induction of *Labisia pumila* var. *Alata* (Kacip Fatimah) Adventitious Roots *in vitro*

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Introduction

Labisia pumila is from the genus Myrsinaceae. There are three different varieties of *L. pumila* found in Malaysia, namely: *L. pumila* var. *alata*, var. *pumila*, and var. *lanceoalata*. Each variety has its own use, but *L. pumila* var. *pumila* and *L. pumila* var. *alata* are commonly used for medicinal purposes. In Malaysia, *L. pumila* is popularly known as Kacip Fatimah. *L. pumila* is a sub-herbaceous plant with creeping stems and is mainly found in the lowland and hill forests in Southeast Asia, particularly Malaysia, Indonesia, Thailand, Laos, Cambodia and Vietnam (Farouk et al., 2008) at an altitude between 300 and 700 m (Zaizuhana et al., 2006). This plant has been utilized by many generations of Malay women for the purpose of inducing and facilitation of labor. It is also used as postpartum medication in the form of mixed preparation to help in the contraction of uterus. This plant is reported to delay fertility and help regain body strengths (Zakaria et al., 1994). Traditionally, Kacip Fatimah extract is prepared by boiling the roots, leaves or the whole plant in water, and the extract is consumed orally (Burkill, 1935). Kacip Fatimah products are easily available as there are many forms of *L. pumila* sold in the market such as drinks, pills, tablets and powder. Present problem faced by the herbal industry is insufficient supply of Kacip Fatimah raw material as availability of *L. pumila* in the forest is becoming scarce due to market demand, its slow growth rate in its natural habitat and logging activities in the forests (Farah Fazwa et al., 2013). To counter this issue, *in vitro* propagation of Kacip Fatimah had been studied. Furthermore, to increase the production process, induction of adventitious roots of *L. pumila* var. *alata* were studied.

One of the techniques used for mass propagation planting material is tissue culture. Tissue culture process consist of four main processes i.e. sterilization, multiplication, *in vitro* or *ex vitro* rooting and acclimatization. However, in this study, mass propagation through adventitious roots is discussed.

Figure 1: Kacip Fatimah tissue culture plantlets.

Figure 2: Kacip Fatimah adventitious roots.

Materials and Methods

Plant materials

Clean culture of *L. pumila* var. *alata* was obtained from FRIM Tissue Culture Laboratory (Figure 1). Leaf, stem and roots were divided and used (Figure 2) to induce adventitious roots.

Culture medium

Six different culture medium with three replicates were used in this study. These include Murashige and Skoog (MS), Woody Plant (WPM), Gamborg B5, ½ MS, ½ WPM and ½ B5 medium. These results will determine the root induction for Kacip Fatimah. Additional plant growth regulator 1-Naphthaleneacetic acid (NAA) at 1 mg/L was also used in this study.

Methods

Leaf, stem and roots from Kacip Fatimah plantlets were inoculated into each medium. About 0.05 g of inoculum/explants was used. Weight of each inoculated flask was recorded and three replicates were prepared for each samples and explants. All cultures were incubated under total darkness with continuous agitation at 100 rpm using rotary shaker. After three months in culture, the cultures were harvested. The roots fresh weight and dry weight were recorded.

Results and Discussion

After three months in culture, it was observed that root induction using root parts as explants showed the best result as compared to leaf and stem explants (Figure 3). Early observation results showed that, new roots started to grow after about two weeks in culture. After 3 months in different culture medium, the flask with root part as explants still produce the most adventitious roots (Figure 4). According to Ling et al. (2013), leaf explant was the best for root inductions as she only use leaf and stem as explant. But from this study, leaf explant was less efficient than root explant as the frequency and amount of roots induced were relatively low when compared with the root explants. Figure 3 showed the different of adventitious roots induced and produced by using different explant.

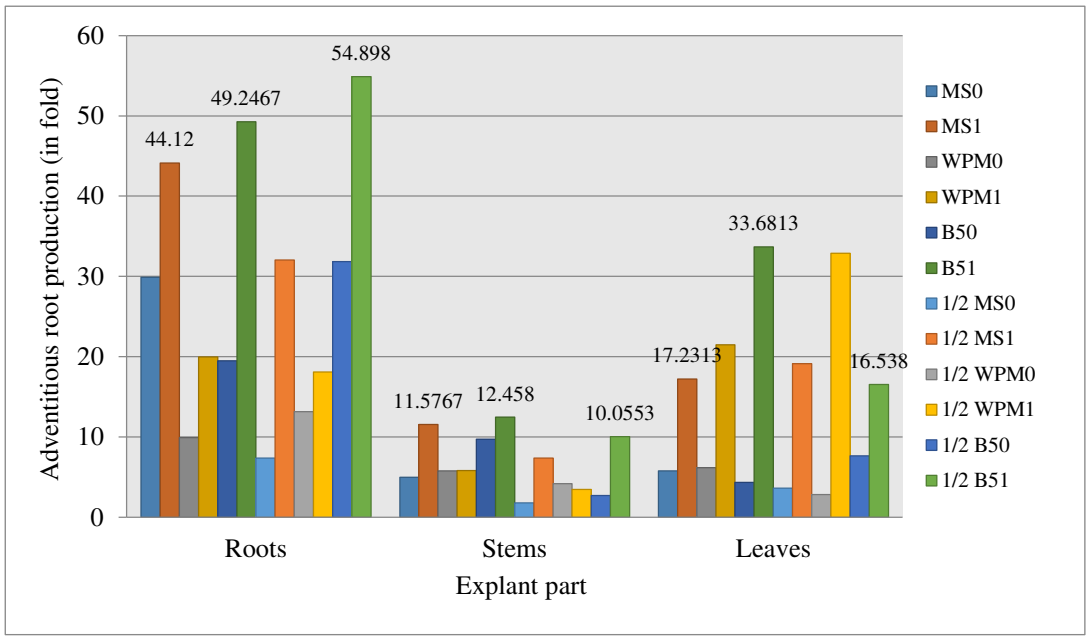

Figure 3: Production of Kacip Fatimah adventitious roots from root, stem and leaf explants.

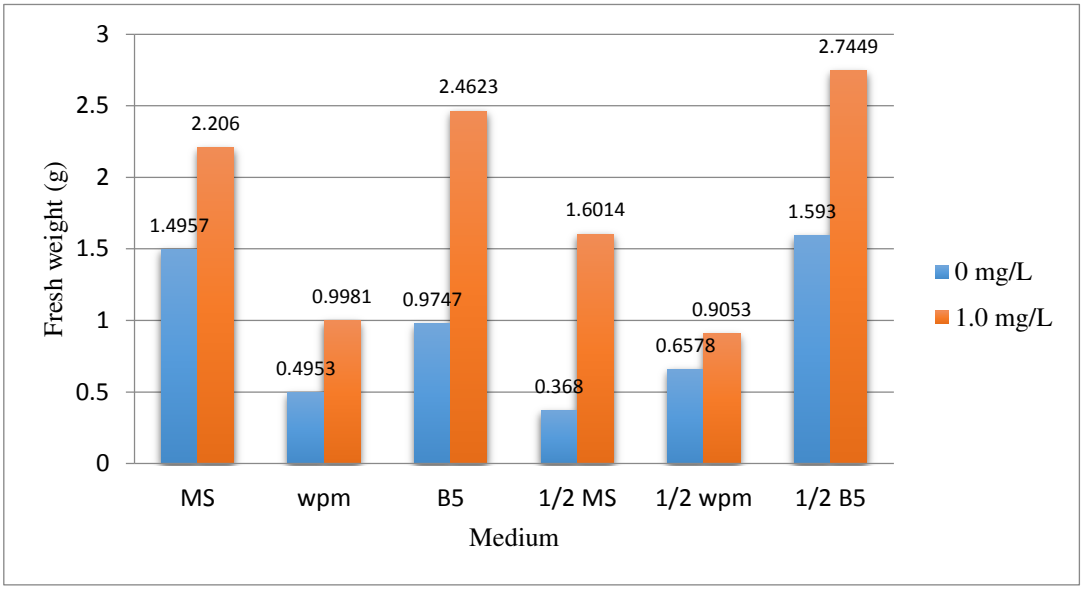

Figure 4: Kacip Fatimah adventitious root in different medium (Fresh weight (g)).

Based on the graph above (Figure 4), ½ B5 medium supplemented with 1 mg/L NAA showed the best growth with 54.8-fold of mean harvest from roots explant in 3 months' time followed by B5 supplemented with 1 mg/L NAA placed second with 49.2-fold of mean harvest and thirdly MS supplemented with 1 mg/L NAA with 44.1 fold of mean harvest. As for the culture using leaf and stem explant, B5 medium supplemented with 1 mg/L NAA produced the most adventitious roots as compared to other medium.

From these results, it showed that producing adventitious roots of kacip Fatimah is easier and economical by using root as explant.

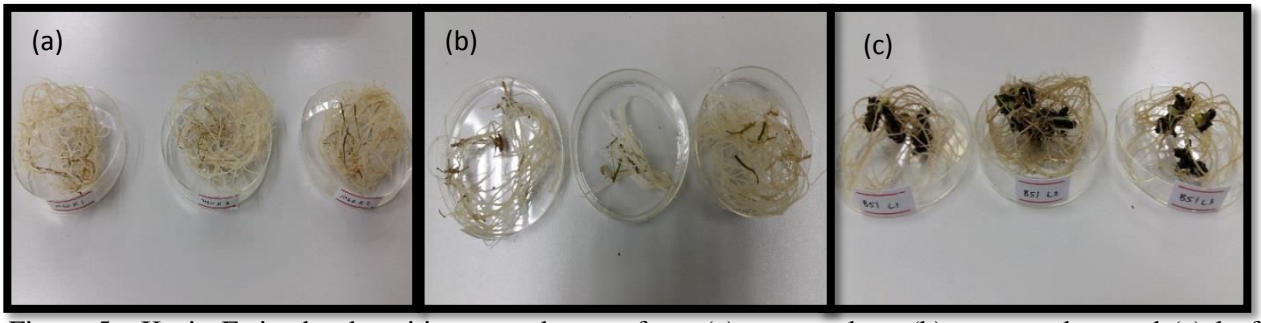

Figure 5: Kacip Fatimah adventitious root harvest from (a) root explant, (b) stem explant and (c) leaf explant.

Medium added with plant growth regulator showed positive result as each basal added with 1-Naphthaleneacetic acid (NAA) hormone produced greater results compared to the auxin free medium. Although, only 1 plant growth regulator which is NAA and only 1 concentration of NAA were tested. But the observation showing that by adding NAA, the roots produced at least 37% more for all the explants. This research finding is in contrast with Ling et al. (2013) where they found out that IBA in MS medium was found to be the most efficient PGR for root induction but it was not tested with B% or WPM medium. Out of the three basal medium, B5 showing the best results compared to others. More studies need to be done by using different auxins and different concentrations of plant growth regulators. As *in vitro* growth and shoot formation was not achieved without adequate concentrations exogenous hormones (Sasikumar et al., 2009). However, using inadequate or excessive amount of growth hormones can cause morphological and physioloical abnormalities (Bouza et al., 1994).

Conclusions

The study showed that all three types of explants which are root, leaf and stem can be used to induce adventitious root. But using root as explant is the best to induce *L. pumila* var. *alata* adventitious root compared to other explants. In term of medium, ½ B5 medium added with 1 mg/L of NAA produced the most adventitious roots for root explant while B5 + 1 mg/L NAA and MS + 1 mg/L NAA taken 2nd and 3rd place. As for leaf and stem explant, B5 medium supplemented with 1 mg/L NAA will be the best. Adding plant growth regulator (NAA) produced better result in inducing adventitious roots but further studies are required in order to confirm the best auxin and its concentration.

Acknowledgements

The authors are thankful to Forest Research Institute Malaysia, FRIM for providing the facilities and fund for this study. We would also like to express our gratitude to staffs of FRIM Tissue Culture Laboratory.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effect of Plant Growth Regulators on Cutting Propagation of Cempaka Putih (*Magnolia alba*) and Cempaka Kuning (*Magnolia champaca*)

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Introduction

Cempaka putih (*Magnolia alba*) and cempaka kuning (*Magnolia champaca*) are fragrant flowering trees with high ornamental values. Besides, they are also used as sources of timber, fragrance and traditional medicine across their range of occurrence (Xia et al., 2008). They are conventionally propagated by approach-grafting, which is labour-intensive and complex, because *M. alba* does not produce seed (Xia et al., 2008) while *M. champaca* produces morphophysiologically dormant seeds with long juvenile period (Fernando et al., 2013).

Therefore, investigation on cutting propagation method for both of the species is needed for sustainable utilisation of these two *Magnolia* species. In fact, many species of the *Magnolia* genus are important trees that have found many uses for human, while being rare and under-utilised (Cicuzza et al., 2007). As the propagation by stem cutting for tropical *Magnolia* is in general still difficult and poorly understood (Ranney and Gillooly, 2014), improvement on the cost-efficient and technically simple method can be beneficial for the utilisation, conservation and improvement of this important genetic resource.

Cutting propagation involves adventitious rooting of detached segment of plant which is controlled by many environmental and endogenous factors. Auxins, such as indole-3-butyric acid (IBA), are the essential plant growth regulators that regulate root formation in woody plants (Pop et al., 2011). Plant cells undergo dedifferentiation due to wounding and endogenous or exogenous auxin and become committed to form root meristemoids, which later develop into dome-shaped root primodia (De Klerk et al., 1999).

Among the classes of plant growth regulators, ethylene is another activator of adventitious root formation along with auxin (Pop et al., 2011). It is hypothesised that ethylene improve adventitious rooting by increasing the sensitivity of cells to auxin (Neto et al., 2009). Exogenous supplementation of ethylene through the application of ethephon solution has been shown to improve adventitious rooting caused by IBA application in *Pinus thunbergii* (Mori et al., 2011) and *Malus domestica* (de Klerk et al., 1999).

In separate experiments for each species, effect of the type of cutting and IBA concentrations are investigated on *M. champaca*, and the effect of ethephon and IBA concentrations are investigated on softwood cuttings of *M. alba*.

Materials and Methods

In the first experiment, softwood and semi-hardwood cuttings were used for *M. champaca*. The cuttings of 10 cm were taken from Faculty of Agriculture, UPM, all leaves cut into halves and cutting base treated with 0 (control), 6000, 12000, and 18000 mg/L IBA for 5 seconds. The cuttings were then planted in

rooting media of 1:1 perlite:vermiculite in RCBD arrangement and kept under 90% shade and intermittent misting system with 15 minutes of mist per hour for 14 hours a day during daytime. After 90 days, the cuttings were measured for rooting percentage, number of roots and length of roots.

In the second experiment, softwood cuttings of *M. alba* were taken from Faculty of Agriculture, UPM. All leaves on cuttings were cut into halves and cutting bases were treated either with IBA directly (control) or with 0.0001% (w/v) ethephon solution for 24 hours before treated with IBA. The cuttings were treated with IBA solutions of 0 (control), 3000, 6000 and 9000 mg/L concentrations for 5 seconds and then planted in the same rooting media and under the same intermittent misting system in RCBD arrangement. After 90 days, the cuttings were measured for rooting percentage, number of roots and length of roots.

Analysis of data was carried out with ANOVA (Analysis of Variance) using SAS 9.4 (SAS Institute Inc.).

Results and Discussion

The type of cuttings and IBA concentrations are both significantly factors for the rooting of *M. champaca* cuttings (Table 1). Most of the semi-hardwood cuttings did not root, probably due to lack of leaves to provide enough nutrients for further growth. On the other hand, softwood cutting is suitable for producing healthy rooted cuttings for *M. champaca*, which is expected for evergreen species (Ranney and Gillooly, 2014).

Previous studies have found that IBA alone is suitable for rooting stem cuttings of a few magnolia species, including *M. virginiana* (Griffin et al., 1999) and hybrids of *M. acuminata* (Sharma et al., 2006) . In this study, different IBA concentrations produced significantly different rooting percentages and length of roots. The highest rooting percentage is observed in softwood cuttings treated with 12000 mg/L IBA (66.7%), closely followed by those treated with 6000 mg/L IBA (60%), with the mean length of roots measuring 7.5 cm and 5.9 cm respectively.

Table 1: Effect of types of cutting and IBA concentrations on rooting percentage, number of roots and length of roots of *M. champaca*.

Treatment		Rooting percentage (%)	No. of roots	Length of roots (cm)
Type of cutting	IBA concentrations (mg/L)			
Softwood	0	6.7^{bc}	0.3^{a}	0.1^{b}
	6000	60.0^{ab}	5.9^{a}	5.9^{a}
	12000	66.7^{a}	4.4^{a}	7.5^{a}
	18000	13.3^{abc}	3.5^{a}	1.5^{b}
Semi-hardwood	0	0.0^{c}	0^{a}	0^{b}
	6000	0.0^{c}	0^{a}	0^{b}
	12000	6.7^{bc}	0.7^{a}	0.2^{b}
	18000	0.0^{c}	0^{a}	0^{b}

Means with the same letter (within a column) are not significantly different at α>0.05 according to Tukey's test.

For *M. alba* cuttings, ethephon treatment and IBA concentrations did not produce significantly different results for rooting percentage, number of roots and length of roots according to Tukey's tests (Table 2). However, the highest rooting percentage of 40% have occurred on cuttings treated with 9000 mg/L

regardless if they are pretreated with ethephon or not, suggesting that higher concentrations of IBA may produce better rooting percentage.

While not a significant factor statistically, it can be observed in this study that combination treatment of ethephon and IBA produced slightly more roots than just IBA, while ethephon alone does not have positive effect on number of root, which is in agreement with the hypothesis that ethylene increases the number of cells responsive to auxin for root initiation (Neto et al., 2009). Considering the low number of roots was produced in *M. alba* cuttings, it might also be beneficial to further optimise the ethephon level to increase the effect on number of roots.

Table 2: Effect of ethephon and IBA concentrations on rooting percentage, number of roots and length of roots of *M. alba.*

Treatment		Rooting percentage (%)	No. of roots	Length of root (cm)
Ethephon Treatment	IBA concentrations (mg/L)			
0-day	0	20.0[a]	0.8[a]	4.2[a]
	3000	16.0[a]	0.6[a]	5.5[a]
	6000	16.0[a]	0.6[a]	3.8[a]
	9000	40.0[a]	1.4[a]	8.7[a]
1-day	0	12.0[a]	0.4[a]	3.4[a]
	3000	28.0[a]	0.9[a]	6.6[a]
	6000	16.0[a]	0.8[a]	4.0[a]
	9000	40.0[a]	2.2[a]	7.5[a]

Means with the same letter (within a column) are not significantly different at α>0.05 according to Tukey's test.

Conclusions

In this study, cempaka kuning and cempaka putih have been successfully propagated by the cutting method which is less labour-intensive compared to the conventional methods. This improvement can benefit the production of planting materials of *Magnolia*.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Viability and Vigour of Mango (*Mangifera indica* L.) var. Chokanan Scionwood as Affected by Different Storage Media and Temperature

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Introduction

Planting material of Chokanan mango is produced by grafting. Wedge grafting is by far the most common technique due to its high success rate, ease of use and is less time-consuming. Often problem arises when the source of scionwood is located far from the nursery and there is a need to transport and store the scionwood. Under normal circumstances, harvested scionwood have to be used immediately due to loss of viability over time. Scionwood storage technology has evolved with the advent of affordable, electric-powered refrigeration and polyethylene-plastic films and bags (Monte, et al., 2002). One of the main factors in maintaining viability of scions during storage is by reducing water loss from the stored scionwood. This is done by placing the scions in a proper wrapping material and kept moist by media that can hold water efficiently. Polyethylene plastic sheeting is a good material for wrapping scionwood as it allows respiration process and at the same time retards the passage of water vapour (Hartmann et al., 2013). The other essential factor in storing the scionwood successfully is the storage temperature. Retaining the dormancy of the stored scions is crucial as using scionwood that have begun to grow while in cold storage will result in grafting failure (Warmund and Starbuck, 2009). Taking into account the above two factors, this study was conducted to determine the effects of different storage media and storage temperature on the viability and vigour of Chokanan mango scionwood.

Materials and Methods

Preparation of scionwood

Collection of scionwoods was done in the morning during 11 to 13th April 2014 at the 10 acres field plot of MARDI Serdang. Healthy terminal shoots, 1-2 months after vegetative flushing were selected from the stockplants aged around 4 years old. The scionwoods were defoliated and standardized to a length of 10-15 cm. The basal ends were sealed with an asphalt based sealant to prevent desiccation.

Storage treatments

Scionwoods were placed in clear, perforated polyethylene zip lock bags with dimension of 25 cm x 15 cm and 1.5 mils thick. The plastic bags were then filled with different fully moistened media; sphagnum moss (SM), sawdust (SD), perlite (P) and subsequently stored for 30 days at different temperatures; 5, 15, 27ºC. Storage of scionwoods in moist paper, placed in semi dark, humid place served as the control treatment.

Determination of viability and vigour of scionwood

After 30 days of storage, the scionwoods were acclimatized to room temperature before being grafted onto 6 month old rootstocks raised from seedlings of *Mangifera indica* var. Telur. The plants were placed under 50% shade provided by black netting and watered twice a day. After 90 days of grafting, based on growth

and flushing cycles (Figure 1), viability and vigor of the stored scionwoods were determined by scoring 0, 1 and 2 with description as follows:
Score 0 : Scionwood not viable, no growth of scion shoots
Score 1 : Scionwood viable, medium vigour with 1 flush
Score 2 : Scionwood viable, high vigour with 2 flushes

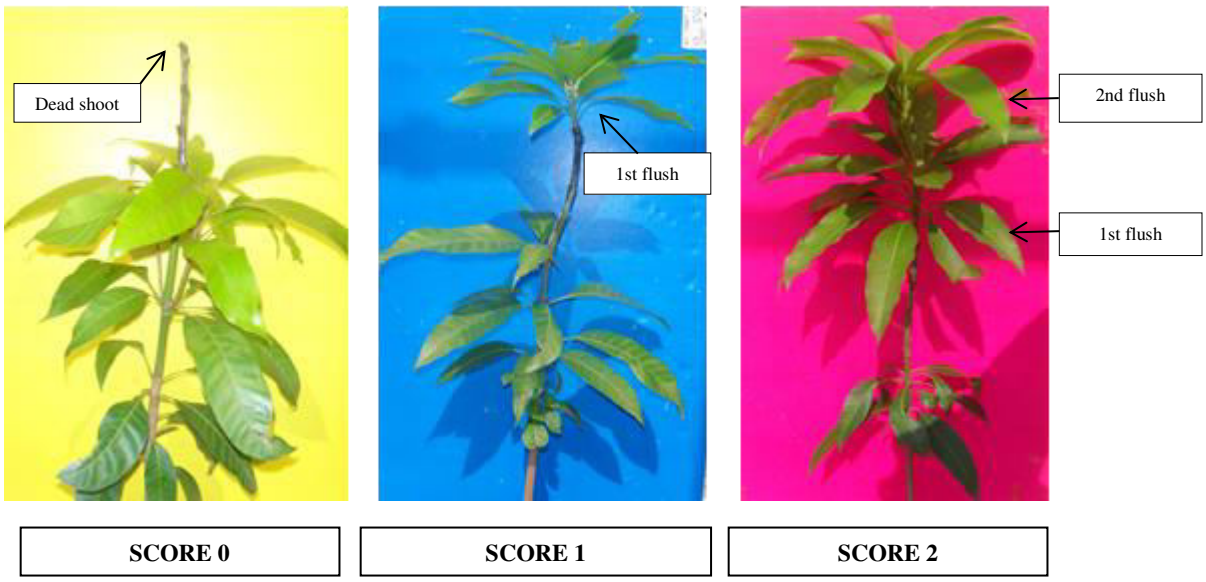

Figure 1: Scoring of scionwood growth based on flushing cycles.

Data collection and analysis

The experiments were arranged in Completely Randomized Design (CRD) with nine treatments (Table 1), four replications of each treatment and five subsamples. Viability percentage (a summation of sample scored 1 and 2) as well as individual percentage of score 0, 1 and 2 were calculated for each treatment and subjected to one-way Analysis of Variance (ANOVA). Differences among treatments were compared using Least Significant Difference (LSD).

Results and Discussion

Scionwood viability

Results show that 100% of the fresh scionwood (0 days of storage) were viable as evidenced by successful graft-take and subsequent growth after 30 days of storage. As for the control, shoots were scored 0, indicating dead scionwood during storage. Perlite at all three levels of storage temperature; 5, 15 and 27ºC, were able to maintain shoot viability with viability percentage of 85, 95 and 100%, respectively. Viability of stored scionwood using other treatments was above 70% except for treatment combination of T7 which is 25% (Table 1).

Table 1: Viability (%) of scionwood stored using different treatment combinations; P = Perlite, S.M = Sphagnum moss, S.D = Sawdust

Treatment		Viability (%)
Fresh scionwood		100
T0	Control	0
T1	P+5	85
T2	P+15	95
T3	P+27	100
T4	S.M+5	70
T5	S.M+15	100
T6	S.M+27	95
T7	S.D+5	25
T8	S.D+15	70
T9	S.D+27	90

Scionwood vigour

In terms of vigour, 95% of the shoots from the fresh scionwoods were scored 2 and the rest was scored 1. For percentage of scored 2, there was no significant difference (P<0.05) between scionwood stored using perlite and fresh scionwood. This means that vigour of scionwood stored using perlite in all studied temperature can be maintained after 30 days of storage without significantly affecting its quality. For the rest of treatment combinations, percentage of shoots that were scored 2 was significantly different (P<0.05) (between 10-45%) compared to fresh scionwood.

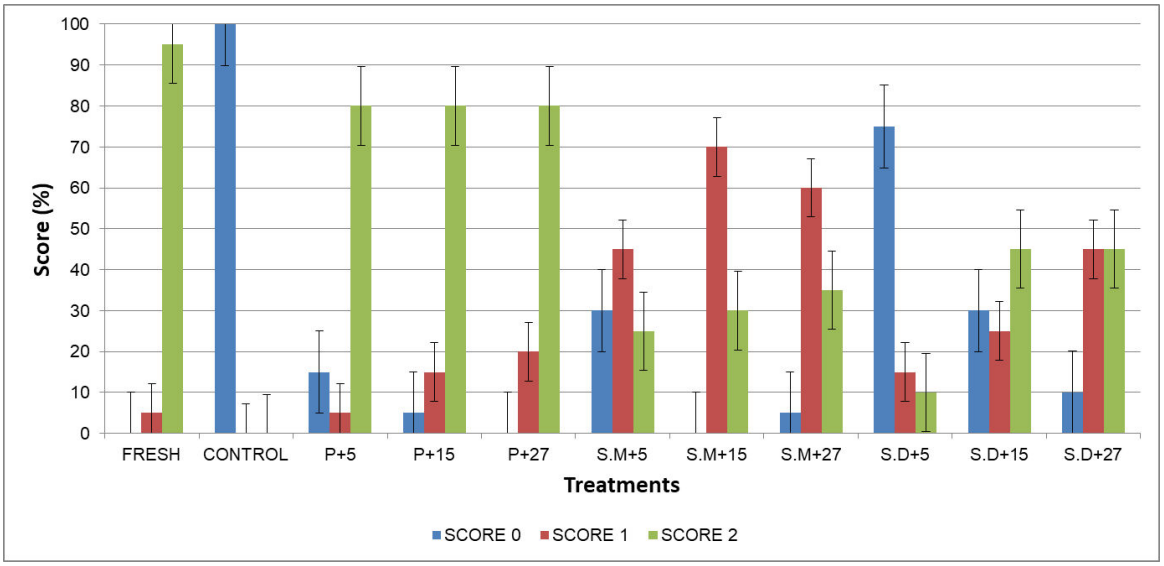

Figure 1: Percentage of score for shoot growth of scionwood stored 30 days using different treatment combinations (p<0.05).

Effects of media on scionwood viability and vigour

In this study, it was observed that perlite was able to maintain viability and vigour of stored scionwood compared to other storage media. Deterioration of stored scionwood is because of dehydration that occurred during storage. When scionwood is dehydrated, biochemical activities in the cells cannot be

carried out. Subsequently, callus regeneration will be retarded and formation of cambial cells, phloem and xylem will be affected (Hartmann et. al., 2013). Thus, there will be no translocation of nutrients and water from the rootstock to the scionwood and the scionwood will eventually die. This means that moist perlite was able to minimize water loss in scionwood during storage. Perlite is a unique volcanic material, comprised of tiny closed air cells or bubbles which provide extremely large surface area. The surface of each particle is covered with tiny cavities that trap moisture efficiently (275 m/L) without getting soggy (Hochmuth and Hochmuth, 2013). These characteristics are essential in ensuring the scionwoods did not dehydrate during storage.

Effects of temperature on scionwood viability and vigour

In terms of storage temperature, 27ºC were able to retain vigour of stored scionwood compared to other temperatures. Salveit and Morris (1990) reported that many plants indigenous to the tropics and subtropics suffered chilling injury upon exposure to non-freezing temperatures below 12°C. Loss of viability and vigour of scionwood stored at 5 and 15ºC can be linked to chilling injury that leads to several physiological deformities such as leakage of ions, decrease in activity of a number of enzymes and increases of metabolites (Atwell et al., 1999). Presumably, these physiological changes which occurred in the scionwood affected graft-take and resulting in not viable scionwood.

Conclusions

Without proper storage, viability and vigour of Chokanan mango scionwood would be lost after 30 days of storage. Combination of perlite and 27ºC storage temperature can maintain viability and vigour of Chokanan mango scionwood after 30 days of storage.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Assessing Economic Benefits of Pollination Services by Natural Pollinators

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Introduction

In natural ecosystem, insect pollinate more than 50% of tropical forest and thus play a major role in maintaining and conserving biodiversity. In agricultural ecosystem, many agricultural crops are dependent on insects for their pollination (Klein et al., 2007). Crops and other cultivars benefit from pollination services of nearby forest or other natural habitats, which provide forage and nesting space for pollinators (Roubik, 1995; Kremen et al., 2002, 2004; Klein et al., 2003, De Marco and Coelho, 2004; Ricketts, 2004). Bees and other pollinating animals supply a valuable input to agricultural production that can increase both the size and quality of harvests (Allen-Wardell et al., 1998). Many plants have evolved intricate relationship with many insect pollinators, without which they would not reproduce and maintain their genetic diversity (Daily et al., 1997). In consequence, natural forest ecosystem plays an important role for pollination services.

Malaysia is rich with native pollinator such as bees, stingless bees and bats. Some of these species could be used to pollinate several agricultural crops such as starfruits, guava, citrus, mango, watermelon, durian and coconut. However, the pollinator numbers are declining and causing global concern for pollination services (Kearns et al., 1998). This could lead to threaten food security and biodiversity (FAO, 2007). In 2000, the Convention of Biological Diversity established an International Initiative for the Conservation and Sustainable use of Pollinators, which aims to promote sustainable use of pollinator diversity in agriculture and related ecosystem.

The objective of this study was to assess the economic benefits of pollination services provided by the natural pollinators in nearby forest to four types of crops which are starfruits, guava, durian and watermelon.

Materials and Methods

The study was conducted in Pahang, one of the states in Peninsular Malaysia. Data on crops production were obtained from the Department of Agriculture (DOA) of Pahang. The data comprised the information of 1,782 farms distributed at all districts in Pahang (Figure 1). Information provided were area planted and harvested (ha), quantity harvested (kg) and production value for year 2014. The information on crops production data provided by the DOA excluded the Taman Kekal Pengeluaran Makanan (TKPM). This paper only concentrated on four types of crops, namely starfruit, guava, watermelon and durian. As these crops were observed could be pollinated by natural pollinators such as stingless bees (Slaa et al., 2006).

Here, we used a bioeconomic approach, which integrated the production dependence ratio on pollinators. Dependence ratio is the level of dependence on pollination for the production of crop or seed. The

estimation of the potential value of pollination services was calculated by multiplying production value of each crop with its pollinator dependence ratio (Gallai et al., 2009) as Equation 1.

Pollination economic value = production value X pollinator dependence ratio -------- (Equation 1)

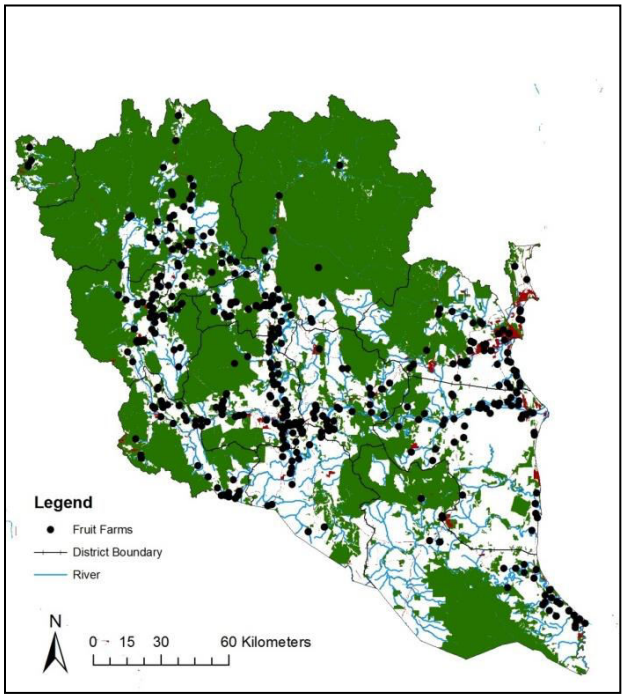

Figure 1: Distribution of crop farms in Pahang.

Some of the pollinator dependence ratio as reported by Norowi et al. (2010) listed in Table 1. Here, we used pollinator dependence ratio published for starfruits, guava, durian and watermelon then multiply with their production values.

Table 1: Pollinator dependence ratio for selected crops in Malaysia (Norowi et al., 2010).

Commodities	Pollinator dependence ratio (USD)
Starfruits	0.65
Guava	0.65
Citrus (Mandarin)	0.05
Mango	0.65
Watermelon	0.96
Durian	0.65
Coconut	0.25

Results and Discussion

A total of 1,782 farms (starfruit, durian, watermelon and guava) with the total area of 2,489.25 ha had been assessed in this study (Table 2). Durian was the most planted crop in Pahang followed by watermelon. The total production were accounted 16,803 kg, with watermelon production was the highest among the four crops assessed (Figure 2). The total production values based on DOA data were RM 37,411.20.

Table 2: Data collected from Department of Agriculture, Pahang.

Crop	Production area (ha)	Quantity harvested (kg)	Production value (RM)
Starfruits	1.21	1450	16,500.00
Guava	1.2	2613	3,761.20
Watermelon	611	10360	14,640.00
Durian	2470	2500	2,510.00

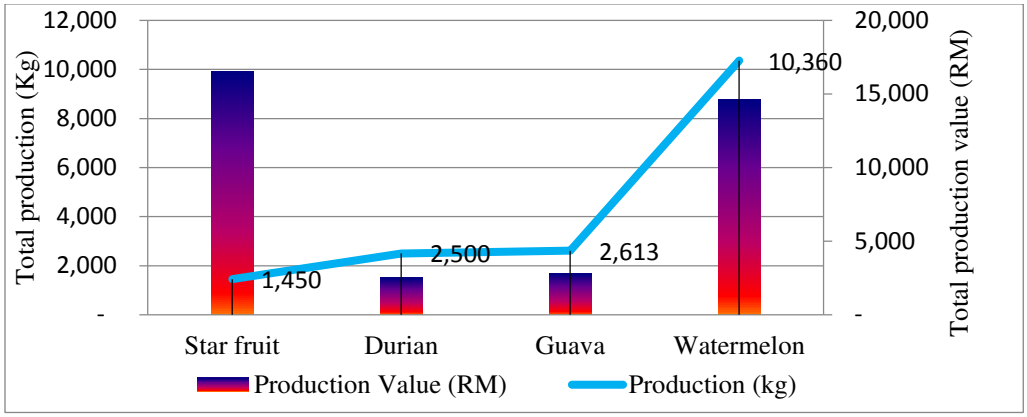

Figure 2: Total production (kg) and production values (RM) for starfruit, durian, guava and watermelon.

Table 3 shows the results on the potential pollination economic value of the crops calculated according to Equation 1. Total pollination economic value was RM28,709.28. This value accounted about 77% of the actual production value of crops. Watermelon pollination estimates values were the highest, with highest dependence ratio which imply that this crops is highly dependence on natural pollinators for their productivity.

Table 3: Pollination economic value.

Crop	Production value (RM)	Pollinator dependence ratio	Pollinator economic values (RM)
Starfruits	16,500.00	0.65	10,725.00
Guava	3,761.20	0.65	2,444.78
Watermelon	14,640.00	0.95	13,908.00
Durian	2,510.00	0.65	1,631.50
Total	37,411.20		28,709.28

This result shows the important impact of pollinator to crops production. The actual values of pollination service will be higher if the services of pollination for other agricultural crops are considered (Samejima et al., 2004). With conservation of the natural pollinators, would help to achieve pollination services thus will benefits most of the agricultural crops.

Conclusions

Economic value of pollination services for four crops estimates about 77% of the total crop's production value. It indicated potential pollination services to some agricultural crops in Malaysia. If other crops are being assessed, the economic value of pollination might be higher. As the recommendation, determination of pollination values should be based on ecological and economic approach to avoid over and

underestimation. To be better valuation on pollination services, the economic approach have to take into consideration in the analysis. These findings only give brief scenario of the impact of pollinators to the production in physically.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effect of Government Intervention on the Production of Paddy in Peninsular Malaysia

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Introduction

The launching of the Agro Food Policy in 1984 allowed the agriculture sector in this country to grow from a low productivity sector to a competitive sector which increases production. Besides, the food commodity is the main focus in order to solve the food supply issue in Malaysia (DOA, 2015). The Ministry of Domestic Trade, Co-operatives and Consumerism has specified rice as one of the unlisted product and control item. In Malaysia, there are two categories of paddy farming: granary and non-granary areas. Granary area refers to the huge irrigation schemes which are more than 4,000 acres and which are documented in the National Agricultural Policy as the primary area for rice production. There are eight granary areas in Malaysia namely MADA, KADA, IADA Kerian Sungai Manik, IADA Barat Laut Selangor, IADA Penang, IADA Seberang Perak, IADA Kemasin Semerak and IADA KETARA. They manage relevant activities in the paddy industry.

The government has provided extensive incentives to escalate paddy productivity after food crisis in 2008. These efforts are also intended to improve the living standard of the rice farmers. Some of the programs are channeled by the government including credit facilities, fertiliser subsidies, irrigation investment, guaranteed minimum price, income support action, subsided retail price as well as research and extension support (training and advisory). In spite of great fiscal outlays for this constituency, rice production is still constantly insufficient to fulfill the market and population needs (Norsida and Sami, 2009). In order to escalate the production of rice in granary and non-granary areas, the infrastructure for irrigation and adequate dominate will need to be improved. Other factors linked to the success in rice production are the use of high quality seeds and high-yielding rice, good practice of farming technologies, and efficient agronomic management (MOA, 2011).

This research was conducted to determine the effectiveness of government policy on the production of paddy in the selected granary areas via the application of Policy Analysis Matrix (PAM). There are three central issues of agricultural policy analysis which can be addressed using PAM results. The first issue is the effectiveness of agricultural systems under existing technologies and prices. The second issue is how the impact of new public investment in infrastructure affects the efficiency of agricultural systems. Lastly considered is the impact of new public investment in agricultural research and technology on the efficiency of agricultural systems (Monke and Pearson, 1989). The prime objective of creating PAM is to determine a few significant policy parameters for policy analysis. Some of the most generally used parameters are nominal protection coefficient input (NPCI), nominal protection coefficient output (NPCO), and effective protection coefficient (EPC) (Khai, 2012).

Materials and Methods

Study area and data sampling

This study will determine the impact of government intervention on the paddy farming production in four granary areas in Peninsular Malaysia: MADA, KADA, IADA Penang and IADA KETARA. Primary data is used as source in this study. The data was collected for the 2013 cropping season. The farmers interviewed were selected randomly from all paddy farmers from each granary area. A total of 5% of farmers involved were from each granary. For this purpose, the non-probability sampling method, which is a purposive sampling technique, has been applied. This method is suitable for non-probability approach as it is fitting for an in-depth interview of the respondents (Given, 2008). The detailed cost of paddy production can be obtained by the in-depth interview as it is appropriate method. The interview questions include output and input information and some major socio-economic characteristic of the farmers, as well as input prices.

PAM framework as an indicator of government intervention

According to the PAM model, the farm budget is divided into four categories. The categories are revenue, cost of tradable and inputs, cost of domestic factors and profits, where profits are the differences between the revenues and the last category of total input costs (Ogbe et al., 2011). Another important component of the analysis is the separation of the input into tradable and non- tradable. Fertilizers, seeds, fungicides, pesticides, insecticides, herbicides are counted in tradable inputs whilst the non-tradable inputs include irrigation fees, land, labor, tractor and sprayer service, capital, and simple farm tools and implements.

The first row of the matrix includes private prices. This represents production costs and revenues in terms of market prices faced by the farmer. Hence, in private prices, the formula to calculate the profits express in term of market prices subtracts the two cost categories (B and C) from revenues (A). The second row of the PAM represents the costs and revenue of farmers, as they are dealing the prices that would emerge from a lack of government policies or market failures.

Table 1: Standard format of PAM.

	Revenue	Tradable input cost	Domestic factor cost	Profit
Private Prices	A	B	C	D
Social Prices	E	F	G	H
Divergences	I	J	K	L

Source: Monke and Pearson (1989).

Ratio indicators for comparison of unlike outputs are:
Nominal Protection Coefficient on Tradable Output (NPC) = A/E
Nominal Protection Coefficient on Tradable Input (NPC) = B/F
Effective Protection Coefficient (EPC) = (A–B)/ (E –F)

Actually, the relationship under the rows of the matrix includes divergence. Divergences make private prices resolve from their social counterparts. By distorting policy intervenes that cause a private market price to diverge from an efficient price or by underlying market forces which have failed to provide an efficient price result the divergence to escalate. There are a few indicators involved to estimate the effectiveness of government intervention on the paddy industry, which are Nominal Protection Coefficient of Output, Nominal Protection Coefficient of Tradable Inputs and Effective Protection Coefficient.

Nominal protection coefficient of output (NPCO)

NPCO measures the difference between the domestic and social cost. It is the domestic market price of a commodity, divided by national opportunity cost in trade.

$$\text{NPCO} = \frac{\text{Revenue in Private Prices (A)}}{\text{Revenue in Social Prices (E)}}$$

When the value is more than one, the NPCO signifies that the private (domestic) price of output is greater than its parity price. Hence, producers are protected for the products and consumers are taxed. On the other hand, when the NPCO is lower than one, the producers are implicitly taxed on the product, either due to market failure of government intervention or/and consumers are subsidized. This makes producers face a negative incentive effect.

Nominal protection coefficient of tradable inputs (NPCI)

NPCI refers to the ratio of the financial values to the social values of the tradable inputs used. If NPCI is greater than one, this suggests that the producers are taxed when they buy tradable input. The domestic input cost is higher than what they cost in terms of global price. If the NPCI is lower than one, tradable inputs are subsidized. The world price equivalent is higher than the domestic input price.

$$\text{NPCI} = \frac{\text{Cost of Tradable Inputs in Private Prices}}{\text{Cost of Tradable Inputs in Social Prices}}$$

Effective protection coefficient (EPC)

According to Sangawongse et al. (1999) Effective Protection Coefficient (EPC) is calculated as the ratio of value added in the private price to value added in social prices. It shows the combined effects of policies in the tradable commodities markets. An EPC greater than one shows a positive incentive effect on commodity policy, resulting in the subsidization of farmers. Farmers are taxed if an EPC is less than one.

$$\text{EPC} = \frac{\text{(Private Revenue - Private Tradable Inputs)}}{\text{(Social Revenue – Social Tradable Inputs)}}$$

Results and Discussion

Characteristic of farm input and output variable

The average area of farmer's paddy field in KADA is 8.25 hectare, while the average area is 2.7 hectare in IADA KETARA, 2.9 hectare in MADA, and 4.5 hectare in IADA Penang. On average, the widest cultivated area was operated by farmers in KADA, followed by farmers in IADA Penang, MADA and finally IADA KETARA. Table 2 shows the highest production costs spent for paying the workers and the cost of rent and land tax. For MADA, RM1068.00 is allocated to pay rent and land taxes, followed by RM1002.00 to pay workers. Expenses for seeds were recorded in third place with RM524.00. For IADA Penang, RM1346 was allocated to pay workers' wages, RM790 to pay rent and land taxes, and RM652.00 to purchase rice seeds. On average, the farmers at KADA had allocated RM 477.00 for labour costs, and to pay rent, land taxes, and harvest costs, and RM292.00 to buy seeds. For IADA KETARA, RM494.00 was used to pay rent and land taxes and was spent on harvesting cost and RM342.00 was used to pay workers.

A majority of the farmers in IADA KETARA work on the farm on their own and are assisted by their family members at times.

On the use of seeds for planting activities, it is shown that the farmers in MADA used the fewest seeds at 131.62 kg per hectare as compared to IADA Penang where the farmers used an average of 286.69 kg per hectare, which puts IADA Penang as the highest amount used amongst the four granary areas. Sometimes, problems with pest such as *Pomacea canaliculata* and *Pomacea incularum*, more commonly known as '*siput gondang*', caused the farmers to use seeds at three times the amount recommended. It was also found that farmers in KADA used at least 5 kg more seeds than farmers in MADA. In terms of fertiliser usage, the farmers in MADA only used 474.69 kg per hectare of fertiliser, while the highest usage of fertilizer was recorded by farmers in IADA Penang with a total usage of 504.44 kg fertilizer per hectare.

Herbicides, insecticides and fungicides are the pesticides used in this study. Farmers in MADA are noted to apply the lowest amount of pesticide to paddy farms at only 1.44 litres per hectare, followed by KADA with 1.65 litres per hectare. IADA Penang is placed as the third highest usage of pesticides with 2.59 litres per hectare and IADA KETARA recorded the highest usage of pesticides at as much as 9.29 litres per hectare. Fuel is one of the crucial inputs in paddy production. In this study, fuel was noted to be used in water pump generators, fertilization, pest control and weed control. MADA farmers had the highest fuel consumption of 44.46 litres per hectare followed by IADA Penang, KADA and lastly by IADA KETARA where the farmers used only 18.90 litres of fuel per hectare. The highest used of labour force was in KADA at 62.17 hours/ha followed by IADA KETARA, MADA and IADA Penang.

Table 2: Statistic of farm input and output.

	MADA	IADA Penang	KADA	IADA KETARA
Size (ha/farmer)	2.9	4.5	8.25	2.7
Seed/ha (kg)	131.62	102.79	136.8	152.13
Seed/ha (RM)	524	652	292	267
Fertilizer/ha (kg)	474.69	504.44	477.29	486.74
Fertilizer/ha (RM)	16	132	32	76
Pesticides/ha (Liter)	1.44	2.59	1.65	9.29
Pesticides/ha (RM)	74	200	112	43
Fuel/ha (Liter)	44.46	43.02	41.57	18.9
Fuel/ha (RM)	82	77	76	36
Workforce/ha (hour)	54.64	40.1	62.17	56.97
Workforce/ha (RM)	1002	1346	477	342
Transportation (RM)	111	149	32	110
Harvesting cost (RM)	364	489	390	417
Tax and rent (RM)	1068	790	353	494
Total cost (RM)	3241	3835	1764	1785
Mean yield (mt/ha)	5	4.8	3.6	3.7
Mean income/ha (RM)	6711	6821	3923	4655

MADA had the highest yield of paddy production and for uses of input. Despite fuel consumption, MADA also demonstrated the lowest overall input application. However, this number is merely a descriptive analysis. In order to compare the competitiveness of every granary, a specified analysis must be conducted

as the analysis of competitiveness will cover many angles of evaluation and is expected to prepare more reliable result. Based on Table 2, the production of paddies in MADA recorded the top value at 5.00 mt/ha, followed by IADA Penang with a mean production of about 4.8 mt/ha. IADA KETARA ranked in third with a production of 3.7 mt/ha and KADA had the lowest paddy production at 3.6 mt/ha. The mean of paddy production for four granary areas studied was 4.3 metric tonnes per hectare. This amount is rather higher than the production of Thai Rice in year 2010 as Kallika et al. (2010) noted in his research that mean paddy production for Thai Rice was 4.261 metric tonnes in 2010. The result for the calculation of the farmer's gross income in monetary terms stated the highest average income was RM6821.00 as obtained by farmers in IADA Penang, followed by MADA's farmers with the average income of RM6711.00 per hectare. Compared to the farmers in the West Coast, the income of farmers in the East Coast area are much lower as the average income is only at RM3810.00 for farmers at KADA while farmers in the IADA KETARA have the average income of RM4655.00 per hectare. As different states hold different selling price which influences the gross income. For MADA, the rice price is RM1099.28 per ton and for IADA Penang its RM1137.50. Meanwhile, the selling price for IADA KETARA is RM1014.27 and KADA has the lowest selling price with only RM806.80 per ton.

Analysis of government policy effect on paddy producers

This section will discuss about the effects of government protection on paddy production by using the indicators of protection for both output and input. Table 3 shows a summary of the results on the Protection Coefficient on paddy in four areas.

Table 3: Protection coefficients for four granary areas in Peninsular Malaysia.

	KADA	KETARA	MADA	IADA PENANG
NPCO	0.64	0.938	1.004	1.044
NPCI	1.147	1.146	1.146	1.147
EPC	0.587	0.909	0.989	1.027

KADA and IADA KETARA had a NPCO of less than 1, showing that the farmers in these granaries are taxed on paddy production. MADA and IADA Penang showed results greater than 1. This result means that the private price of output is greater than its parity price and therefore the farmers in MADA and IADA Penang are positively covered in paddy production. However, IADA KETARA showed a value closer to 1 at 0.938. Compared to NPCO, the NPCI for every area was more than 1 with KADA and IADA Penang has NCPI recorded at 1.147 which is similar to MADA and IADA KETARA with NCPI recorded at 1.146. These values indicate that all paddy producers in every granary area are taxed on buying tradable inputs. It is noted that government policies increase input cost for paddy production through various taxation levels.

Table 3 also shows the result for EPC. Based on the table, only IADA Penang showed results greater than 1 at 1.03, while MADA and IADA KETARA showed results close to 1 at 0.99 and 0.91, respectively. The lowest value was presented by KADA at only 0.59. The EPC result shows only the farmers in IADA Penang are covered under positive incentives while other farmers are not protected through policy interventions taxed. The production of paddies in MADA, IADA KETARA and KADA is not protected and is taxed at 1% for MADA, 9% for IADA KETARA, and 41% for KADA. In point of fact, the EPC is a more consistent indicator of effective incentives than NPC. This is because EPC recognizes the full impact of a set of policies which involve both output price enhancing (import tariffs) and cost reducing (input subsidies) effects. The EPC not only captured out the impact of protection on input and output, but also revealed the degree of protection accorded to the value-added process in the production activity of the

relevant commodity. Kanjunt et al. (1999) in a study of economic analysis of Karen farming system in the Mae Chaem watershed, noted different results where the value of EPC for paddy rice and upland rice was greater than 1, resulting in a net subsidy for producers.

Conclusions

This study on the effects of government protection on paddy production showed that an EPC value stated in this research are less than one for every granary area except IADA Penang. These results indicate that government policies provide negative incentives for paddy producers in MADA, KADA and IADA KETARA, while a value greater than one for IADA Penang indicates that the farmers in this area are protected through policy intervention in the paddy production. IADA KETARA proved to be the best granary area overall. For government intervention based on PAM analysis for indicators of comparative advantage, in terms of NPCO and EPC, IADA Penang was the best granary, while in terms of NPCI, IADA KETARA was noted as the best.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Chapter 3

Seed Technology and High Quality Planting Material

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Propagation of Kuini (*Mangifera odorata*) by Different Grafting Techniques

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Introduction

Kuini (*Mangifera odorata*) is a mango variety belongs to Anacardiaceae family that is native to South East Asia (Kiew, 2002). It is found in Malaysia, Indonesia, Thailand, Philippines and Vietnam (Orwa et al., 2009). *Mangifera odorata* possibly represents hybrid form between *M. indica* and *M. foetida* (Teo et al., 2002; Kiew, 2002). *Mangifera odorata* is a medium-sized tree, 10-15 m, rarely exceeding 20 m height; globose or broadly ovoid crown; straight bole, grey bark, and containing irritant sap (Orwa et al., 2009). In West Java, kuini is a popular fruit, having local economic significance in areas where *M. indica* cannot be grown satisfactorily because of the wet climate (Orwa et al., 2009). About 70% of the kuini fruit part is edible (Orwa et al., 2009). The kuini fruit is light orange in colour, juicy and sweet when ripe (Campbell, 2007; Orwa et al., 2009; Ledesma and Campbell, 2014). Kuini fruits were used for making chutney and for pickles with salt (Orwa et al., 2009). It is usually propagated by seed, rarely by grafting; marcotting is possible but difficult (Campbell, 2007). For commercial purpose, grafting is the most appropriate method because it maintains the genetic characters from the propagated variety (Mandal et al., 2011; Ram et al., 2012). However, the common methods used are time consuming and low in success. One of the major requirements to increase production of kuini planting materials would be the rapid multiplication and distribution of superior clones. However, the success of grafting depends on different factors such as influence of environmental parameters, method of grafting, time and rootstocks (Shantagouda et al., 2008). The suitable grafting technique is necessary to meet the increasing demand for planting materials of this fruit species. Hence, the objective of the study was to evaluate the effects of different grafting techniques on success and growth of kuini planting materials.

Materials and Methods

Mangifera indica cultivar Telur was used as source of rootstocks. Four months old of Telur rootstocks were used for grafted with kuini scion using top wedge grafting, side wedge grafting and budgrafting techniques under netted structure (Figure 1a). The scion shoots of kuini (10-15 cm length) at the same size with the rootstock were collected on the same day for grafting purposes (Figure 1b). Forty rootstocks with three replications were used for each technique for grafted with kuini scion. Grafted plants were covered with clear plastic bags. Grafted plants were watered twice daily and general plant protection measures were carried out by applying fungicides and pesticides to control pests and diseases. Success rate was recorded at three months after grafting (MAG) while graft survival rate was recorded at six months after grafting. Monthly, the graft growth parameters such as shoot height, graft diameter and number of leaves were also measured.

Success rate of the grafts was calculated after three months of grafting by using the following formula and express in percentage:

$$\text{Success rate (\%)} = \frac{\text{No. of grafted plants in the experiments}}{\text{Total no. of grafted plants}} \times 100$$

Survival rate of the grafts was calculated after six months of grafting by using the following formula and expressed in percentage:

$$\text{Survival rate (\%)} = \frac{\text{No. of grafts remained alive at the end of experiment}}{\text{No. of successful graft}} \times 100$$

Figure 1: (a) 4-month old rootstocks of *M. indica* var. Telur and (b) Kuini scions.

Results and Discussion

Different grafting techniques showed highly effect on the success and survival rates of the Kuini planting materials after three and six months of grafting (Table 1). The highest percentage of success of grafts was found in the plants grafted by the top wedge grafting technique (85.25%) and the lowest was in the budgrafting (40.55%) after three months of grafting. The highest survival rate was notice in the graft produced by top wedge grafting (75.00%) (Figure 2) and the lowest was in the budgrafting (30.23%) after six months of grafting. From the results, it was found that the efficacy of the top wedge grafting technique was the best in comparison with the other grafting techniques under this study. Reddy and Melanta (2001) reported that top wedge grafting technique recorded a maximum graft union success for grafting mango in India. The highest success and survival of Kuini scion produced by top wedge grafting might be due to the quick graft union process after grafting. According to Islam et al. (2004), top wedge grafting is the slanting cut onto the rootstocks, the single option for the matching of the cambium layer, the long cambial layer connection, the easiness of the wrapping of the wrapping materials hindering the entrance of the rain water might be cause of higher success and survival rate of grafts produced by this technique through rapid proliferation and intermingling of vascular tissues. The highest success of top wedge grafting might be due to stronger and complete union of top grafted before sprouting (Alam et al., 2006; Simon et al., 2010). This result is in close conformity as obtained by Singh et al. (2012). However, the lower graft union success could be attributed to the lack of intimate contact of cambial region of both stock and scion and to interference of exudation of latex (Hartman et al., 1997).

Table 1: Effect of grafting techniques on graft success and graft survival rates of kuini.

(Grafting techniques)	Graft success (%)	Graft survival (%)
	3 MAG	6 MAG
T1	85.25	75.00
T2	40.55	30.23
T3	61.23	50.22

**MAG= Months after grafting; T1= Top wedge grafting; T2= Budgrafting; T3= Side wedge grafting.*

Table 2: Effect of grafting techniques on shoot height (cm), graft diameter (mm) and number of leaves of kuini.

(Grafting techniques)	Shoot height (cm)			Graft diameter (mm)			Number of leaves		
	30 DAG	60 DAG	90 DAG	30 DAG	60 DAG	90 DAG	30 DAG	60 DAG	90 DAG
T1	3.53	5.53	7.04	5.58	6.49	7.58	5.42	9.24	11.12
T2	1.21	2.42	3.20	5.32	6.01	7.40	2.34	3.22	5.30
T3	3.00	3.98	5.89	5.38	7.38	8.01	6.85	8.40	9.70

**DAG= Days after grafting; T1= Top wedge grafting; T2= Budgrafting; T3= Side wedge grafting.*

The growth of the kuini scion was greatly affected by different techniques of grafting (Table 2). The highest mean shoot height (7.04 cm) of the kuini scion was noticed in the plants produced by top wedge grafting followed by side wedge grafting (5.89 cm) while kuini scion grafted using budgrafting produce the lowest mean shoot height (3.20 cm) after 90 days of grafting. The highest mean graft diameter was recorded in the plant produced by side wedge grafting (8.01 mm) followed by top wedge grafting (7.58 mm). The lowest mean graft diameter was recorded in the plant produced by budgrafting (7.40 mm) after 90 days of grafting. The highest growth of graft diameter in the plant produced by side wedge grafting might due the quick graft union process between scion and rootstock that enhanced the translocation of the food materials from leaves to the rootstock through scion (Islam et al., 2004).

At 90 days after grafting the highest mean number of leaves (11.12) was produced by top wedge grafting and the lowest mean number of leaves (5.30) produced by bud grafting (Table 2; Figure 3). The highest growth of shoot height and mean number of leaves in the plants raised by top wedge grafting might be due to the scion inducing adequate callus formation, quick dissociation of the barrier zone and a rapid intermingling and interlocking of the vascular tissue (Hartman et al., 1997). Thus, translocation of water and minerals to the scion from the root through the rootstock was enhanced. It produced more food materials and ultimately excellent growth of the scion, giving the highest mean of shoot height and leaves number.

Figure 2: Survival of kuini scion grafted by top wedge grafting technique after 6 months of grafting.

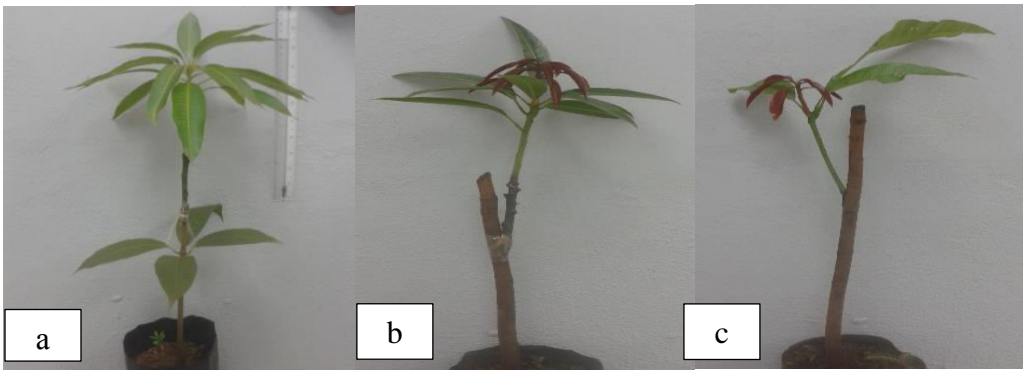

Figure 3: Kuini scion grafted on rootstock using (a) top wedge grafting, (b) budgrafting and (c) side wedge grafting after 90 days of grafting.

Conclusion

Based on the results, top wedge grafting technique is the best approach to propagate *M. odorata* as it increased the success and survival rates as well as improved growth of the planting materials of the species.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Evaluation of Seed-borne Pathogens Incidence in *Carica papaya* L.

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Introduction

Carica papaya L. is one of the most economically important fruit crops that currently being cultivated throughout tropical and subtropical areas all over the world for consumption, either fresh or processed products. Nevertheless, many papaya diseases caused by fungi, bacteria and viruses result in great yield losses. Seed-borne pathogens have some detrimental effects on seed such as reducing seed viability, vigour, germination capability, shortening longevity of storage, and causing physiological changes. Some seed-borne pathogens are also seed-transmitted, which can cause severe diseases in the field that may usually result in yield losses (Neergard, 1979). Healthy or pathogen free seeds are considered as the key factor for desired plant population, good harvest, a successful crop establishment and production. Health of seeds can be affected by direct infection by pathogens or through contamination (Rashid and Fakir, 2000). For a good crop, good seed germination is essential, which indicates that the seed should be pure, viable and healthy which can contribute towards increased germination as well as yield. The most effective disease management strategy is accomplished by using seed testing or seed detection assays to screen and eliminate infested seed lots before planting. Moreover, early identification and listing of plant pathogens in an area allows for control development and management strategies in order to avoid crop losses and to prevent the spread of plant diseases to new areas (Ekhuemelo and Ebenezer, 2013). A lot of research has been done for the control of papaya diseases in the field or postharvest, however, minimal study has been conducted to assess papaya seeds for seed-borne pathogens. Thus this study was carried out to determine the seed-borne pathogens associated with the seeds of selected local varieties of papaya in Malaysia.

Materials and Methods

Preparation of seed samples

Seeds of 3 papaya varieties of Sekaki, Eksotika and Eksotika II were randomly selected. Seed samples were surface disinfected with 0.1% (v/v) mercuric chloride before assayed.

Detection and identification of seed-borne fungi

The presence of seed-borne fungi and bacteria associated with the selected seed samples was detected using standard blotter and agar plate methods. Four hundred seeds were plated on 3 layered moistened filter papers in 9 cm petri plates (25 seeds per plate) with 8 replications (2 plates per replication) in blotter method. While, another 400 seeds were plated on potato dextrose agar (PDA) and nutrient agar (NA) in 9 cm petri plates (25 seeds per plate) with 8 replications (2 plates per replication) in agar plate method (ISTA, 2017). Petri plates were incubated for 7 days under room temperature and percentage of fungal incidence was recorded. At the end of the incubation period, fungi and bacterial pathogens growing out from the seeds on both blotter and agar medium were examined and identified based on morphological

characteristics of colony and sporulation structures of fungi under a compound microscope using reference manuals by Watanabe (2010) and Mathur and Kongsdal (2003) and molecular techniques.

Experimental design

The experiments were conducted in Completely Randomized Design (CRD). All data were subjected to analysis of variance (ANOVA), where the significant ($p<0.05$) differences between means were determined by Least Significant Difference (LSD). The SAS (version 9.2) software was used to perform all analyses.

Results and Discussion

Four hundred Sekaki, Eksotika and Eksotika II papaya seeds were tested and a total of 9 bacterial pathogens; *Enterobacter cancerogenus, Bacillus cereus, Pseudomonas monteilii*, *E. cloacae, P. aeruginosa*, *P. alcaligenes*, *B. Megaterium*, *P. nitroreducens* and *Staphylococcus saprophyticus* were detected with percentage of incidence as shown in Table 1. The results also revealed that seedborne fungi; *Aspergillus flavus, A. niger*, *Bipolaris sp.*, *Choanephora infundibulifera*, *Cochliobolus lunatus*, *Coprinellus radians*, *Curvularia lunata* and *Geotrichum candidum* were present in tested papaya seeds (Table 2). The bacterial and fungal pathogens that gave more than 5% incidence were considered as predominant.

Table 1: Infection percentages of seed-borne bacteria isolated from papaya.

Bacteria	Incidence (%)			Total incidence (%)
	Sekaki	Eksotika	Eksotika II	
Enterobacter cancerogenus	37.0	3.4	6.7	47.1[a]
Bacillus cereus	12.0	0	0	12.0[b]
Pseudomonas monteilii	6.9	1.7	3.4	12.0[b]
Enterobacter cloacae	4.0	1.4	1.4	6.8[c]
Pseudomonas aeruginosa	5.1	0	1.7	6.8[c]
Pseudomonas alcaligenes	6.8	0	0	6.8[c]
Bacillus megaterium	1.7	3.4	0	5.1[d]
Pseudomonas nitroreducens	0	1.7	0	1.7[e]
Staphylococcus saprophyticus	0	0	1.7	1.7[e]

Means followed by the same letter(s) are not significantly different (LSD test at P<0.05).

Table 2: Infection percentages of seed-borne fungi isolated from papaya.

Fungi	Incidence (%)			Total incidence (%)
	Sekaki	Eksotika	Eksotika II	
Aspergillus flavus	12.5	0	12.5	25[a]
Aspergillus niger	19.5	0	0	19.5[a]
Bipolaris sp.	17.5	0	0	17.5[ab]
Choanephora infundibulifera	12.5	0	0	12.5[b]
Cochliobolus lunatus	8.0	0	1.5	9.5[c]
Curvularia lunata	7.5	0	1.0	8.5[cd]
Coprinellus radians	4.0	2.0	0	6.0[d]
Geotrichum candidum	0.5	0	1.0	1.5[e]

Means followed by the same letter(s) are not significantly different (LSD test at P<0.05).

The isolated bacteria and fungi indicated the possible diseases that could affect papaya seedlings emerging from such infected seeds. *Enterobacter* spp. were previously reported to cause soft rot which they induced whole plant and leaf wilt, defoliation and discoloration of vascular tissue (Wang et al., 2010). *Pseudomonas aeruginosa* has been reported to cause diseases in a variety of plants including root rot (Gao et al., 2014). Fungi from the genus *Aspergillus* produced mycotoxins could make changes in the chemical ingredients inside the seeds, reduce nutritive value and viability of seeds and even cause seed death (Duan et al., 2007). This means seeds heavily infected with fungi may not germinate and could attack the emerging seedlings. A study reported by Park and Cho (2014), *Cochliobolus lunatus* and *Choanephora infundibulifera* led to seedling blight diseases. Seeds attacked by *C. lunatus* usually fail to germinate resulting in poor stand development while *Geotrichum candidum* could cause root rot disease. Besides, *Bipolaris* spp. have been reported as pathogens causing leaf spot disease and *Choanephora infundibulifera* has been reported to cause seedling blight (Rott et al., 2000).

The decreased in seed germination and vigour index was directly associated with seed-borne pathogen infection and causing seedling death (Al-kassam and Monawar, 2000). Furthermore, seed-borne fungi could be present on the seed surface and also inside the seed as the causal agents of diseases invading the roots, stems, and leaves. Seed moisture content, temperature and degree of invasion of seeds by pathogens were among the factors that influenced the development of seed-borne pathogens (Anjorin and Mohammed, 2009). Besides, fungi could spread from seed to placenta of the fruit; penetrate the developing ovules or young seed with unlignified testa at any point on their surface. Infection of seeds could also occur directly from the mother plant and could also be mechanically attached to the surface of the testa, and then remaining dormant until the seed germinated (Sariah and Zainun, 1988).

Conclusions

The present study revealed that seed-borne pathogens were associated with papaya diseases were found in seed lots used in this study, which greatly may influence seed quality and germination capability. Thus, seed health testing to detect seed-borne pathogens is an important step in management of papaya diseases, prevention of disease spread and can be a useful guide to strategic disease control.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Cryopreservation Techniques of *Citrus aurantifolia* (Key Lime)

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Introduction

Citrus species, which include mandarin (*Citrus reticulata*), pomelo (*Citrus maxima*), kalamansi lime (*Citrus microcarpa*) and sweet orange (*Citrus sinensis*) have high nutritional value and economically important in the South East Asia countries including Malaysia. *Citrus aurantifolia* commonly known as limau nipis or key lime is one of the important citrus species among the family Rutaceae and is widely grown in this country (Cho et al., 2002). The fruit is mainly used to flavor food and drinks. It is also valued for its medicinal properties. At present, genetic resources of this plant are mainly conserved in the field. It is propagated by seeds and through vegetative means. The main issue with the plants conserved in the field gene-bank is attacked by diseases such as witches broom disease of Mexican lime (WBDL) associated with *Candidatus Phytoplasma aurantifolia*, which is a major cause of *C. aurantifolia* losses (Al-Yahyai et al., 2012; Mollayi et al., 2015). In Iran, WBDL caused about 50% loss of its lime acreage in 22 year time (Al-Yahyai et al., 2012). Due to this disease, over half a million trees in about 7000 hectares have been destroyed to control the disease from spreading (Mardi et al., 2011).

In this circumstance, seed storage can be useful to conserve the genetic diversity. However, the seeds of many citrus species showed non-orthodox seed storage behaviour which is categorized as intermediate or recalcitrant seed (Malik et al., 2012). It can only withstand partial dehydration (Hor et al., 2005). They cannot be stored under conventional gene-bank conditions due to cold-sensitivity and longevity cannot be increased through desiccation (Graiver et al., 2012). For such semi-recalcitrant seeds species, they need special treatments to preserve the genetic diversity for long-term.

Cryopreservation seems a convincing alternative method to store tropical fruit tree species. Cryopreservation employs storage at ultralow temperatures using liquid nitrogen (-196°C). At this temperature, all cellular division and metabolic processes are suspended, with minimal impact on genetic stability. Theoretically, plant material can thus be stored without any sub-culturing for an unlimited period of time. Moreover, cultures are stored in a small volume, protected from contamination and require very limited maintenance (Withers and Engels, 1990; Maxted et al., 1997). Due to various problems and limitations encountered with other approaches, cryopreservation currently offers the only safe and cost-effective option for long-term conservation of genetic resources of recalcitrant and intermediate seed species. Yet, there are also many reports of failures attempts of cryopreserving the embryos of the recalcitrant seeds (Hor et al., 1990; Normah et al., 1996, 1997, 2000). Cho et al. (2002) observed desiccated cryopreserved embryonic axes of *C. aurantifolia* treated with various sucrose concentrations (0.1-0.7 M) in pre-culture media resulted in survival ranging from 7.5%-92.5%. Further research on increasing recovery rates by improving the plant source quality and optimizing conditioning treatments need to be done. The key of success to cryopreservation is achievable through dehydration tolerance (Panis et al., 2005). Therefore, optimum dehydration stage in cryopreservation is an important criteria that needs to be studied. The objectives of this study are to observe the effect of desiccation on the survival of seeds after cryopreservation and to study the effect of exposure time to a crycoprotectant plant vitrification solution 2 (PVS2) on the survival of excised *C. aurantifolia* embryonic axes.

Materials and Methods

Plant materials

Fruits of *C. aurantifolia* were obtained from the local supermarket. Only the matured fruits were used in the experiments. Experiments were performed in the laboratories of the Mygenebank Complex in MARDI Serdang. The average relative humidity in the laboratory area was around 48%.

Desiccation–cryopreservation of C. aurantifolia whole seed

Seeds were surface-sterilized in 10% sodium hypochlorite for 10 min and rinsed 3 times in sterile distilled water, followed by 70% ethanol for 30 seconds. The seeds were then rinsed 3 times in sterile distilled water and soaked in 1% sodium hypochlorite for 5 min before rinsed 3 times in sterile distilled water. Seeds were then desiccated using a desiccator chamber filled with silica gel. Desiccation periods ranged between 0 to 8 days. At the end of each desiccation period, seeds were transferred in the 2 mL cryo-vial and attached with the cryo-cane, immersed directly in liquid nitrogen (LN) for at least 1 hour. They were rewarmed for 1 min in a water-bath at 38-40°C. The seeds were germinated on the filter paper in petri dish at room condition 25 ± 1°C. Observations were made at every 2 days interval for 30 days. Seeds that which germinated into normal seedlings were considered viable. Experiments were performed with 10 intact seeds per experimental condition and replicated four times. Data were analyzed by ANOVA using Duncan's Multiple Range Test (DMRT) for data comparison and mean survival percentages are presented in the table with their standard deviation.

Cryopreservation of C. aurantifolia zygotic embryonic axes by vitrification

Seeds were surface sterilized as described previously. The testas were removed aseptically from the seeds and embryonic axes were then separated from the cotyledons with a scalpel blade and directly cultured on pre-culture media for 24 hours on basal Murashige and Skoog (MS) (1962) medium pH 5.8 supplemented with 0.3 M sucrose, 2 M glycerol and 2 gL^{-1} gelrite. The protocols followed by Malik and Chaudhury method (Malik and Chaudhury, 2006) with minor modification. Then, the embryonic axes were transferred in cryovial contain loading solution (liquid MS medium supplemented with 2 M glycerol and 0.4 M sucrose, pH 5.8) for 20 min, at 25°C. The loading solution was then replaced with 0.5 ml of 100% cryoprotectant solution PVS2 for 0, 10, 20, 30 min, at 25°C. The cryoprotectant solution PVS2 was liquid MS medium supplemented with 30% glycerol, 15% ethylene glycol, 15% DMSO, and 0.4 M sucrose with pH 5.8. The embryonic axes were then either transferred to recovery medium (-LN) or plunged in liquid nitrogen (+LN). After at least 1 hour of storage at -196°C, the embryonic axes were rewarmed in a water-bath at 38-40°C for 1 min, unloaded in 0.5 mL 1.2 M sucrose for 20 min and transferred on recovery medium consist of solid MS supplemented with 1 gL^{-1}activated charcoal, 0.17 $gL^{-1}NaH_2PO_4$, 1 mgL^{-1}each BAP and NAA, 3 gL^{-1}gelrite. The cultures were kept under 16h light/ 8h dark photoperiod at room condition 25 ± 1°C.

Results and Discussion

Desiccation-cryopreservation of C. aurantifolia whole seed

Table 1 showed the seed moisture contents (MC) and germination percentages of *C. aurantifolia* with (+LN) or without (-LN) plunging in liquid nitrogen. Results showed *C. aurantifolia* seeds can be desiccated below 10% MC with or without liquid nitrogen and still can survive with germination percentage ranged from 35% to 60% in liquid nitrogen (cryopreserved) and 50% to 90% without liquid nitrogen (control). The highest survival percentages of cryopreserved seeds were obtained (60%) after 3 and 4 days of desiccation, corresponding to seed moisture contents of 6% and 7.9%, respectively. Survival of cryopreserved *C. aurantifolia* seeds achieved at the broader range of moisture content 16.3% and 4.5%. The seeds germination percentage decreased by day after desiccation and the lowest germination percentage that is 50% discovered after 7 days of desiccation. This study confirms the previous study of Cho et al. (2002) on the seed tolerance to desiccation. Highest survival was found (60%) after 3 and 4 days of desiccation before cryopreserved. There are no survivals for cryopreserved seeds that were desiccated less than 2 days of period. Water removal from the seeds or embryonic axes plays a major role in preventing freezing injury in the cells especially in *C. aurantifolia* seed (Gonzalez-Arnao et al., 2008). From the results achieved in this study, citrus embryonic axes may have some tolerance to desiccation and it is possible to be direct cryopreserved with partial drying too.

Table 1: Effect of desiccation period on the moisture content (%) and the survival of control (-LN) and cryopreserved (+LN) *C. aurantifolia* seeds. Values followed by the same letter within column are not significantly different at the 0.05 probability level by DMRT.

Desiccation period (days)	Seed moisture content (%)	Survival rate (%)	
		+LN	-LN
0	54.4 ± 5.6	0[a]	95 ± 2.8[a]
1	32.2 ± 5.9	0[a]	100[a]
2	16.3 ± 3.8	50 ± 14.1[b]	92.5 ± 3.5[a]
3	6.0 ± 3.0	60 ± 28.3[b]	75 ± 21.2[a]
4	7.9 ± 2.6	60 ± 28.3[b]	90 ± 21.2[a]
7	4.5 ± 2.0	35 ± 21.2[b]	50 ± 7.1[a]

Cryopreservation of C. aurantifolia zygotic embryonic axes by vitrification

The initial seed moisture content of *C. aurantifolia* used in this study was 58.79 ± 4.81% and the seed germination percentage was 97 ± 5.77%. After cryopreservation, survival was achieved after 10 to 30 min exposure to PVS2 but there is no significant different between time of exposure (Table 2). No survival was obtained for 0 min exposure to PVS2 after cryopreservation. The survival of cryopreserved embryonic axes *C. aurantifolia* ranged from 10% to 13% while non-cryopreserved ranged from 20% to 27% only and can be considered low. Highest survival can be seen after 20 to 30 minutes exposed to PVS2 before cryopreserved. Factors like sensitivity to PVS2 solution or the size of embryonic axes might limit the survival of embryonic axes with and without liquid nitrogen exposure. The size of *C. aurantifolia* embryonic axes used in this study is less than 1 mm^2. Many of previous studies emphasized the significance of the explant size for cryopreservation success (Kulus and Zalewska, 2014). The smaller the explant is, the higher the chances of survival due to deep penetration of the cryoprotectant (Kulus and

Zalewska, 2014). However if the target size of embryonic axes is too small it may damage over the process of extraction.

Table 2: Effect of exposure time to the osmoregulator PVS2 and the survival of *C. aurantifolia* embryonic axes cryopreserved through vitrification technique (+LN) and non-cryopreserved (-LN). Values followed by the same letter within column are not significantly different at the 0.05 probability level by DMRT.

Time exposure to PVS2	Survival rate (%)	
	+ LN	- LN
0 min	0[a]	20 ± 20[a]
10 min	10 ± 10[a]	27 ± 15.28[a]
20 min	13 ± 11.55[a]	23 ± 25.17[a]
30 min	13 ± 15.28[a]	23 ± 20.82[a]

Conclusions

In conclusion, the highest survival percentages of cryopreserved seeds were obtained (60%) after 3 and 4 days of desiccation, corresponding to seed moisture contents of 6% and 7.9% respectively while for the embryonic axes vitrification, the highest survival can be seen after 20 to 30 min exposed to PVS2 which ranged from 10%-13% survival. There are other factors other than the desiccation sensitivity and vitrification solution exposure effect seems to exist that limits the embryonic axes tolerance to liquid nitrogen exposure. These factors still need to be studied and defined before the protocol can be employed.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Seed Quality of *Capsicum annuum* L. during Maturation

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Introduction

Chilli is the most important vegetable grown in Malaysia and has very high demand due to its culinary functions. It is being consumed fresh and in many different forms of processed products such as sauces, pastes, powders, and flakes. At 2014, self-sufficiency level (SSL) of chilli is Malaysia was recorded at 52%. On the other hand, SSL of other vegetables like tomato, cucumber, brinjal, long bean, and lady's finger were recorded more than 100% (MOA, 2015). Due to its high demand and low supply, the market price is always high. Therefore, a lot of farmers prefer to grow chilli to meet market needs and to attain higher profit. However, until today the importing volume is still high.

Seed quality can potentially influence crop yield through affecting seedling establishment especially when it is grown under unfavourable environmental conditions (Tekrony and Egli, 1991). According to Perry (1982), seed maturity is one of the main components in seed quality and a prerequisite for successful germination and seedling emergence. Therefore, it is very important to harvest seeds at their maximal quality. However, times of occurrence of maximal seed quality are greatly debated.

The maximum seed dry mass (physiological maturity) which occurs at the end of seed filling period defines the best harvesting time to obtain maximum seed quality (Shaw and Loomis 1950; Harrington 1972; Tekrony and Hunter 1995; Tekrony and Egli 1997). The seeds harvested after the end of seed filling period will also have lower viability and vigour because of seed ageing (Harrington 1972). However, other research reports showed that maximum seed quality was attained some time after the end of seed filling period on various crops such as brinjal (Demir et al., 2002), tomato (Demir and Ellis, 1992a; Demir and Samit, 2001), sweet corn (Wilson and Trawatha, 1991), bean (Senhewe and Ellis, 1996), muskmelon (Welbaum and Bradford, 1988), soybean (Zanakis et al., 1994), pumpkin (Neto et al., 2015) and sweet pepper (Demir and Ellis, 1992b). It showed that maximum seed dry mass did not coincide with the occurrence of maximum seed quality. Ellis and Pieta Filho (1992) suggested that mass maturity is more appropriate term to refer to the end of seed filling phase because only maximum seed dry mass was observed but not maximum seed quality.

Fruit physical characteristic like fruit colour often being observed during the studies of the occurrence of maximum seed quality. Kwon and Bradford (1987), Demir and Samit (2001) and Dias et al. (2006)-had studied changes in fruit colour of tomato during seed development and maturation. Suprakarn et al. (2005) suggested harvesting fully ripe fruits (brownish colour) of purple variety of brinjal for the production of high quality seeds. Studies done by Vidigal et al. (2011) on sweet pepper suggested that fruits that completely red should be harvested for high quality seed production. Therefore this study was aimed to determine the occurrence of maximum seed quality of a new potential line of chilli cv. Line 5, and its fruit colours during seed maturation.

Materials and Methods

Planting materials

Line 5 chilli is an open pollinated inbred line developed by Malaysian Agricultural Research and Development Institute (MARDI) that has good processing qualities for the production of sauces. Seeds were sown in seedling trays using peat moss as medium. At forty five days after sowing, six hundred seedlings were planted in 16 cm x 16 cm black polybags under 4 m x 150 m protected structure equipped with a fully automatic fertigation system at MARDI Klang, Selangor. Fertilizer and water were supplied daily through the irrigation system following recommendation by MARDI for chilli production. Three hundred flowers at full anthesis were tagged from August 3 until August 29 in 2016. Fruits were harvested at 34, 38, 40, 42 and 44 days after anthesis (DAA) and changes in fruit colour were recorded. Seed moisture content (wet basis) was determined immediately after extraction, in duplicate samples, by drying at 103 ± 3°C for 17 hours (ISTA, 2015) and seed dry matter content was calculated and expressed in mg per seed (mg/seed). The remaining seeds were further dried using sun-drying to targeted moisture content (MC) of 9%. Weight of water removed (W_f) was estimated using target moisture content formula as per below. Seeds were weighed from time to time and drying was stopped when W_f was reached.

$$W_f = W_i \times (MC_i - MC_f)/(100 - MC_f)$$

where:

W_f = Weight that should be reduced from initial weight to obtain MC of 9%
W_i = Initial weight of seed
MC_i = Initial moisture content
MC_f = Target moisture content, which is 9%

After drying process was done, moisture content of randomly 50 seeds taken at each harvesting time was determined using the slow oven method (ISTA, 2015) to confirm if the target moisture content had achieved.

Seed germination

Four replicates of 100 seeds from each treatment were placed on top of wet tissue paper in sandwich boxes (18.5 x 13x 7 cm), then placed in a germination chamber for 14 days (ISTA, 2015). Germinating seeds were recorded every day for 14 days. Final germination percentage (%) was calculated and recorded after 14 days of placement on wet tissue paper.

Seed vigour performances

Seed vigour parameters such as mean germination time (MGT), germination speed index (GI), time to obtain 50% germination on total seed tested (T_{50}) were calculated at the end of germination test. MGT was calculated according to the equation of Ellis and Roberts (1981). GI was calculated as described in Association of Official Seed Analyst (AOSA, 1983). The T_{50} was calculated according to the formula of Coolbear et al. (1984) modified by Farooq et al. (2005).

Electrical conductivity

A conductivity meter, CON510 (EUTECH INSTRUMENTS, SINGAPORE) was used to measure the seed leachates. Four replicates of 50 seeds from each treatment were weighed and soaked in 50 mL deionized water at 25°C for 24 hours before readings were taken. Results were expressed in $\mu S\ cm^{-1}\ g^{-1}$ (ISTA, 2015).

Statistical design and analysis

The experimental layout was a completely randomized design (CRD) with four replications. Statistical procedure was carried out using the SAS software and data were analysed using ANOVA. Treatment means were compared using the Least Significant Difference (LSD) test at 95% probability level.

Results and Discussion

During maturation, water content of seeds extracted from freshly harvested fruits decreased gradually from 34 DAA until 40 DAA, then slightly increased and remained constant at 42 and 44 DAA (Figure 1). Seed moisture content remained high even after maximum seed dry mass was attained at 40 days after anthesis (DAA). Similar results where seed water content remains high during maturation were reported in other fleshy-fruited vegetables such as sweet pepper (Demir and Ellis, 1992b), tomato (Demir and Ellis, 1992a), brinjal (Demir et al., 2002), cucumber (Nakada et al., 2011), pumpkin (Neto et al., 2015) and zucchini (Silva et al., 2017). According to Welbaum and Bradford (1988), in fleshy-fruit vegetables, osmotic solutes primarily sugars accumulate, thus creating a low water potential in the fruits that maintains high seed water content, but below the threshold required for germination.

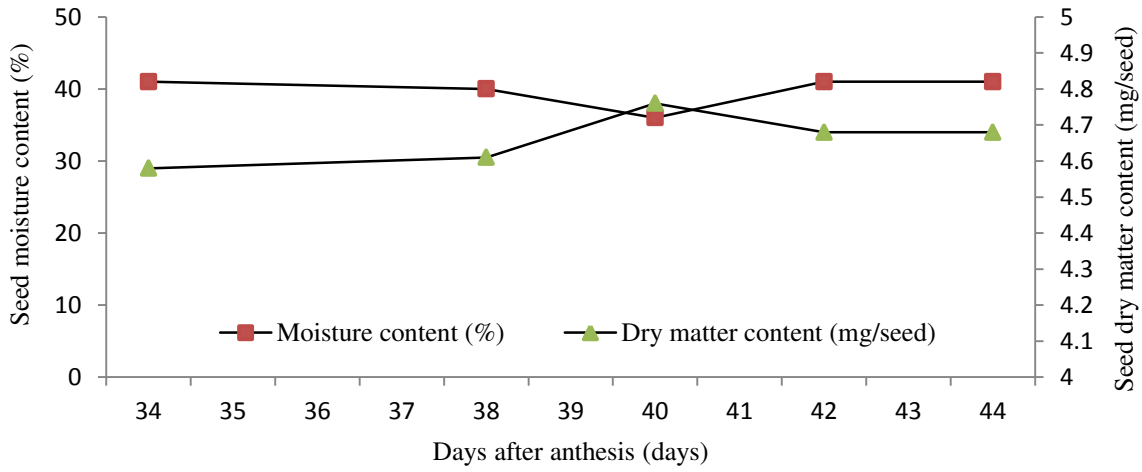

Figure 1: Moisture and dry matter content of chilli seeds cv. Line 5 harvested at different maturity.

While seed moisture content gradually declined from 34 to 40 DAA, seed dry mass continued to accumulate and was recorded maximum at 40 DAA (4.76 mg/seed). Thereafter, it was recorded decreased at 42 DAA and remained constant at 44 DAA. Results suggested that the end of seed filling period (physiological maturity) occurred at 40 DAA and fruits were observed with bright red colour. Studies done by Yisa et al. (2013) on two cultivars of chilli in Nigeria reported that maximum seed dry mass was

obtained at 48 DAA where fruits were observed with full red colour. Meanwhile, studies done by Dias et al. (2006) on sweet pepper in Brazil and Demir and Ellis (1992a) on tomato in United Kingdom reported that maximum seed dry mass was obtained approximately at 75 and 50 DAA and fruits were observed with 90 and 50% red colour, respectively.

Fruit colour is a useful indicator in estimating the best time to harvest but it highly depends on cultivar, crops and growing locations. Therefore, it is very important to identify the time of the occurrence of the maximum seed quality of each individual cultivar and to observe changes in its fruit physical characteristics such as fruit colour, firmness, and skin texture during maturation. In this study, fruits harvested at 34 DAA were observed with red colour and dark green signals (Figure 2). At 38 DAA, fruit colour changed to bright red with orange signals. Meanwhile, at 40, 42 and 44 DAA, fruits were observed completely in bright red, red and dark red colour, respectively.

Figure 2: Colour of chilli fruits, cv. Line 5 harvested at different maturity of 34, 38, 40, 42 and 44 days after anthesis (DAA).

Table 1: Germination percentage, germination speed index, time to obtain 50% germination on total seeds tested and mean germination time of chilli seeds, cv. Line 5 during maturation.

Factor	Germination percentage (%)	Mean germination time (MGT)	Germination speed index (GI)	Time to obtain 50% germination on total seed tested (T_{50})	Electrical conductivity (EC) ($\mu S\ cm^{-1}g^{-1}$)
Maturity stages					
34	81[a]	9.44[a]	17.46[a]	9.95[a]	56.53[a]
38	81.5[a]	9.16[a]	18.25[a]	8.52[ab]	57.26[a]
40	86.9[a]	9.72[a]	17.28[a]	8.83[ab]	66.23[a]
42	92.5[a]	9.41[a]	19.65[a]	8.31[ab]	28.9[b]
44	89.5[a]	8.98[a]	20.61[a]	7.55[ab]	27.8[b]
F value					
Maturity stages	1.09ns	0.89ns	1.03ns	1.16ns	23.11**

***Significantly at 1% probability level. Means in each column with the different letters within the same column indicate significant differences at P≤0.05% level according to LSD test.*

Results revealed that germination percentage, germination speed index (GI), mean germination time (MGT), and time to obtain 50% germination on total seed tested (T_{50}) were not affected significantly ($P<0.05$) by maturity stages. However, electrical conductivity (EC) was affected significantly by maturity stages (Table 1). Seeds harvested from 34 DAA and onwards have high germination percentage (>80%) and recorded maximum when seeds harvested after physiological maturity stage (at 42 DAA with 92.5%).

Many other studies done on various crops have found similar results where the time of the occurrence of maximum seed quality does not coincide with physiological maturity (maximum seed dry mass) especially in fleshy-fruit vegetables (Oliveira et al., 1999; Demir and Ellis, 1992a,b; Demir et al., 2002).

Electrical conductivity of chilli decreased during seed maturation and recorded ranged from 66.3 to 27.8 $\mu S\ cm^{-1}g^{-1}$ (Table 1). In this study, electrical conductivity was significantly different but not significantly correlated to germination percentage and seed vigour performances during seed maturation. Similar result was obtained by Silva et al. (2017) in zucchini where seed germination and seed vigour of seeds harvested from mature fruits at 49, 56 and 63 DAA remain high and not significantly different even though electrical conductivity was significantly different and decreased. Similar results were obtained by Dias et al. (2009) and Demir and Ellis (1992b) on sweet pepper, Demir and Ellis (1992) on tomato and Neto et al. (2015) on pumpkin seeds harvested from mature fruits. However, when seeds harvested at younger age or immaturely, results on high electrical conductivity coincided with the lowest germination percentage and seeds vigour performances (Vidigal et al., 2011). Therefore, further study should be done by adding few more harvesting times before 34 DAA with 4 days interval to investigate significant relationship of electrical conductivity with low germination percentage and seed vigour performances during seed development.

According to Vidigal et al. (2011), maximum seed quality can be determined by the combination of germination percentage and seed vigour performances. In this study, maximum seed quality was attained at 44 DAA which is 4 days after physiological maturity. Results are not consistent with the hypothesis proposed by Harrington (1972) where seed quality will be maximal at physiological maturity, then decreases thereafter. These results are in line with the results obtained in many other studies done on various fleshy fruit vegetables (Welbaum and Bradford, 1988; Demir and Ellis, 1992a; Demir and Ellis, 1992b; Demir and Samit, 2001; Demir et al., 2002; Neto et al., 2015).

Conclusions

Chilli seeds, cv. Line 5, attained maximum seed dry mass at 40 DAA when seed moisture content was observed with 37% and fruits were completely bright red. Seed quality was not affected significantly by maturity stages however seed vigour does. Therefore, for the production of high quality seeds, it is recommended to harvest fruits at 42 to 44 DAA.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

In vivo and *in vitro* Germination of *Musa violascens* and *Musa borneensis* Seeds

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Introduction

Musa is a large perennial monocotyledonous herbaceous plant genus in Musaceae family native to the Indo-Malesia, Asian and Australian tropics. It has five taxonomic sections with two of them contain edible bananas. Banana and plantain are considered as an important food crop for people as it ranked fourth globally after rice, wheat and maize. Banana is either consumed raw as fruits or cooked, steamed, boiled, grilled, baked, and fried to make into chips, dessert, snack and food like curry and soup. The plant can be used as silage, rope, cordage, garlands, shelter, clothing, smoking materials and ceremonial and religious uses (Nelson et al., 2006). The edible cultivated varieties were derived from the hybridization between two wild species namely *Musa acuminata* Cola and *Musa Balbisiana* Cola which gave rise to different genomic groupings namely AA, AAA, AB, ABB/BBB, ABBB (Hakkinen and Hong, 2007; Suhana et al., 2012). The increasing areas of banana plantations in tropical countries are threatened by the continuous pests and diseases problems such as the appearance and spread of the virulent form of Sigatoka leaf spot disease. Thus, awareness has been increased among banana breeders in order to produce new cultivated varieties with the disease-resistant characteristics (Pancholi et al., 1995). However, breeding programs are difficult to be done in cultivated *Musa.* This is due to the nature of fruits from cultivated *Musa* species which are normally sterile and very low fertility. Cultivated *Musa* species are known as its parthenocarpic characteristics where fruits are produced without pollination and fertilization thus seeds are not form (Nelson et al., 2006)**.**

Recently, wild banana is seen as an important and potential plant to be used in order to produce new disease resistant cultivated varieties through breeding programme. Suhana et al. (2012) has identified that some of wild banana conserved in the germplasm collection are resistant to Fusarium wilt disease. Wild banana produces fertile and viable seeds which can be used as source of material in the breeding work. However, the germination of banana seeds is extremely non uniform with some are very difficult to germinate. In addition, factor that affects the germination behaviour in wild banana seeds was unclear (Asif et al., 2001). This situation could hinder the breeding work as several current research programs require a continuous supply of small and uniform plants which can be obtained through seeds (Ahmed et al., 2006). Upon harvest, some banana seeds show no sign of dormancy and are readily germinated. However, they become dorman after drying. *Musa gracilis* with the moisture content of 46% resulted 70% germination after moist heat treatment for 7 days. When seeds were dried to 12% moisture content, no germination was recorded even after 3 months of germination period. In addition, germination tests have been done on various banana species resulted in range of responses which species and varieties showed dormancy characteristics. Banana seeds show varying degrees of dormancy and they respond differently to various breaking dormancy treatment (Chin, 1996). Stotzky et al., (1962) stated that difficulties in germinating banana seeds of *Musa balbisiana* lie in the seed coat and scarification of the seed coat promoted germination. Different opinion was raised by Puteh et al. (2011). They suggested that seed dormancy of *M. acuminata* Colla is may be due to physiological in nature. As a consequence, a study on the germination potential of two wild banana species namely *Musa violascens* and *Musa borneensis* on

different germination substrates was undertaken. Scarification treatment was also applied on the seeds in order to ensure that if there is physical dormancy from the seed coat. *In vitro* embryo culture was also tested in order to confirm whether there is dormancy lies on the embryos. *Musa borneensis* is wild banana species, native to the island of Borneo, having a diploid chromosome number of 2n = 20. *M. violascens* is also a wild banana species, native to Peninsular Malaysia having a diploid chromosome number of 2n = 20 (Wong et al., 2002).

Materials and Methods

The bunch of mature unripe *M. violascens* (Figure 1) and *M. borneensis* (Figure 2) fruit were harvested and kept at ambient condition in the air conditioned laboratory (25 ± 2°C) for ripening. Upon ripening, the seeds were extracted from the fruits, washed and cleaned under running tap water. The seeds were blotted dry with tissue paper and were left dry for 2 hours in the laboratory conditions.

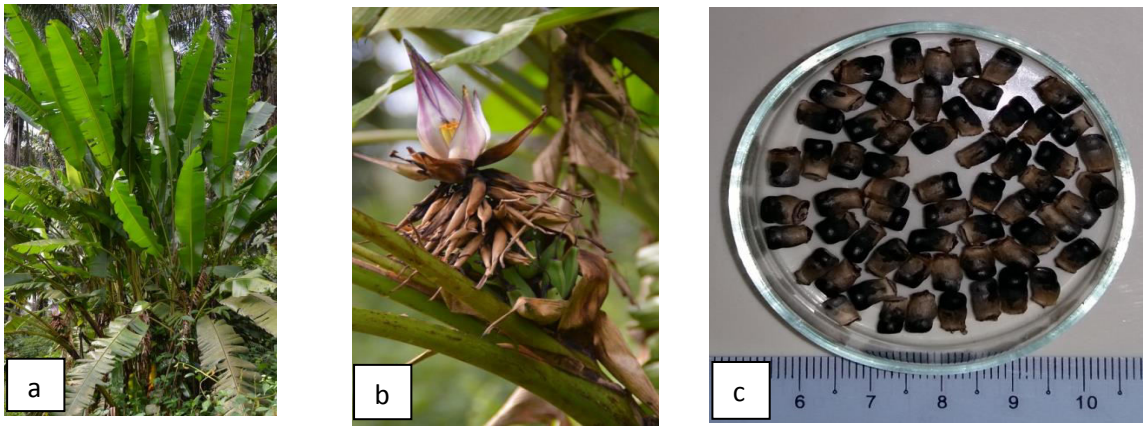

Figure 1: *Musa violascens* plant (a), fruit bunch (b) and seeds (c).

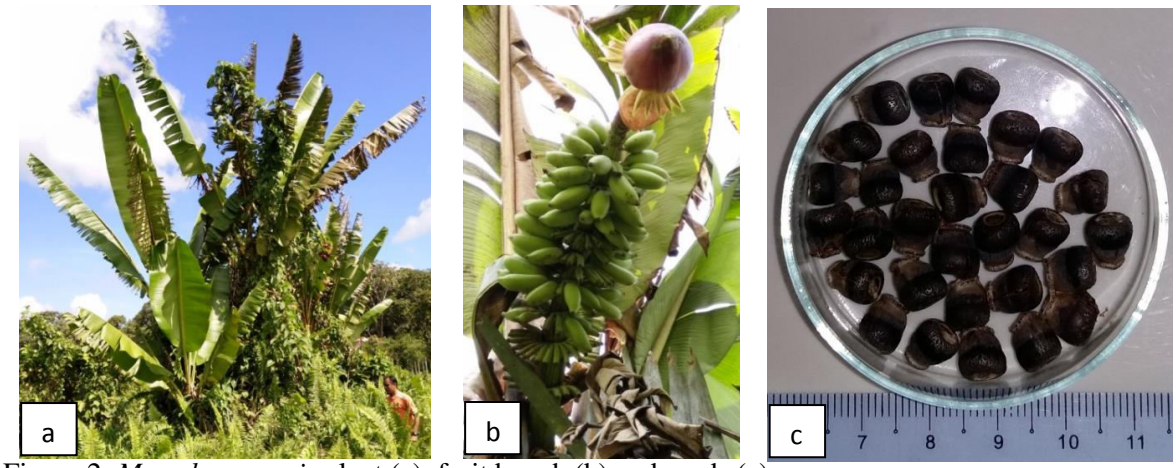

Figure 2: *Musa borneensis* plant (a), fruit bunch (b) and seeds (c).

In vivo germination

The seeds were sown on the 3 different germination substrates namely sand, soil and filter paper. The seeds were planted in germination boxes filled with moistened sand at the depth of 2.5 cm and covered with a thin layer of sand for about 1 cm. The same condition to those described above was applied on the soil. Distilled water were used to moist the substrates. The germination boxes were placed in the germination room at natural condition with average temperature between 22 - 32°C with 80% relative humidity and 12 hours sunlight. The seeds also were sown in petri dish on a wet filter paper. The dishes were placed at ambient room temperature 25 ± 2°C with 12 hour light. An additional experiment was carried out for the scarification tests for breaking dormancy. In this recent study, attempts to break dormancy were done by chipping a very tiny portion of the seed coat with the surgical blade prior to sowing on the moistened sand, soil and filter paper using the similar method to those described above. The germination was watered with distilled water. Seeds were sown in three replications with ten seeds per replications for all germinating substrates. The germination of these seeds were observed at interval of 1, 2 and 3 months.

In vitro germination

Sterilization of seeds

The seeds were washed under running tap water for 30 min. In the laminar air flow, the seeds were soaked with 30% (v/v) commercial bleach (Clorox) and tween 20. The seeds were further disinfected with 70% (v/v) ethanol and 5% (v/v) Clorox mixed with tween 20 followed by rinsing with sterilized distilled water for 3 times. The seeds were then blotted dry on the sterilized filter paper in the petri dish. Seeds were cut longitudinally and tiny white mushroom-like embryo were excised.

Culture medium

The excised embryos were cultured aseptically into culture tube contained of 20 mL of full strength Murashige and Skoog (MS) semisolid medium supplemented with myo-inositol (100.0 mg/L), glycine (2.0 mg/L), nicotinic acid (0.5 mg/L), pyridoxine HCL (0.5 mg/L), thiamine HCL (0.1 mg/L), ferrous sulfate (27.8 mg/L), Na_2 EDTA (37.3 mg/L) and sucrose (40 g/L). The pH of the medium was adjusted at 5.7 to 5.8 with 0.1 M NaOH and 0.1 M HCL and autoclaved at 121°C and 15 p.s.i for 15 min. 4 g/L gelrite were added to solidify the medium. Cultures were kept under 18/6 h photoperiod and 60% relative humidity at 25 ± 2°C for the embryos to germinate. Embryos were cultured in three replications with ten embryos per replications. The germination of these embryos were observed at interval of 1, 2 and 3 months.

Data analysis

All data were analyzed by analysis of variance (ANOVA) using Statistical Analyses System Software (SAS) release 9.4. This study adopted the Completely Randomized Design (CRD). Means was differentiated at $P \leq 0.05$ level of significance using Least Significant Different Test (LSD).

Results and Discussion

There were no significant different on germination percentages of *M. violascens* in both sand and soil media after 2 and 3 months (Figure 3). Filter paper was not a suitable germination substrates for wild banana seeds as none of the seeds from both species were germinated even for the period of up to 3

months (Figure 3 and 4). This might due to in ambient condition that being used in germinating seeds on filter paper as according to Chin (1996) the most common and effective treatment is the use of alternating temperature by germinating seeds under natural conditions in the glasshouse with day/night temperatures of 35-38/20°C. *M. violascens* recorded lower germination as compared to *M. borneensis* which were 36% and 53% respectively after 3 months. In addition, *M. violascens* also germinated later than *M. borneensis* where germination were observed only after 2nd month of sowing as compared to 1st month in *M. borneensis.* Among all the substrates tested, soil was found to be the most preferred substrates for germinating seed of *M. borneensis.* Although germination percentage of seeds on sand were higher as compared to soil during the 1st month (Figure 4 and Figure 5), but the percentage significantly increased in soil as the duration was prolonged. In this current study, there was variable germination responses seen among species tested. Different germination pattern was also observed on another species where only 2% of wild *M. acuminata* ssp Malaccensis germinated in soil mixture after 40 - 50 days in greenhouse condition (Asif et al., 2001). Although soil resulted the highest germination percentage, however 53% was still considered low. Scarification treatment was done by chipping the seed in order to break seed dormancy thus increase the germination percentage. Unfortunately, chipping was not the right method of breaking dormancy in *M. violascens* and *M. borneensis* seeds as all seed on all substrates for both species were fail to germinate. This could be chipping process which might injured the seed.

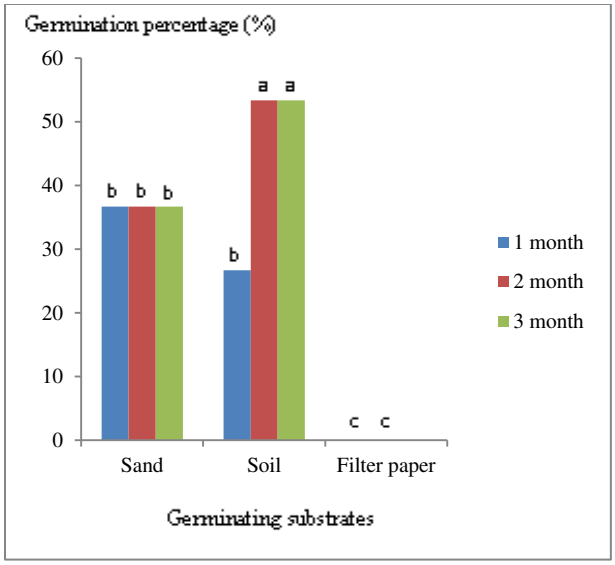

Figure 3: Germination percentage of *M. violascens* seeds on sand, soil and filter paper after 1. 2 and 3 months.

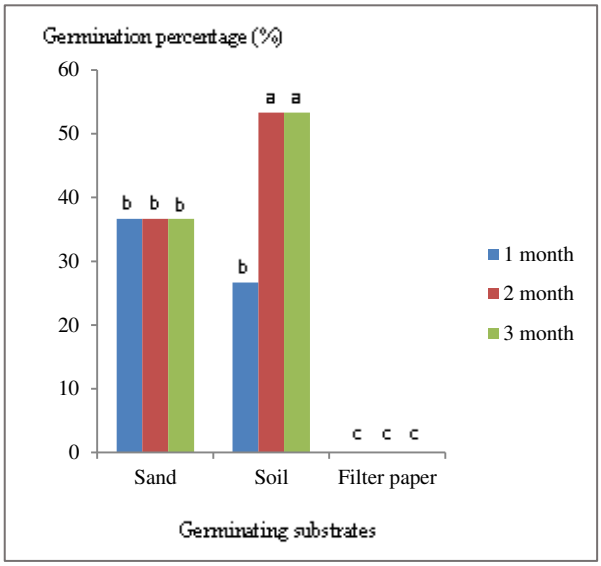

Figure 4: Germination percentage of *M. borneensis* seeds on sand, soil and filter paper after 1, 2 and 3 months.

Means with different letters within the same figure are significantly different at p≤0.05.

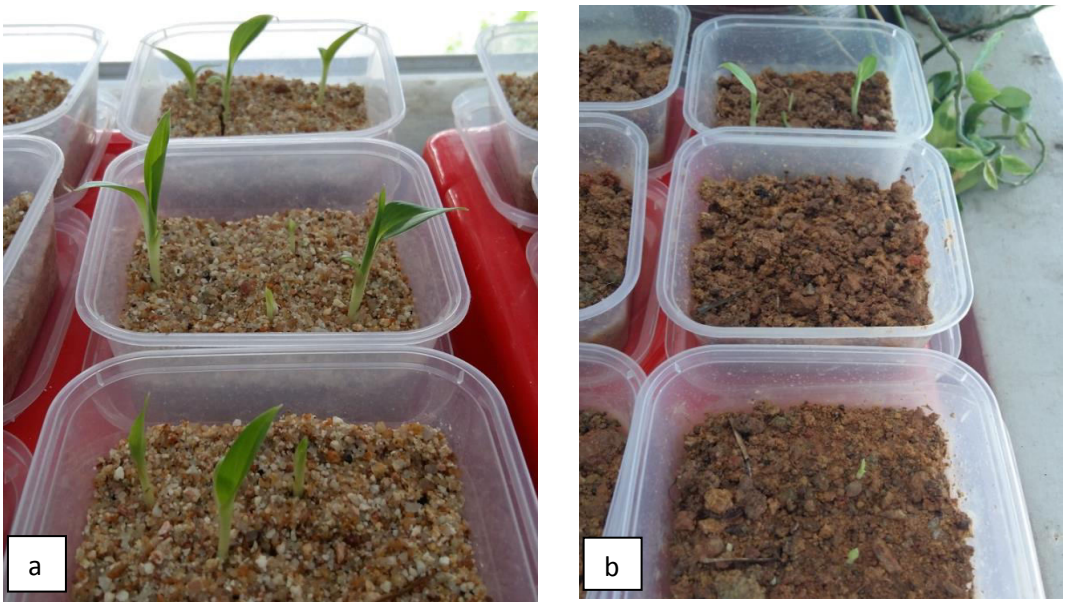

Figure 5: One-month old seedlings of *M.borneensis* in sand (a) and soil (b).

Higher germination percentages were recorded which were 43% and 86% for *M. violascens* and *M. borneensis* respectively when cultured on MS media as compared to the germination on sand and soil for all germination period (Figure 6 and 7). Embryos of *M. violascens* started to germinate earlier when cultured which was during the second weeks of culture (Figure 8) as compared to sand and soil (2 months). Similar trend was found by Pancholi et al. (1995) where *in-vivo* seed germination reached 78% but only after 9 months. Because of this delayed and intermittent germination, embryos were excised from seeds and inoculated onto half-strength Murashige and Skoog (1962) medium and after 2 weeks, 82% of embryos were germinate. Seeds of *M. balbisiana* sown in seed tray in a peat-based mixture or in petri dish on wet filter paper or cotton did not germinate even after five months. 40-66% embryo germinated *in-vitro* after 2 weeks (Ahmed et al., 2006). Only 2% of wild *M. acuminata* ssp malaccensis germinated in soil mixture after 40-50 days greenhouse condition compared to up to ≥ 90% germination in culture media which took only 1 week (Asif et al., 2001). In this recent study, there was no treatment applied during culture such as soaking or addition of plant growth regulator as according to Pancholi et al. (1995), the addition of GA_3 did not improve the germination percentage in *Musa* and GA_3-free treatment gave the highest germination percentage. Study done by Asif et al. (2001) proved that embryo germination of *Musa* was achieved without soaking. Gelling agent and light have no significant influence in seed germination. According to Uma et al. (2011), culture conditions have no significant influence in seed germination of *Musa* provided that the seed should fully matured during harvest.

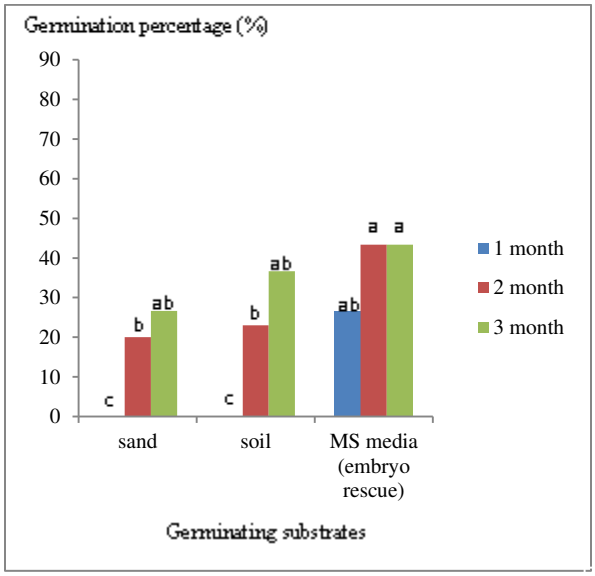

Figure 6: Germination percentage of *M. violacens seeds* on sand and soil, embryos on MS media after 1, 2 and 3 months.

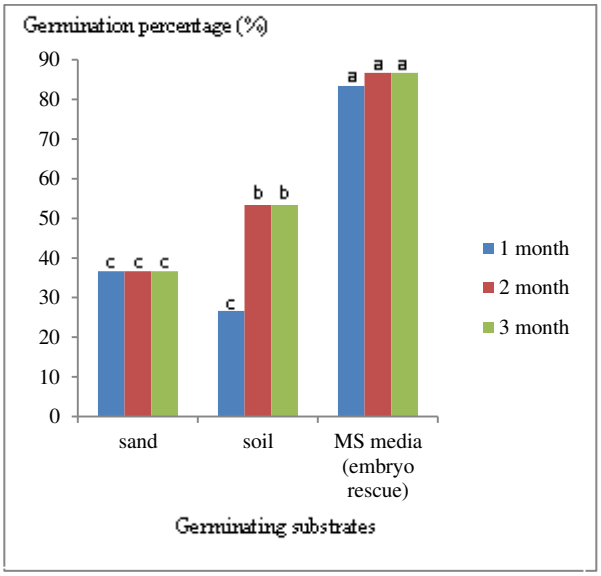

Figure 7: Germination percentage of *M. borneensis* seeds on sand and soil, embryos on filter MS media 1, 2 and 3 months.

Means with different letters within the same figure are significantly different at p≤0.05.

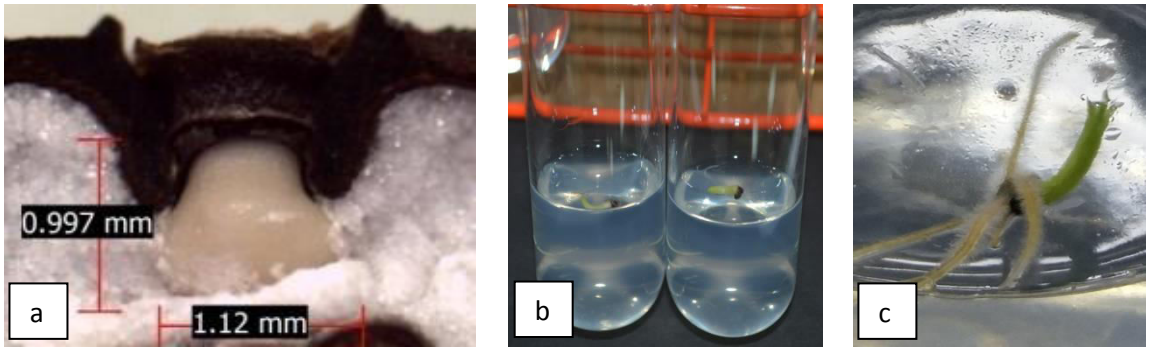

Figure 8: Tissue culture. Embryo (a), after 2 weeks (b) dan after 3 weeks (c).

Conclusions

It was concluded that different types of germination substrates, germination method and banana species resulted in different germination potential. *In vivo* germination was prolonged and intermittent while *in vitro* embryo culture enhanced the speed and proportional success of germination.

Acknowledgements

We thank the Global Crop Diversity Thrust and Millennium Seed Bank Partnership for providing the fund on the project entitled Collection of Crop Wild Relatives in Malaysia that made this work possible.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Germination of *Chromolaena odorata* L. Seeds as Affected by Light

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Introduction

Plants can be classified in terms of their responses to light for germination as follows: (i) those that require light to germinate, (ii) those that require darkness to germinate and (iii) those that have a large percentage of seeds neutral to light. These groups have been named positive photoblastic, negative photoblastic and neutral photoblastic by Baskin and Baskin (2014). *Chromolaena odorata* which belonging to the plant family Asteraceae is a vigorously growing dicotyledonous weed with a lifespan of more than three years. Under favorable weather conditions, the plant can grow up to 25cm in height in a week and within six months 2-3m in height forming an almost impenetrable shrub. The whole plant is hairy and granular and emits a pungent odor when crushed (Sheldrick, 1968). On good open land *C. odorata* forms a much branched stem (Gunasekara, 1994). Distribution of *C. odorata* is limited to areas with a rainfall of 200 cm and above and where temperature ranges from 20°C to 37°C (Timbilla and Braimah, 2000). Traditionally, fresh leaves or a decoction of *C. odorata* have been used for many years for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Le, 1995). *Chromalaena odorata* is an ornamental plant that has been used in shifting slashand-burn agriculture to compete with *Imperata cylindrica* (alang alang or cogon grass), which is harder to control (GISD, 2015). This species competes effectively with other plants and crops and may become the dominant species. Initially *C. odorata* spreads by seeds, but after establishment it may also reproduce vegetatively from lateral branches. It can also re-grow after slashing and burning. The ripe seeds are wind dispersed, although adherence to the fur of animals and machineries are also important modes of seed dispersal over long distance (Hoevers and M'Boob, 2003). Its stems branch freely with lateral branches develops in pairs from the axillary buds. The flower heads are borne in terminal corymbs of 20 to 60 heads on all stems and branches. The flowers are white or pale bluish-lilac, and form masses, covering the whole surface of the bush. The seeds of Siam weed are small (3-5 mm long, ~1 mm wide, and weigh about 2.5 mg/seed (Vanderwoude et al., 2005). It was reported that this species able to produce about 80000 to 90000 seeds per plant and require light to germinate. *Chromolaena odorata* is spread mainly by seeds, yet little is known about the environmental factors that affect germination and emergence of this species. The aim of the present study was to analyze the effect of light on germination of *C. odorata* seeds.

Materials and Methods

Seeds of *C. odorata* were collected from three different locations in Kuala Terengganu, Terengganu. Infected and infested seeds were removed, only clean seeds were selected randomly for further experiments. There were three replicates of 0.1 g each for seed moisture content (MC) determination. A germination test was performed with four subsamples of 50 seeds each, distributed on paper towels, moistened with water and placed in germination room at 28°C. The germination process was done in two conditions i) without light ii) with light (using white fluorescent lamps) in order to understand

photosensitivity of *C. odorata* seed. Assessment was performed according to the rules for seed analysis by International Seed Testing Association (ISTA). The seedlings that presented open cotyledon leaves were counted and the results were expressed in percentage. Observation was done two days of time interval for at least 30 days.

Results and Discussion

Seed moisture content is the most reliable indicator of seed maturity and harvest timing in grass seed crops (Thomas et al., 2010). The low water content in the seeds indicates the maturity level of seeds that are suitable for collection. Collection of *C. odorata* seed also takes into account this factor. The moisture content in seed after collection has been taken and the data on seed moisture content of *C. odorata* seeds are presented in the Table 1. The moisture content of all seeds collected from different seedlots number gave 14 to 26%. The percentage of those moisture content is suitable as a guidance for the maturation of *C. odorata* seed for harvesting as suggested by the previous researcher. McNeal and York (1964) studied the effects of drying sorghum seed on viability. They concluded that sorghum to be used for seed should be harvested when the moisture content is about 20%.

Table 1: Seedlots and moisture content of *C. odorata* used in the experiment.

Seedlots number	Moisture content (%)
TKT 1	14.3552
TKT 13	17.5106
TKT 20	26.7786

Seeds germinated under light (white fluorescent lamps) had a higher germination percentage for all locations (TKT 1, TKT 13 and TKT 20). Seeds collected from location TKT 1 gave 72% of germination followed by seeds collected from TKT 13 and TKT 20, 37.5 and 25.5%, respectively (Figure 1). Seeds germinated in dark conditions showed lower percentage for all locations which is between 5 to 32.5%.

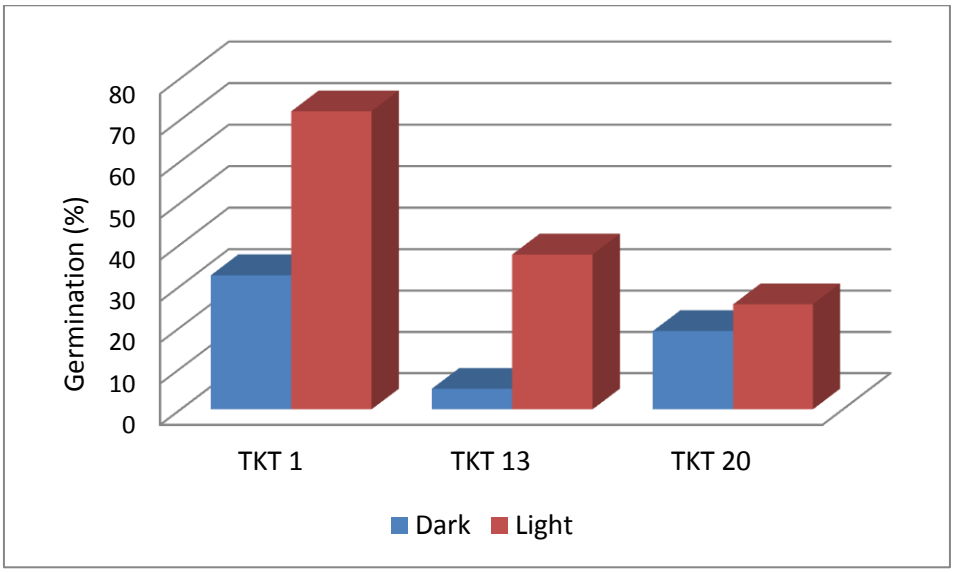

Figure 1: Percentage of seed germination of *Chromolaena odorata* as influenced with or without light.

Light and temperature are the main factors that promote germination in soils with good water availability. In determining the effect of light conditions on germination, we found a differential response by *C. odorata* seeds when exposed to light. This result is in concurrence with Yamauchi et al. (2004), where seeds exposed for short periods of time to red light increased transcription levels of genes related to GA metabolism and seed germination. Thus, light should be provided to *C. odorata* seeds to improve germination rates. In a dark treatment, fewer seeds would germinate and emergence would be somewhat delayed. Light is one of the main cues initiating germination in weeds but its effect can interact with other factors in the seed's environment. According to Smith (1975), the phytochrome is responsible for germination in light sensitive seeds. Furthermore, Takaki (2001) considered that all seeds possess phytochrome; however, those that germinate as much in the light as in the dark present phytochrome A and those that germinate only in the light contain phytochrome B. Based on the results of the current study, it appears that exposure to light intensity is important factor promoting germination of *C. odorata* seeds. To the present of study revealed that this species preferred open sunny habitats for growth. Seeds can use light to detect if they are close to the soil surface, this is especially important in the case of smallseeded species like *C. odorata* because small seeds have limited resources and these seedlings could not emerge successfully if they germinate too deep in the soil (Fenner and Thompson 2005). It has been suggested that light response and seed mass coevolved as an adaptation to ensure germination of small-seeded species only when they are close enough to the soil surface as to be able to emerge (Milberg et al. 2000).

Conclusions

The data discussed is part of the study conducted on *Chromolaena odorata* seeds. The results showed that *C. odorata* seeds germinate higher with the presence of light. Our findings support the hypothesis that small seed mass and light requirements are coevolved as an adaptation to ensure germination. These observations can help in handling the *C. odorata* seeds for planting, especially in handling seeds from future selected mother trees in the production of high quality plant materials.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Chapter 4

Post-Harvest Technology and Quality Control

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Anti-fungal Activity of Betel Leaf Extract against Stem-end Rot Disease of Papaya during Cold Storage

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Introduction

Papaya (*Carica papaya* L.) fruit belonging to the family Caricaceae, has a high demand due to its affordable price, easy availability as well as its nutrient value (vitamin A, C, minerals, fibers) and dietary antioxidants such as Beta-carotene, B-cryptoxanthin and Lycopene (Rivera-Pastrana et al., 2010). Even though it is native to tropical America, it is widely grown as a commercial fruit crop in many tropical and sub-tropical regions around the world (Bautista-Baños et al., 2013). In Malaysia, papaya is one of the main fruit products that gives high economic value for farmers and export market. Currently, 'Sekaki' is the leading cultivar for domestic consumption and export market, where Hong Kong is the major export destination of Malaysian papaya followed by Singapore, United Arab Emirates and Brunei (Rahman, 2008).

The rapid ripening and softening process limits the storage, handling and transport potential of papaya (Ravneel Prasad, 2015). It has a high percentage of postharvest losses combined with diseases by microorganisms, especially fungi. According to Alvarez and Nishijima (1987), papaya postharvest losses due to diseases are ranged from 1-93% depending on postharvest handling and packing procedure. There are three general types of diseases; fruit surface rots, stem-end rots and internal fruit infections. A research with 'Sekaki' cultivar in Malaysia, showed that among the diseases the highest disease incidence and severity were recorded for anthracnose caused by *Colletotrichum gloeosporioides*, followed by stem-end rot caused by *Botryodiplodia theobromae* (Rahman, 2008). Netto et al. (2014) mentioned that disease incidence of stem-end rot caused by fungi, *Lasiodiplodia theobromae* can reach around 70-80%, with a resulting reduction in commercial value of the fruit, thus it makes very important disease of papaya worldwide.

Botryodiplodia theobromae invade the severed peduncle after harvest as well as spores may invade through crevices between the peduncle and the papaya flesh or invade through small wounds that occur at the harvest and cause stem-end-rot disease (Alvarez and Nishijima, 1987). Traditionally control of papaya postharvest fungal diseases depends on a series of chemical fungicides, where two most common fungicides, thiabendazole and benomyl are known to control the disease up to 50% (Hewajulige and Wilson, 2010). However, prolonged application of single fungicide on fresh fruits and vegetables may lead to development of resistant strains and reduce the effectiveness of fungicide against the target organisms. At the same time due to the carcinogenicity, long degradation time and high residual toxicity harming to consumers' health and environment, most researches are focused at finding alternative to synthetic fungicides for postharvest disease management (Ali et al., 2016).

The utilization of natural products, especially the plant extracts, has been shown to be effective against many plant pathogens and considered to be safe for consumers and environments (Bussaman et al., 2012). *Piper betel*, a member of the Piperaceae, is also known "Sirih" in Malaysia and Indonesia, is one of the invaluable medicinal plants where its leaves have been used for many medicinal purposes (Guha, 2006). *P.*

betel leaves contain a wide variety of biologically active compounds, which are responsible for its antioxidant, antifungal, antibacterial and chemo preventive activity (Ali et al., 2010). Up to date there is no report about the use of betel leaf extracts to control postharvest diseases of papaya. The aim of the current work is to elucidate the effect of betel leaf extract to control the growth of stem-end rot pathogens in papaya and to apply it at a suitable concentration to inhibit the spore germination and mycelial growth of the pathogen.

Materials and Methods

Preparation of betel leaf extract

Betel leaf extract in propylene glycol was obtained from Indonesia (Haldin Pacific Semesta Company) and used as 100% original containing 200 ppm total phenolic compounds. Betel leaf ethanolic extract was prepared locally following the method by (Bussaman et al., 2012). Fresh betel leaves were bought from Semenyih, Selangor and washed thoroughly using tap water. After air-dried, the leaves were dried in a hot-air oven at 45-50°C for two days. Dried leaf samples were ground using small grinder and the leaf powder was placed in glass bottles. The powdered samples were stored at 4°C until required.150 g of leaf powder was added to 450 mL of 80% (v/v) ethanol (thus ratio between leaf powder and solvent was 1:3) and the mixture was agitated for 72 h on a rotary shaker (120 rpm). The obtained extracts were centrifuged at 8,000 rpm for 10 min. The extract was filtered through Whatman filter paper No.1 and transferred to 500 mL round-bottom flasks. Finally, the solvent ethanol was evaporated from extract using rotary evaporator at 45°C (Bussaman et al., 2012). The crude extract was dissolved in distilled water in same volume equal to the ethanol volume used to macerate. This extract was used as the betel leaf ethanolic extract 100% for *in vitro* studies.

Isolation and identification of pathogen

Symptomatic tissues (1 cm^2) from the skin of papaya were surface-sterilized using 1% (v/v) sodium hypochlorite for 3 min, followed by three washes with purified water and drying on sterile paper. The edges of the surface- sterilized tissues were cut off and the tissues were cultured on Petri dishes containing potato dextrose agar (PDA) and incubated at room temperature (26 ± 2°C). Once mycelial growth was observed, the colonies were re-isolated on fresh PDA dishes to obtain pure cultures. The isolates obtained were then identified based on their morphological and cultural characters. Re-isolations were carried out continuously on PDA slants to maintain inoculum. Two slants of inoculum were sent to CABI (Microbial Identification Service), UK for molecular identification.

Pathogenicity test

Koch's postulate was carried out to test pathogenicity. Healthy papaya fruits were surface sterilized using 0.5% (v/v) sodium hypochlorite solution followed by washing with purified water. Conidia were harvested from 10 days old culture of *L. theobromae* by filtering through four layers of cheesecloth. Sterile purified water was used to adjust the conidial concentration to 1×10^6 conidia mL^{-1}, using a hemocytometer. A sterile sharp tooth pick was used to make 2 mm deep artificial wounds at the base of the fruit and each wound was inoculated with 20 µL of conidial suspension using a sterile syringe needle. The fruits were placed in a cardboard box for incubation for 10 days at 26 ± 2°C and observed daily for lesion development.

In vitro antifungal assay

PDA plates were amended with different concentrations (6%, 8%, 10% and 12%) of two types of original betel leaf extracts, separately. One was betel leaf ethanolic extract and other one was betel leaf extract in propylene glycol. The "poison food technique" (Sivakumar et al., 2002) was carried out to evaluate antifungal activity of betel leaf extract on the inhibition in radial mycelial growth of *L. theobromae* on PDA medium. 5 mm diameter of *L. theobromae* plugs obtained from actively grown pure culture were placed in the center of PDA dishes containing the different concentrations of Betel leaf extracts. Petri dishes containing only PDA were used as controls. The petri dishes were incubated at room temperature (26 ± 2°C) and radial mycelial growth was assessed every two days until the mycelium in control dishes reached the edge of the plate. The effect of the treatments was assessed by comparing growth (mm) of the fungal mycelium and the percentage inhibition in radial mycelial growth (PIRG) was calculated using formula described by Al-Hetar et al. (2011).

In vitro spore germination inhibition test was performed following the method of Ali et al. (2016). Using a sterile, bent glass rod, 0.5 mL of spore suspension of *L. theobromae* (500 spores per plate) were spread on the PDA plates with each treatment. The hemocytometer was used to determine the density of the spore suspension while sterile distilled water was added to adjust the desired concentration of the spore suspension. For control dishes contained PDA only. After 24 h, the number of germinated spores was counted in 10 microscopic fields of 100 spores in 20 replicated plates under a light microscope at 10× magnification. Percentage of inhibition in germination was calculated by the method of Cronin et al. (1996).

Studies of scanning electron microscopy (SEM)

PDA plates were amended with different concentrations (6%, 8%, 10% and 12%) of both types of betel leaf extracts for inoculate conidial spores and mycelium plugs separately. After inoculation, the mycelium plugs were incubated for 6 days and spores were incubated for 2 days at room temperature (26 ± 2°C). After incubation, a thin layer of 0.8 cm^2 squares of PDA with conidial spores and PDA with mycelium were cut off separately from each treatment plates and mounted on stubs to get SEM images, immediately. The SEM images were taken at ×500 and ×2500 magnification for conidia and ×1200 magnification for mycelia using FEI Quanta 400F, Field Emission Scanning Electron Microscope.

Statistical analysis

Four replicates were used for each treatment with 5 plates in each replicate. The treatments were conducted in a completely randomized design (CRD) throughout the experiment. Data were subjected to analysis of variance (ANOVA) using Genstat Software 15th Edition. Means were compared by Least Significant Difference (LSD) tests as $P<0.05$.

Results and Discussion

Lasiodiplodia theobromae Pat. colonies grew rapidly on PDA plates, covering the entire surface of the Petri dishes abundantly within 5 days. The mycelium was white at the beginning and then turned to dark greenish- gray and later became black when matured. Initially the conidia were hyaline, unicellular, oblong in shape, thick-walled with granular content. When conidia matured they were two-celled, light brown in color. According to CABI, the ITS rDNA sequence obtained from the sample of pure culture of

pathogen, produced top matches at 100% identity to sequence of *L. theobromae* (synonym *Botryodiploda theobromae*).

Pathogenicity test

Initially, water soaked wide margins were produced at the base of the papaya fruit by the pathogen. With the progression of the infection, the lesion margin remains translucent as the rest of the infected tissues turned to wrinkled, black and dry and white mycelium formed at the stem end on the affected area at an advanced stage of infection. Similar results were reported by Rahman et al. (2008) and Netto et al. (2014).

In vitro antifungal assay

Inhibition of radial mycelial growth of *L. theobromae* was observed after 5 days of incubation at 26 ± 2°C using two types of betel leaf extract (locally prepared betel leaf ethanolic extract and betel leaf extract in propylene glycol which was bought). The growth was decreased significantly ($P<0.05$) by the different types of betel leaf extract at all concentrations tested as compared to the control (Figure 1). The maximum percentage of inhibition in growth was observed as 97.91% with 12% locally prepared betel leaf ethanolic extract (where 91.87% was observed with 10%), followed by 90.77% with 12% betel leaf in propylene glycol extract. The germination of *L. theobromae* conidia was significantly ($P<0.05$) inhibited by both types of betel leaf extracts tested at all concentrations compared to the control (Figure 2). The maximum percentage inhibition in conidial germination 93.91% and 90.26% of *L. theobromae* was observed in 12% betel leaf ethanol extract and 12% Betel leaf propylene glycol extract. High sensitivity of conidia of *L. theobromae* to betel leaf ethanolic extract was observed with completely inhibited in germination at 12% concentrations.

Even though the germ tube of few conidia grew for 24 hours in 10% betel leaf ethanolic extract and 12% betel leaf propylene glycol extract, but they ruptured after two days, thereby no mycelium colonies could be seen after incubation in these plates. Antimicrobial activity of betel leaf extract has been detected towards many bacteria and fungi. (Ali et al., 2010b; Sugumaran et al., 2011; Hoque et al., 2012). The active ingredient of piper betle oil which is obtained from the leaves are primary a class of allyl benzene compounds, chavibetol, chavicol, estragole, eugenol, methyl eugenol and hydroxycatechol (Sugumaran et al., 2011). *Piper betle* exhibited more effective anti-bacterial and antifungal properties and hydroxychavicol is one of the major constituents of *P. betle* (Ali et al., 2010b).

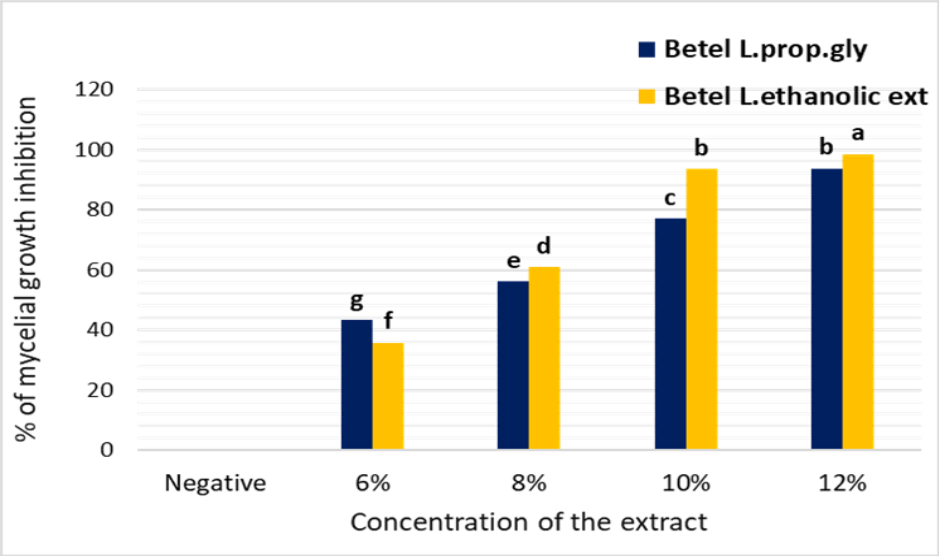

Figure 1: Effect of various concentrations of betel leaf extracts on mycelial growth inhibition of *L. theobromae*.

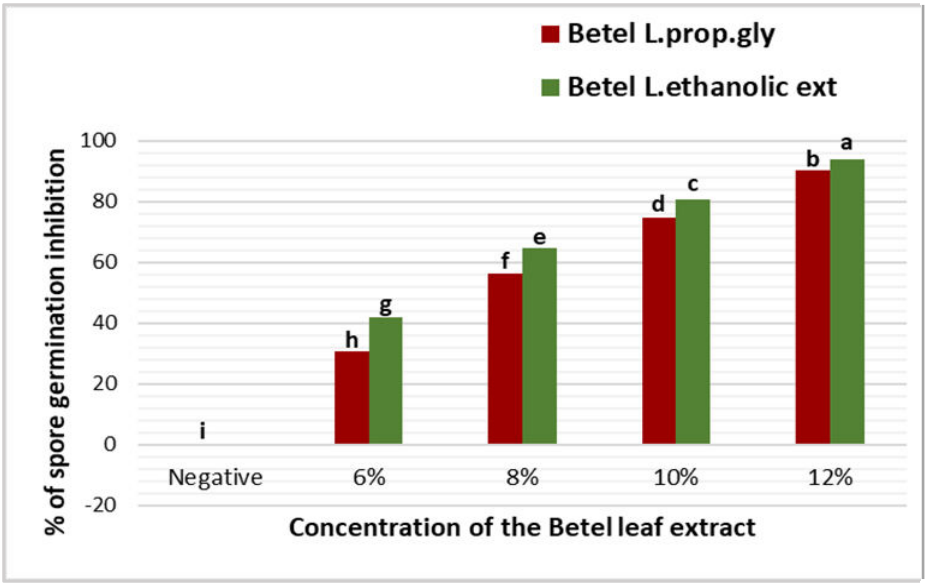

Figure 2: Effect of various concentrations of betel leaf extracts on spore germination inhibition of *L. theobromae.*

Studies of scanning electron microscopy (SEM)

The results of scanning electron microscopy (SEM) images showed disruption and distortion of fungal mycelium and fungal conidial spores grown in 8%, 10% and 12% of the both types of betel leaf extract. Refer to Figure 3, ruptured and deformed conidia with sunken walls may due to leakage of cell membrane (indicated with arrow in respective figures). Further confirmed the earlier findings that hydroxychavicol alters the cell membrane structure, resulting in the disruption of the permeability barrier of microbial membrane structures Ali et al., 2010b).

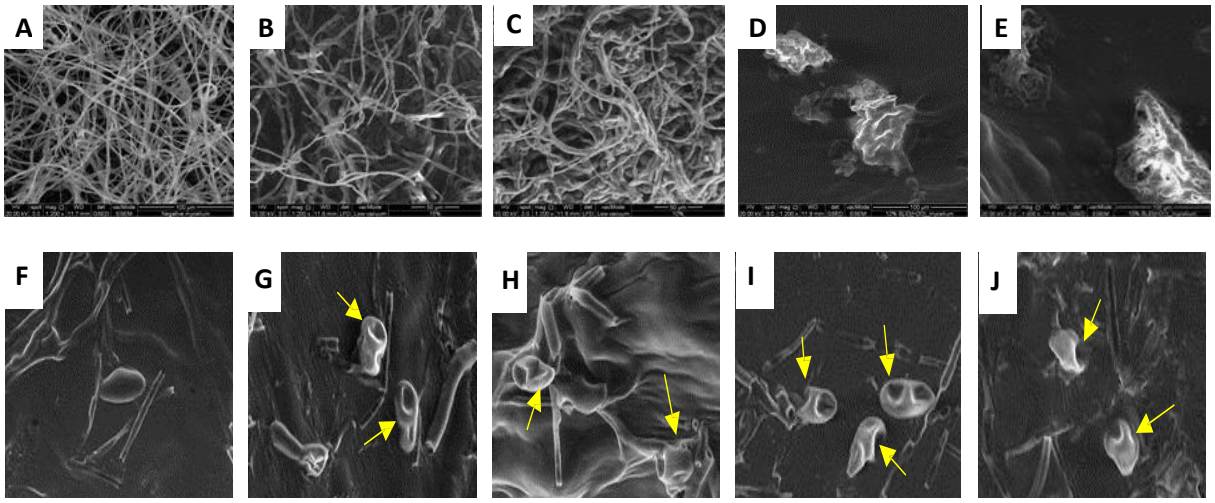

Figure 3: Images of scanning electron microscope of mycelial growth and spore germination on PDA amended with both types of betel leaf extracts. Negative control-: mycelia (A), where conidial spore germination (F). Betel leaf propylene glycol-: mycelia in 10% (B) and in 12% (C), where conidia in 10% (G) and in 12% (H). Betel leaf ethanolic extract-: mycelia in 10% (D) and in 12% (E), where conidia in 10% (I) and in 12% (J).

Conclusions

The results suggest that betel leaf extract possesses bioactive compounds containing antifungal properties that can be useful to control *Lasiodiplodia theobromae* (*Botryodiplodia theobromae*) spore germination and mycelial growth. Antifungal properties of betel leaf ethanolic extract and betel leaf propylene glycol extract could be fruitfully utilized in postharvest stem-end rot disease and to extend shelf-life of papaya and other fruits. Betel leaf extract can be used as an eco-friendly "green fungicide" instead of synthetic fungicide.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Different Storage Temperatures on Physicochemical Characteristics and Quality of Melon Manis Terengganu (*Cucumis melo* var. Inodorus cv. Manis Terengganu 1)

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Introduction

In general, there are three types of melons in Malaysia, namely watermelon (*Citrullus lanatus*), rockmelon (*Cucumis melo* var. Cantalupensis), and honeydew (*Cucumis melo* var. Inodorus). These melons are widely cultivated and easily obtained in Malaysia. Even though there are more than 500 varieties of melons and 150 varieties of watermelons all over the world, only Super Dragon (watermelon), Jade Dew (honeydew) and Glamour (rockmelon) are the most popular ones in Malaysia (Rasmuna and Nik, 2016). Among these three types of melon, rockmelon is sweeter and has more compact pulp than watermelon. Melons not only boost our health esteem, but can also become an important part of our healthy diet with its refreshing characteristics (Rasmuna and Nik, 2016).

Under *C. melo*, there are seven sub species known as Cantaloupensis, Reticulatous, Inodorous, Flexuosus, Conomon, Chito and Dudaim (Boyhan et al., 2014). Cantaloupe melons have the highest amount of beta-carotene or also called pro-Vitamin A, are an essential nutrient required for eye health, and may have the potential, as an antioxidant to reduce the risks associated with cancer, heart disease, and other illnesses. Recently, a new developed variety of melon has been introduced specifically for Terengganu known as Melon Manis Terengganu (MMT) and has been developed by the Green World Genetics (GWG) Sdn. Bhd, Selangor. MMT has been claimed to have high nutritional value and can be further processed into various products such as melon ice cream, jelly, juice and jam (Utusan Malaysia, 2017). Approximately, around 666 metric tonnes of MMT can be produced from 222,000 melon plants which worth about RM2 million per harvest and can reached to RM6 million in a year (New Straits Times, 2017).

As a new developed variety, MMT postharvest biology and technology's information is still scarce. Proper postharvest handling practices are essential in reducing postharvest losses and maintaining the fruit quality attributes. A number of studies on the effects of different postharvest treatments on the changes in physicochemical characteristics in various fruit crops were conducted. This showed that the important of postharvest aspects in Malaysian fruit industry particularly in MMT. In postharvest, the abiotic and biotic factors play a major role in maintaining the quality of the fresh produce. Among all these factors, temperature plays an important role in preserving the quality of the fresh produces. The most important factor in reducing postharvest losses and preserving quality are by monitoring appropriate temperature during processing, transportation, marketing and post-purchasing (Al-Ati and Hotchkiss, 2002). Therefore, this research focuses more on the effects of different storage temperatures on physicochemical characteristics and quality of MMT.

Materials and Methods

A total of 135 melon fruits at uniform maturity stage of (1⁄2 of full slip) and free from any defects and decay were chosen for the experiment. The fruits were purchased from the Taman Kekal Pengeluaran Makanan (TKPM) Besut, Terengganu and immediately transported to the Postharvest Technology Laboratory, Universiti Malaysia Terengganu. The experiment was arranged in a complete randomized design (CRD) with three replications. The fruits were placed in the basket and then stored at different storage temperatures viz.; 5ºC, 10ºC, 15ºC, 20ºC and 25ºC served as control. Each replicate consists of nine fruits. The fruits were stored for 24 days and the parameter evaluations were done on every three days interval, day 0, 3, 6, 9, 12, 15, 18, 21 and 24. Postharvest parameters assessed were the percentage weight loss, fruit color, total soluble solids concentration, fruit firmness, titratable acidity, ascorbic acid concentration and total phenolic content of MMT fruits. Sensory evaluation of MMT also been assessed for every three days interval. Thirty panellists were selected for sensory evaluation test and the score given by the panellist were calculated to identify the level of acceptance and quality of the fruit. The data were subjected to one-way analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by Tukey for minimum significant difference at $P \leq 0.05$ (SAS Institute Inc., 1991).

Results and Discussion

The effects of different storage temperature on MMT fruits were noticeable on the percentage of weight loss (Figure 1). In general, the weight loss of the MMT was below 3% at the end of three weeks of storage. Keeping the melon in high humidity environment able to retard the promotion of the enzyme-substrate interaction due to the maintenance of full cellular hydration and reduction in cell disorganizations and thereby prolong the postharvest life of the fruit (Lester and Bruton, 1986). Cohen and Hicks (1986) claimed that the appearance of melon with less than 4% weight loss was considered acceptable and there was no sign of physical shrivelling to the fruit. In line to this, the percentage weight loss of MMT fruits can be considered acceptable either stored at ambient or low temperature storage for 24 days. Furthermore, this slight moisture loss did not cause obvious deleterious changes in other quality parameters such as peel and pulp colours, fruit firmness and titratable acidity (Table 1 and 2, Figures 2, 3 and 4) and also sensory evaluation.

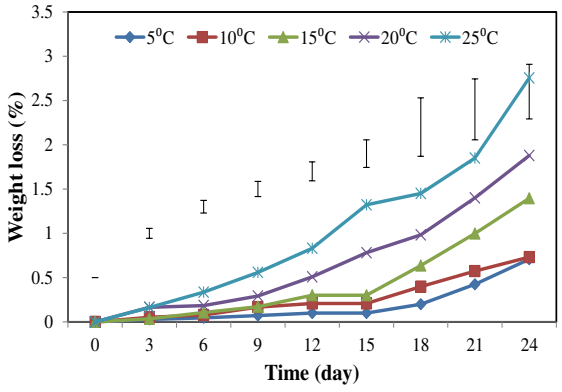

Figure 1: Effects of different storage temperatures on percentage weight loss of Melon Manis Terengganu. Vertical bars represent $HSD_{0.05}$.

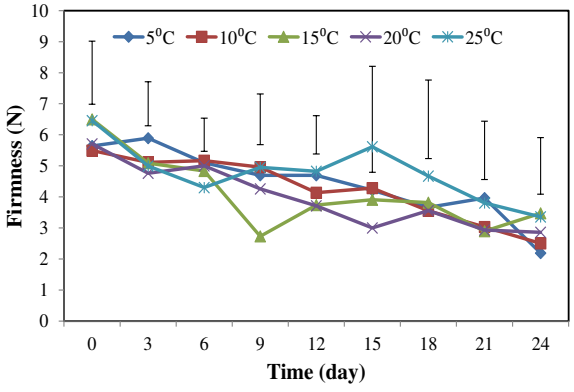

Figure 2: Effects of different storage temperatures on fruit firmness of Melon Manis Terengganu. Vertical bars represent $HSD_{0.0.5.}$

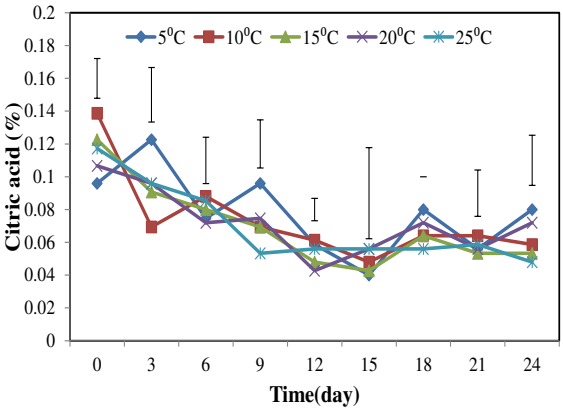

Figure 3: Effects of different storage temperatures on tiratable acidity of Melon Manis Terengganu. Vertical bars represent $HSD_{0.05}$.

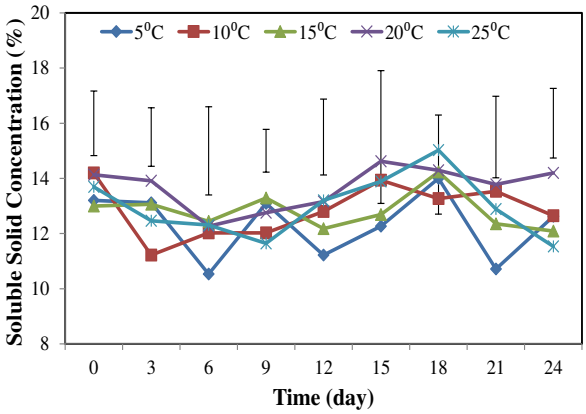

Figure 4: Effects of different storage temperatures on soluble solids concentrations of Melon Manis Terengganu. Vertical bars represent $HSD_{0.05}$.

Table 1: Effects of different storage temperatures on skin colour of Melon Manis Terengganu.

Treatment/Day	0	3	6	9	12	15	18	21	24
Lightness (L*)									
5^0 C	69.1	72.3	73.3	72.4	73.2	72.7	72.9	73.3	73.6
10^0 C	72.2	73.5	73.1	73.9	74.9	74.9	74.2	74.3	72.5
15^0 C	72.1	72.3	68.1	72.7	73.2	73.9	72.6	72.9	74.5
20^0 C	72.1	73.5	74.0	73.3	74.1	73.7	73.5	73.9	74.1
25^0 C	70.5	73.4	75.8	72.7	75.0	74.0	71.0	71.6	74.3
$HSD_{0.05}$	11.68^{ns}	2.78^{ns}	5.43^{***}	3.65^{ns}	2.82^{ns}	5.64^{ns}	4.10^{**}	3.67^{ns}	4.40^{ns}
Chromaticity a*									
5^0 C	-4.44	-0.42	1.75	0.19	6.33	-0.57	2.56	-3.07	5.61
10^0 C	-1.51	4.87	-0.85	-0.30	5.01	5.10	5.63	0.74	6.35
15^0 C	-1.15	-0.80	1.16	-0.58	3.91	4.66	6.76	0.15	6.84
20^0 C	0.50	-0.55	-1.44	0.62	5.71	5.00	7.60	3.53	10.74
25^0 C	-1.10	-0.49	-1.46	0.50	0.67	7.46	8.55	4.62	4.71
$HSD_{0.05}$	2.57^{***}	15.65^{ns}	6.11^{ns}	3.98^{ns}	5.20^{**}	8.82^{**}	4.64^{***}	5.16^{***}	9.58^{ns}
Chromaticity b*									
5^0 C	67.7	76.5	77.8	73.1	72.7	67.5	71.9	73.0	74.4
10^0 C	76.3	71.8	74.9	74.1	70.9	69.8	73.0	76.1	73.4
15^0 C	74.3	71.3	70.1	69.6	67.7	70.8	67.8	73.6	73.3
20^0 C	73.4	73.4	74.2	72.8	73.9	69.6	71.3	75.3	75.7
25^0 C	74.4	73.5	74.5	72.0	68.7	72.7	65.4	76.4	71.0
$HSD_{0.05}$	8.08^{**}	8.80^{ns}	29.63^{**}	5.68^{ns}	6.90^{ns}	12.5^{ns}	20.7^{ns}	6.8^{ns}	8.59^{ns}
Hue angle (h°)									
5^0 C	90.9	90.3	88.7	89.9	89.5	90.5	88.0	92.5	85.7
10^0 C	91.1	85.9	90.7	90.2	86.0	86.0	85.6	89.5	85.1
15^0 C	90.9	90.7	86.4	90.5	86.7	86.2	84.2	89.9	84.7
20^0 C	89.6	90.5	91.1	89.5	85.6	85.9	83.9	87.3	81.9
25^0 C	93.7	90.4	91.1	89.6	85.0	84.2	82.3	86.5	86.3
$HSD_{0.05}$	1.99^{***}	12.72^{ns}	8.72^{ns}	3.19^{ns}	4.01^{**}	6.62^{**}	5.82^{**}	4.26^{***}	6.92^{ns}

*ns= not significant ($p>0.05$), **= very significant ($p<0.05$) and *** = highly significant ($p<0.01$).*

Peel and pulp colour development of MMT were not evident as shown in Table 1 and 2. Regardless of storage temperatures, the lightness (L*) and chromaticity b* in pulp and peel remain constant, throughout the experiment. Meanwhile, chromaticity value a* and hue angle (h°) of peel and pulp of MMT were fluctuated throughout 24 days experiment. These fluctuations occur might be attributed to the various pigments at varying concentrations such as chlorophyll, carotenoid and flavonoid. Similarly, Syahidah et al. (2015) have reported that the fruit flesh is not uniform in color which is probably due to different stages of maturity throughout the whole fruits. Furthermore, Supapvanich et al. (2011) claimed that although the lightness, whiteness index and chroma changed during storage, these might not give any influence on the changes in the both intact and fresh-cut melon fruit. As melon ripen, the peel colour change from a light yellowish- green to a deeper yellow. These changes can be seen in all MMT stored at various temperatures. However, the changes were not evident. Moreover, chromaticity is related to the quality of the color and it was used to determine the color saturation or intensity or purity (Syahidah et al., 2015). Similarly, Zainal Abidin et al. (2013), stated that the chromaticity of fresh-cut cantaloupe whilst in storage, there was no significantly different and did not affect the surface browning and loss of yellow colour. This indicates that the color of fresh cut cantaloupe became less saturated with the increasing storage time. In addition, Miccolis and Salveit (1995) have reported that the external color and flesh color did not markedly change during storage. Thus, the color of the MMT skin and flesh were not markedly affected by the different storage temperatures.

Table 2: Effects of different storage temperatures on flesh colour of Melon Manis Terengganu.

Treatment/Day	0	3	6	9	12	15	18	21	24
Lightness									
5^0 C	65.6	64.9	66.6	64.8	67.7	73.1	70.0	68.6	70.0
10^0 C	64.3	67.8	67.5	65.4	65.7	71.7	65.8	65.1	67.9
15^0 C	66.6	68.4	71.6	66.2	66.9	72.7	70.4	67.2	69.9
20^0 C	65.8	66.7	70.3	64.4	66.0	71.3	67.9	63.1	69.9
25^0 C	66.5	65.5	67.0	67.1	66.6	69.5	66.8	67.2	72.7
$HSD_{0.05}$	1.89^{ns}	3.39^{ns}	3.11^{ns}	2.67^{ns}	3.20^{ns}	3.16^{ns}	7.2^{ns}	2.00^{**}	5.76^{ns}
Chromaticity a*									
5^0 C	3.67	4.25	4.77	2.96	8.35	7.82	9.27	0.62	8.51
10^0 C	5.35	0.68	4.72	3.06	8.92	7.29	8.48	5.03	6.59
15^0 C	4.00	2.69	2.00	2.05	4.72	7.71	8.05	3.59	9.02
20^0 C	6.05	2.45	3.31	4.42	8.85	9.06	10.43	5.68	9.00
25^0 C	4.75	0.63	4.46	2.09	9.40	9.30	10.71	3.87	8.02
$HSD_{0.05}$	2.45^{**}	3.97^{ns}	4.18^{ns}	4.62^{ns}	5.69^{ns}	7.88^{ns}	4.31^{ns}	6.07^{ns}	6.21^{ns}
Chromaticity b*									
5^0 C	28.5	26.7	25.6	24.8	21.5	21.6	21.6	24.9	22.3
10^0 C	27.9	26.1	28.7	27.9	22.9	20.8	21.2	26.0	20.8
15^0 C	27.6	27.5	32.8	25.5	24.4	21.8	19.5	25.6	21.5
20^0 C	27.5	30.7	25.3	28.7	24.4	22.6	23.5	25.5	22.2
25^0 C	28.3	26.0	27.8	25.2	23.5	24.5	23.2	24.1	21.1
$HSD_{0.05}$	3.40^{ns}	11.76^{ns}	34.37^{**}	4.73^{ns}	5.45^{ns}	9.00^{ns}	6.15^{ns}	3.09^{ns}	9.11^{ns}
Hue angle (h°)									
5^0 C	82.6	81.0	79.5	83.2	69.8	70.1	66.7	88.8	69.2
10^0 C	79.1	88.8	80.7	83.8	69.9	70.9	68.2	79.0	72.9
15^0 C	81.8	84.4	86.2	85.5	78.3	70.9	67.7	82.0	67.2
20^0 C	77.6	85.3	82.5	81.3	70.0	68.2	66.1	77.5	67.9
25^0 C	80.5	88.8	80.9	85.5	68.4	69.2	65.2	81.2	69.3
$HSD_{0.05}$	4.92^{**}	8.75^{ns}	8.76^{ns}	9.65^{ns}	11.6^{ns}	14.73^{ns}	7.95^{ns}	13.72^{ns}	9.26^{ns}

*Means not significant ($p>0.05$), **means very significant ($p<0.05$) and *** means highly significant ($p<0.01$).*

As shown in Figure 2, the firmness of all MMT fruits stored at different temperatures decreased throughout the 24 days of storage. The loss of texture in fruits and vegetables closely related to the loss of turgor pressure, membrane degradation and disassembly of cell wall polymers (Brummell, 2006). Ranwala et al. (1992) claimed that the softening of melon is related with the modification of pectin and hemicellulose polymers and the loss of non-cellulosic neutral sugars. On the other hand, melon has lack of polygalacturonase activity during ripening and this might play role in pectin polymerization (Rose et al., 1998). In addition, Hadfield et al. (1998) supported a role of polygalacturonase in ripening-associated pectin degradation although only a small amount of the polygalacturonase activity was detected. Possibly, this is the reason on why the firmness of the MMT fruit was declined as the storage period prolonged.

For SSC, no apparent effect of temperatures was observed (Figure 3). Syahidah et al. (2015) claimed that during the maturing and ripening of fruits, the SSC of sugar usually increases and it can act as an indicator of maturity and the stage of ripeness. However, Miccolis and Saltveit (1995) reported that no significant effect of temperature on SSC during storage of different cultivars of melon. Moreover, Cohen and Hicks (1986) also had proved that the same results on Gold Star, Superstar and Saticoy cantaloupe cultivar which was stored at 5 or 12ºC for two to nine days and then continued to store at 20ºC for another two to five days. In addition, the best sugar level for fresh-cut Cantaloupe is between 10-13% (Beaulieu and Gorny, 2001). In the present study, the MMT still had the best sugar level even though the fruit were stored for three weeks at different storage temperature. Similarly, TA also had a decreasing trend as storage period prolonged (Figure 4). This was in agreement with the report of Zainal Abidin et al. (2013).

Sensory quality attributes and nutritive value of fruit play a vital role in consumer purchasing decision and also influence further consumption. Taste, aroma, texture and appearance are the most important quality attribute measured. For sensory evaluation, the physical and chemical parameters are considered to reflect the taste (SSC and TA), aroma (volatile), texture, and color (L*, a*, b*) of the fruit sample. Color has been known as one of the key factor affecting the product appearance (Kays, 1999; Olivas and Barbosa Canovas, 2005). This is because it was assumed by consumers to influence the taste and sweetness (Clydesdale, 1993). Interestingly, after day 3 onwards, all panellists like both colour of MMT, peel and pulp either stored at low temperature or ambient environment.

Texture is an essential characteristic that is highly desirable because consumers typically relate texture with freshness (Fillion and Kilcast, 2002). For texture attribute, there was no significant difference between all MMT fruits stored at different storage temperatures for 24 days of experimental period (Figure 5). Most of the panellists neither like nor dislike the texture of MMT. At harvest and after 3 days storage, all of the panel agreed at score 5 or like the texture of MMT regardless of storage temperature. Similarly, cantaloupe tends to reduce in hardness during storage (Bett, 2002). In contrast, Bett et al. (2011) reported that the cantaloupe stored at 10°C for 7 days exhibited a significant decrease in hardness after 5 days of storage and this may be due to the senescence processed occurred during storage period. In addition, the hardness of the fruit was also markedly decreased during storage (Beaulieu et al., 2004). Overall, all the texture attributes had no significant effect on the different storage temperature.

The aroma and taste of the melon were influenced by the ester compound as well as to a certain extent by sulfur compounds (Homatidou et al., 1992). The major compound associated with Honey Dew melon aroma are ethyl 2-methylbutyrate, ethyl butyrate, ethyl hexanoate, hexyl acetate, 3-methylbutyl acetate, benzyl acetate, (Z)-6-nonenyl acetate, and possibly (E)-6-nonenol and (Z,Z)-3,6-nonadienol (Buttery et al., 1982). Similar result was reported by Madrid and Cantwell (1993), the aroma of the honeydew pieces was declined at 5°C. Meanwhile, high CO_2 condition helped in reducing the aroma loss by retarding the development of off-odors which usually related to microbial growth as claimed by Portela and Cantwell,

(1998). In the present study, MMT fruity aroma was at natural condition as all panellists could not described the aroma of the fruit. All of the panel score 4, neither like nor dislike the aroma of MMT fruit regardless of storage temperature.

Fruit quality is usually estimated by the soluble solid concentration (SSC). However, it has been claimed that the SSC alone is insufficient for index of eating quality (Yamaguchi et al., 1977). Irrespective of storage temperatures, the sweetness of MMT was highly preferred by the panellist throughout 24 days of experiment (Figure 5). This reflects to SSC in MMT as shown in Figure 5. The SSC values were ranging between 11% and 14%. Similarly, Beaulieu and Gorny (2001) reported that the best sugar level for fresh-cut Cantaloupe is between 10-13%. In general, the SSC values of MMT fruit reduced as the storage period extended. Moreover, the amount of sugar is thought to decline during storage or to be buffered due to an increase in titratable acidity (Beaulieu and Baldwin, 2002). According to Lamikanra et al. (2000), the pH, titratable acidity, and SSC did not change during two weeks of the fresh-cut storage at 4°C. Thus, the panellist preference of sweetness level was not affected by the different storage temperature of the MMT fruit. According to Gadže et al. (2011) sour taste was related with organic acids and pH. In present study, the sourness taste of MMT fruit was at the range score of 3 and 4 which indicates that the panel cannot describe the sourness of the MMT on day 0 but they dislike the sourness of MMT on day 3 onwards.

Overall acceptance is the combination of the panellist's preference level towards colour, texture, aroma, sweetness and sourness of the MMT fruit. According to Lester (2006), consumer highlighted that flavour, sweetness and texture were the key factor in assessing the 'overall acceptance'. Figure 5 shows the overall acceptance attributes of MMT fruit treated with different storage temperature. The panel's overall acceptance level remained constant between score 4 and 5 which indicates that MMT stored at different temperatures for 24 days remain fresh and acceptable to be sold.

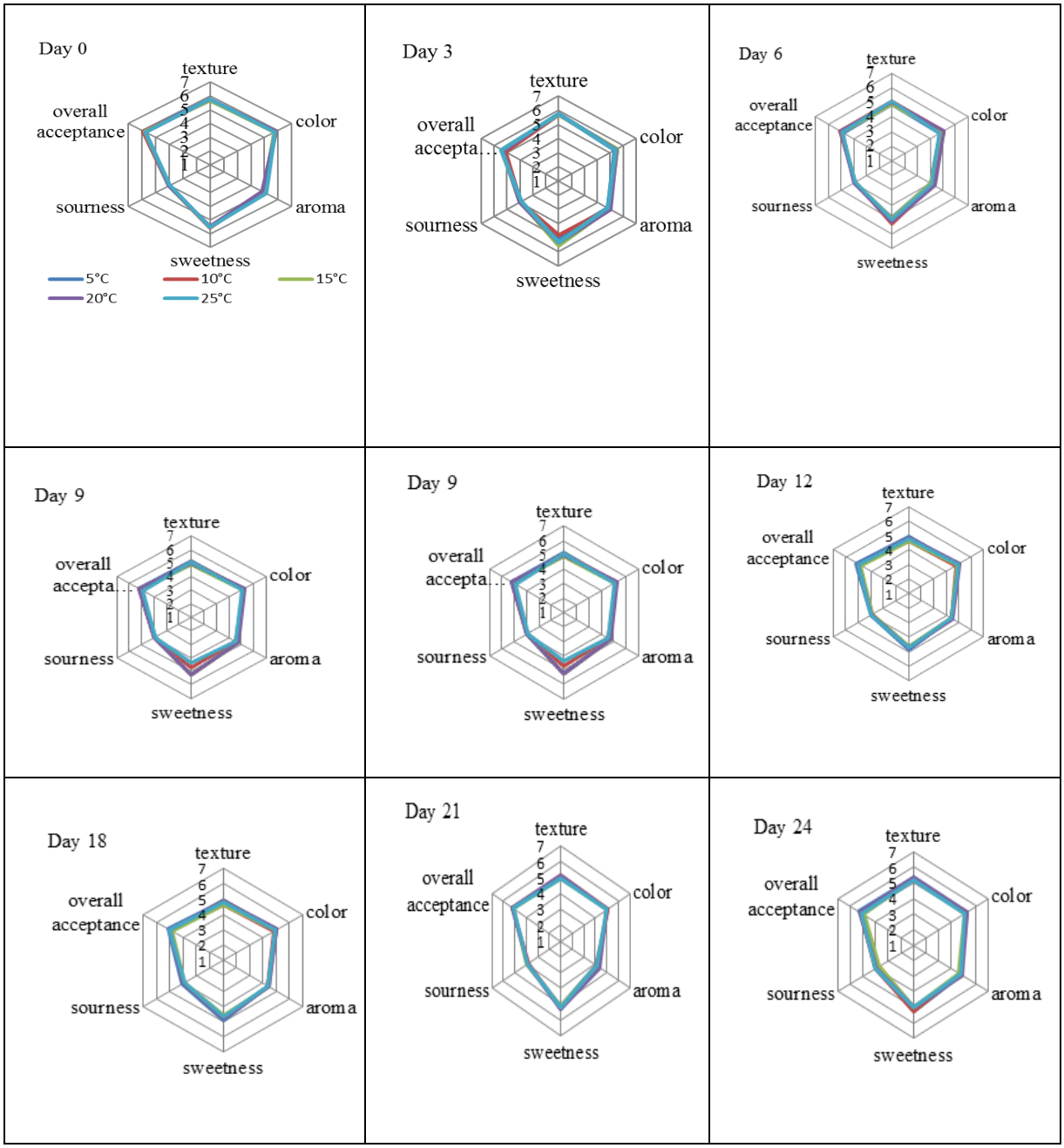

Figure 5: Effects of different storage temperatures on sensory attributes and overall acceptance of Melon Manis Terengganu during 24 days storage.

Conclusions

MMT fruit stored at different temperatures for 24 days remain acceptable to be sold. Interestingly, fruit stored at 25°C had similar internal and external quality to the fruit stored at low temperature storage. In addition, MMT fruits either stored at low or normal temperatures had the same preference of taste, color, texture and aroma. Moreover, 30 panellists agreed that MMT fruits stored at ambient temperature had the

same level of overall acceptance with other fruits stored at low temperature. However, the fruits stored at low temperature may have better preferences of postharvest quality.

Acknowledgements

The authors wish to thank Universiti Malaysia Terengganu for financial support.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Postharvest Quality Assessment of Open-field Cultivated *Ficus carica* L. var. Brunswick Fruits at Different Days of Harvesting

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Introduction

Figs (*Ficus carica* L.) are Mediterranean native crops that recently gains popularity in Malaysia. Because of the thin epidermis layer, figs are highly perishable and unsuitable for storage. The ostiole end of the fruits also tends to crack when mature and it can provide entry site for fungal decay (Kong et al., 2013). Fruits harvested at later stage can encounter excessive softening, tissue maceration and exudation of syrupy liquid from the ostiole (Freiman et al., 2012). Harvesting at the right stage will help to optimize shelf life of figs without compromising fruits quality. According to Marei and Crane (1971), figs fruits developments can be divided into three phases where at Phase 1, fruits will grow rapidly from small bud. At Phase II, fruits will remain same size before entering Phase III, which is ripening phase. Most figs farm in Malaysia are cultivated under rain shelter structure (RSS) to protect the trees and fruits from extreme weather. Lim et al. (2014) reported that Asian pear trees grown under rain shelter showed higher fruit yield, fruit weight and quality and also reduce disease incidence on leaves and fruits compared to open field system but the downside of installation of RSS is it can increase the production cost. Open field cultivation can cause damage or reduce fruits quality when expose to hot and humid weather. Considering this problem, this study was conducted to evaluate quality of the fruits cultivated in open field at different days of harvesting as a guideline for the figs growers.

Materials and Methods

This experiment was conducted under open field figs farms in Sintok, Kedah. *Ficus carica* L. var. Brunswick was selected because of its availability and commonly grown by growers. Fruits were tag from the budding stage and counted as day 1. Fruits were observed weekly and harvested on day 55, 60, 65 and 70 after budding. Harvested fruits were brought to postharvest lab in MARDI Sintok. Quality assessment was conducted on the day of harvesting to evaluate the total soluble solid (TSS), pH, titratable acidity (TA), ascorbic acid and TSS/TA ratio. Total soluble solid was measured by using digital handheld refractometer, ATAGO CO. LTD PAL-1 while pH was taken by using pH meter model HANNA Instrument HI2211. Titratable acidity content was measured by titrating 20 mL extract from sample with 0.1 M 1-1 NaOH until reach 8.2 pH while for ascorbic acid, 10 mL extract from 10 g and 100 mL 3% metaphosporic acid were titrated with standard dye until extract turn into faint pink colour. Statistical analysis was performed by using ANOVA and difference of means was determined by using Duncan Multiple Range Test at 5% level.

Results and Discussion

Table 1 shows that fruits harvested on day 55 were not suitable for fresh consumption because of total soluble solid (TSS) content are very low (1.75°Brix) compared to fruits harvested at day 60 (9.53°Brix), 65 (9.98°Brix) and 70 (9.25°Brix). This could be due to the harvesting period on day 55, the fruits were still in Phase II of growing stages where the fruits have not starts ripen yet (Marei and Crane, 1971; Freiman et al., 2012). Ascorbic acid content was significantly different for fruits harvested on day 70 compared to fruits that harvested at earlier stages fruits. However, from visual observation, the outer part of fruits had started that it starts to deteriorate with several dark spots found on the epidermis of the fruits (Figure 1). In terms of TA, only fruits harvested on day 55 are significantly differ with other treatments with slightly lower. TSS/TA ratios were use to indicate fruits taste by calculating the ratio of TSS and TA where the higher score it gets, indicate better fruit taste. If the fruits contain high TSS or TA only, it could produce bland taste. From Table 1, data showed that the TSS/TA ratio of fruits harvested on day 65 higher as compared to the others

Table 1: Values of total soluble content (TSS), pH, total titratable acidity (TA), vitamin C (AA) and TTS/TA ratio for figs harvested on day 55, 60, 65 and 70 after budding.

Day of fruits harvested	TSS (Brix °)	pH	TA (%)	AA (mg/100g)	TSS/TA
Day 55	1.775^{b}	4.77^{ab}	29.514^{b}	0.987^{b}	0.0619^{b}
Day 60	9.525^{a}	4.53^{b}	41.824^{a}	0.986^{b}	0.237^{a}
Day 65	9.975^{a}	5.1^{a}	41.861^{a}	0.949^{b}	0.241^{a}
Day 70	9.25^{a}	4.99^{ab}	48.782^{a}	1.66^{a}	0.189^{a}

**Means within columns followed by the same letter(s) are not significantly difference based on DMRT test at P≤0.05.*

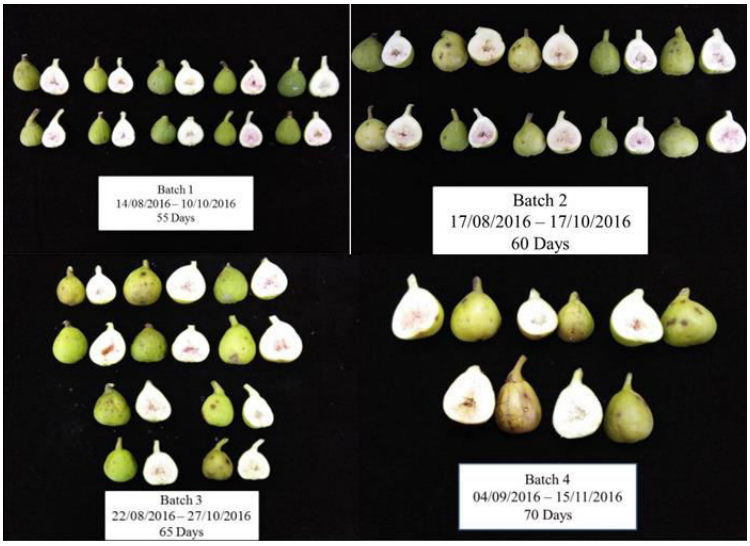

Figure 1: Figs harvested on day 55, 60, 65 and 70 after budding shows changes on skin colour and quality.

Conclusion

In conclusion, better quality fig fruit was observed on harvesting period day 65 considering the higher TSS and TSS/TTA ratio with a good visual appearance. Figs fruits will start ripen after 55 days from budding stage. The suitable harvesting time for fig var. Brunswick was between day 60-65. This finding can be used as guide for fig growers since this variety only change colour slightly after ripen.

Acknowledgements

We are grateful to Figs Direct Sdn Bhd for supplying planting materials for open fields at MARDI Sintok and to all members of fig working group in MARDI Sintok.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Influence of Temperature and Packaging on Postharvest Quality of Lowland Cabbage (*Brassica olearaca* var. Capitata)

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Introduction

Cabbage (*Brassica olearaca* var. Capitata) has greatly increased in demand and well recognized as a very important market vegetable. In Malaysia, cabbages are produced from the very limited highland areas in Cameron Highlands and Kundasang and also are imported from China due to high demand (Perangkaan Agromakanan, 2014). The technology of lowland cabbage cultivation has also been emphasized to cater for the high local demand of this temperate vegetable thus, enabling to reduce the extreme usage of limited land areas in the Cameron Highlands (Rozlaily et al., 2016). Good quality cabbages have firm heads and the outer leaves are bright green, crisp, fresh and free from blemishes (Boyette et al., 1999).

Temperature management is the most effective tool for extending the shelf life of fresh horticultural produce (Cantwell and Trevor, 2002). According to Prange 2004, cabbage intended for long-term (5-6 months) storage is stored at 0°C with 98-100% relative humidity in order to reduce moisture loss and yellowing. The utilization of low temperatures, plastic films, and edible coatings are some of the options to increase the shelf life of vegetables. Packaging can prevent the produce from drying by creating an atmosphere with a high relative humidity. Packaging slows maturation and delays changes in acidity, soluble solids, texture, colour and polygalacturonase (Nakhasi et al., 1991) and reduces water loss (Wall and Berghage, 1996). Similar findings have been made with pak choi stored in plastic film packaging which had effectively reduced moisture loss and wilting and was considerably more effective than manual misting or treating leaves with anti-transpirant chemicals (O'Hare et al., 2001). This finding is also similar to a study carried out on parsley where perforated packaging retained flavour and aroma better than in non-perforated packaging (Heyes, 2004). Therefore, the aim of this study was to determine the suitable temperature and the effect of different packaging on the quality of cabbage during storage.

Materials and Methods

Lowland cabbage at commercial maturity (12 weeks after transplanting) was harvested from the MARDI farm in Selangor. They were selected, being of uniform size and free of physical damage and fungal infection. Then, the cabbage was trimmed to remove outer wrapper leaves so the head has a clean, compact, and fresh appearance. After trimming, the cabbage heads were air dried before being packed in three different packaging; unpacked as control, paper wrapping and stretch film wrapping. Samples were stored at two temperatures; 5°C and 10°C for 6 weeks. The cabbage was sampled weekly to determine the changes in quality.

Postharvest quality evaluation included physical (visual appearance, colour; lightness (L*), hue (h°) and chroma (C*) and chemical (pH, soluble solids concentration (SSC), titratable acidity (TTA) and ascorbic acid content) characteristics. The quality of cabbage was judged visually and the criteria used were retention of original colour, freshness and severity. The colour of cabbage was measured using a

chromameter (Model CR-400 Minolta, Japan). Each colour value, L*, C*, and h°, was expressed as the means of three measurements. SSC was determined with a digital refractometer (Model DBX-55, Atago Co., Ltd, Japan). The pH values were measured using a pH meter (Hanna Instruments pH 211 Microprocessor pH Meter, RI-USA). TTA was determined by titrating 20 mL of extraction with 0.1 mol L^{-1} NaOH to pH 8.2. Ascorbic acid was determined by extraction of 10 g of sample with the addition of 100 mL of 3% metaphosphoric acid. Then, 10 mL of extract was titrated immediately with a standard dye solution to first permanent pink endpoint. The experimental design was a completely randomized design in a factorial arrangement of treatments (three packaging types, each at 5°C and 10°C x 7 storage durations) with three replications. The obtained data was analysed using analysis of variance (ANOVA). The means were separated by Duncan multiple range test at the 5% level of significant.

Results and Discussion

Major changes in the visual quality attributes of lowland cabbage during storage at different temperatures included changes in colour, dryness of the leaves and root growth. During storage at 5°C with stretch film wrapping, results showed that cabbage maintained acceptable visual quality during four weeks. After four weeks, the outer leaves became less green, dry and developed brownish lines. At this point, if the cabbage was marketed, the outer leaves would be trimmed, exposing less green leaves. Deterioration in the visual quality of cabbage stored at 10°C with stretch film wrapping occurred more rapidly than at the lower temperature. After two weeks the external leaves became brownish yellow and roots began to grow. A similar finding was obtained on visual quality of unpacked cabbage and paper wrapping, where the outer leaves turned yellow and root started to grow after two weeks storage (data not shown).

Colour parameters were significantly ($p \leq 0.05$) affected by storage period. L* and C* values were not significantly different between types of packaging. While, hº value of cabbage was higher when storage was at 5°C compared to 10°C, except when stored in stretch film at 10°C (Table 1). Colour of cabbage with stretch film wrapping at both storage temperatures showed more green hue during storage compared to unpacked and paper wrapping were more lighter and yellowish-green due to rapid loss of green colour of leaves. In addition, significant differences were observed in L*, C* and hº among all the treatments during storage. There were also no significant interaction between packaging and storage period in L* and C* values, however, the hue value showed significant interaction.

Table 1 showed that percentage of weight loss of cabbage during storage in unpack at both storage temperatures were significantly higher than the other treatments. Percentage of weight loss of cabbage packed with stretch film wrapping was lower (0.02%) than unpacked (0.10-0.11%) and paper wrapping (0.07%) after six weeks when stored at 5°C and 10°C. Stretch film wrapping was very effective in retaining weight loss of cabbage during storage. For result of interactions, percentage of weight loss gave highly significant difference between packaging and storage period. Similar finding was reported by Porter et al. (2004) in which weight loss of Chinese cabbage increased during storage, attaining 2.1% after 9 weeks of storage at 2°C. Cabbage stored at both temperatures showed changes in SSC and pH levels in different types of of packaging, while TTA and ascorbic acid content were not significantly different (Table 1). Storage duration significantly decreased SSC. Values of pH were significantly increased and TTA was decreased during storage. Ascorbic acid content of cabbage was not affected by packaging as observed at 10°C and 5°C but tended to decrease with the storage period. The decrease in ascorbic acid content during storage could be related with the increase in enzymatic activity and senescence process of cabbage. Meanwhile, there were highly significant interaction between the types of packaging and storage period in SSC, pH, TTA and ascorbic acid content.

Table 1: Changes in soluble solids concentration (SSC), pH, total titratable acidity (TTA), ascorbic acid content, colour of leaves and percentage of weight loss of lowland cabbage (*Brassica olearaca* var. Capitata) in different types of packaging (unpack, paper wrapping and stretch film) at 5°C and 10°C storage temperatures for 6 weeks.

Factor	SSC (%)	pH	TTA (Citric acid %)	Ascorbic acid content (mg/100 g)	Colour of leaves			Weight loss (%)
					L*	C*	h°	
Packaging								
Unpack, 10°C (T1)	4.35^{bc}	6.26^{bc}	0.08^{a}	6.28^{a}	73.25^{a}	24.24^{a}	109.54^{d}	0.09^{a}
Paper wrapping, 10°C (T2)	4.25^{c}	6.24^{c}	0.08^{a}	6.28^{a}	71.22^{a}	25.48^{a}	110.87^{cd}	0.07^{b}
Stretch film, 10°C (T3)	4.35^{bc}	6.29^{b}	0.08^{a}	6.42^{a}	69.99^{a}	26.81^{a}	113.56^{ab}	0.02^{c}
Unpack, 5°C (T4)	4.57^{a}	6.26^{bc}	0.08^{a}	6.98^{a}	72.08^{a}	26.30^{a}	112.32^{bc}	0.11^{a}
Paper wrapping, 5°C (T5)	4.43^{ab}	6.28^{bc}	0.09^{a}	6.85^{a}	71.77^{a}	27.38^{a}	112.48^{b}	0.07^{b}
Stretch film, 5°C (T6)	4.49^{ab}	6.38^{a}	0.08^{a}	6.30^{a}	71.43^{a}	28.58^{a}	114.09^{a}	0.02^{c}
Storage Period								
Week 0	4.70^{b}	6.12^{d}	0.10^{ab}	5.61^{d}	67.19^{d}	33.22^{a}	116.11^{a}	0.00^{g}
Week 1	4.98^{a}	5.99^{e}	0.09^{b}	4.58^{e}	72.88^{ab}	28.26^{b}	114.6^{abc}	0.03^{f}
Week 2	4.52^{c}	6.08^{d}	0.11^{a}	6.14^{cd}	68.44^{cd}	26.17^{bc}	115.23^{ab}	0.05^{e}
Week 3	4.51^{c}	6.37^{c}	0.06^{d}	8.80^{a}	73.80^{a}	25.86^{bc}	113.04^{c}	0.07^{d}
Week 4	3.65^{f}	6.59^{a}	0.05^{e}	7.47^{b}	74.17^{a}	27.24^{cd}	109.86^{d}	0.08^{c}
Week 5	4.35^{de}	6.50^{b}	0.05^{e}	6.36^{cd}	74.15^{a}	24.70^{c}	109.83^{d}	0.11^{b}
Week 6	4.24^{e}	6.33^{c}	0.07^{c}	6.67^{c}	70.74^{bc}	19.80^{d}	106.79^{e}	0.12^{a}
Interaction								
Packaging x Storage Period	**	**	**	**	NS	NS	**	**

Means separation within columns and factor by Duncan's multiple range test at P≤0.05.
L= lightness; C*= chroma; h° = hue angle.*
*NS, *,** Non significant; highly significant at P≤0.05; highly significant at P≤0.01.*

Conclusions

Overall, lowland cabbage packed with stretch film wrapping can be stored at 5°C for 4 weeks. The quality of the stretch film wrapped cabbage was better as compared to unpacked (control) and paper wrapping. As storage temperature increased (10°C) cabbage visual quality attributes deteriorated very quickly. Unpacked cabbage had serious weight loss, yellowing and dryness of the leaves after 2 weeks during storage at 10°C. Paper wrapping should be avoided because it allowed rapid deterioration and root growth.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Gamma Irradiation on Postharvest Quality of MD2 Pineapple

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Introduction

Malaysia has a high potential for the export of fresh tropical fruits especially pineapple var. MD2 to foreign countries. MD2 is a commercial hybrid for international market. It has a sweet taste, attractive skin and flesh colour, high content of vitamin C, but is highly susceptible to chilling injury. However, the infestation of quarantined pests can seriously disrupt marketing of fresh agricultural products. Quarantine disinfestations treatment is very important in order to prevent the establishment of pest associated with a commodity to be imported into a country or region where it does not occur or where its presence is restricted. Several studies have investigated the application of irradiation treatments to disinfest various fruits (Camargo et al., 2007).

In 1986, the Food and Drug Administration (FDA) of United States approved the use of radiation treatments of up to 1 kGy (100 krad) on fruits and vegetables. It has been known for many decades that irradiation is effective in killing, sterilizing or preventing further development of a wide variety of insect pests of quarantine importance on perishable fruits and vegetables. Irradiation also can be used on fruits and vegetables for extending the storage life and reducing decay. Research has shown that the doses required for sterilization of most insects is below 0.75 kGy, whilst the dosages required for effective decay control are often greater than 1 kGy.

Irradiation has proven to be effective for controlling postharvest losses and extending the shelf life by delaying the ripening and senescence of climacteric fruits (Mostafavi et al., 2010). In order to meet the requirement of quarantine protocol, an effect of treatment on postharvest quality also has been investigated. Therefore, this study was conducted to determine the effect of gamma irradiation on postharvest quality of pineapple variety MD2 when exposed to 200 and 400 Gy at the Sinagama Irradiation Facility in Dengkil, Selangor.

Materials and Methods

Pineapple var. MD2 was purchased from a private farm in Johor. Fruits were preconditioned at 10°C overnight. Then, the fruits were sorted and packed in corrugated fiber boxes and irradiated with gamma rays at doses of 0 Gy (control mobile (CM) and control static (CS)), and 200 and 400 Gy, and then stored at 10°C for 21 days. Gamma irradiation was conducted in MINTec Sinagama, Malaysian Nuclear Agency (NM), Dengkil, Selangor, using a Cobalt-60 source. Target doses were monitored by using a Fricke dosimeter, manufactured by Agensi Nuklear Malaysia, located at several positions in the treated boxes. Treated and control fruits were evaluated at 7, 14 and 21 days after treatment for physical and biochemical changes. Characteristics evaluated include physical appearances, titratable acidity, soluble solids concentration, pH, ascorbic acids, flesh colour and texture. The experimental design was a completely randomized design arranged in a factorial experiment of four gamma irradiations x three storage life), with three replications. Statistical analyses of the treatments responses were conducted using analysis of variance and Duncan multiple range test (p<0.05) was conducted to compare treatment means.

Results and Discussion

Evaluation of postharvest quality of MD2 pineapple revealed no significant interactions between gamma radiation and storage life. Gamma irradiation at both 200 and 400 Gy did not cause any effect when compared to control in terms of their flesh colour, texture, and soluble solids concentration after 21 days of storage (Tables 1). Gamma irradiation at doses up to 0.6 kGy had no effect on skin or flesh colour and soluble solids content of both Nam Dokmai and Chok Anan mango cultivars (Uthairatanakij et al., 2006). Ascorbic acids contents in the 400 Gy treated fruits were significantly lower than in control mobile (CM) and 200 Gy fruits (Table 1). The results are in line with Gyorgy and Pearson (1967) who reported that irradiation at doses below 1 kGy may cause only minor and insignificant chemical changes and very little loss of vitamin C content occurs and it is not nutritionally significant. Radiation treatment reduced ascorbic acid and β-carotene in guava with no significant changes in sugars, pectin, and citric acid (Rosario et al., 2013). Gamma irradiation has been shown to cause a decline in the ascorbic acid content of strawberry and 'Autumn Bliss' raspberries (Hussain et al., 2012; Tezotto-Uliana et al., 2013).

The 400 Gy dose significantly reduced the pH of the pineapple (Table 1). Titratable acidity, soluble solids concentration, and chroma value were significantly ($p \leq 0.05$) less in early harvest of pears and the hue is increased by irradiation suggesting that there are differences in radio tolerance of early and late harvest pears (Abolhassani et al., 2013). However, ascorbic acid and titratable acidity in mango and papaya are slightly changed during ripening with no difference in the total sugar content between the irradiated and non-irradiated fruits (Thomas and Beyers, 1979). Data for pH presented in Table 1, indicated that there was a slight variation of pH throughout storage period in the gamma irradiated pineapple. The variation of pH could be due to variation of acidity occurring during the storage period.

Table 1: Changes in flesh colour (L*, C*, h°), soluble solid concentration (SSC), pH, total titratable acidity (TTA), ascorbic acid content and texture of pineapple var. MD2 from different treatments (control static, control mobile, 200 Gy and 400 Gy) at 10°C storage temperature.

Factor	SSC (%)	pH	TTA (% citric acid)	Ascorbic acid content (mg/100g)	Colour of flesh			Texture (N)
					L*	C*	h°	
Gamma irradiations								
Control static	12.83[a]	3.84[b]	0.86[a]	56.38[ab]	71.47[a]	35.14[a]	93.02[a]	11.48[a]
Control mobile	12.61[a]	3.83[b]	00.88[a]	58.99[a]	71.74[a]	37.09[a]	93.70[a]	12.71[a]
200 Gy	12.45[a]	3.85[b]	0.79[b]	60.39[a]	70.50[a]	34.42[a]	93.68[a]	12.31[a]
400 Gy	12.55[a]	3.97[a]	0.78[b]	52.24[b]	70.29[a]	36.80[a]	92.98[a]	12.41[a]
Storage life (W)								
7	13.15[a]	3.99[a]	0.76[b]	54.16[b]	69.93[b]	34.33[b]	94.22[a]	11.13[a]
14	12.15[b]	3.90[b]	0.85[a]	55.76[ab]	69.61[b]	36.22[ab]	93.11[ab]	12.30[a]
21	12.52[b]	3.72[c]	0.87[a]	61.08[a]	73.46[a]	37.03[a]	92.71[b]	13.26[a]
Interaction								
Gamma irradiation x storage life	NS	NS	NS	NS	NS	NS	NS	NS

Each value was the mean of three replicates. Means with the same letter(s) are not significantly different at 5% level ($p<0.05$) according to Duncan multiple range test. L= lightness; C*= chroma; h° = hue angle; NS=Non-significant.*

Conclusions

In conclusion, MD2 pineapple irradiated at 200 Gy could maintain the storage life for up 21 days at 10°C. It is sufficient enough dosage to maintain the quality, meanwhile, it could kill the quarantine pest. Therefore, this study contains useful commercial information and points to further research on the effect of gamma irradiation on pineapple fruit quality.

Acknowledgements

The authors wish to acknowledge the valuable assistance of Ms. Norsiah, Ms. Siti Nur Raihan, Ms. Siti Ilyani, Ms. Syafiqah, Ms. Zainab, Mr. Kamal, Mr. Hassan and Mr. Samsudin for carrying out the research.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Palm Kernel Shell Charcoal on Ethylene Production of Berangan Banana (*Musa* sp. AAA Berangan)

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Introduction

Banana is considered as the eight most important food crop worldwide while in developed countries, it ranks as the fourth most important fruit (Ploetz, 2015). In Malaysia, banana is the second most commonly grown fruit crop and is exported to Singapore, Brunei, Hong Kong and Middle East (Mak et al., 2004). Improper postharvest storage temperature during transportation from farm to the above export markets were the major factors influencing banana loss in supply chain. Normally, it takes 3-12 days to reach any export destination and banana stores at their optimum temperature, 13-18^{o}C, with relative humidity of 85-95% and only can last for 7 days or less (Thompson et al., 1996). Ritenour et al. (1997) reported that banana has a short market life due to its climacteric state that produces high amount of ethylene during the ripening phase, associated with high respiration rate that leads to softening, colour and composition change and aroma production. This hormone is very effective even at low as part-per-million to part-per-billion. Thus, the high production of ethylene in banana lead to rapid ripening and deterioration during the transportation (Ritenour et al., 1997) and reach the destination in poor condition. For that reason, there is an urgent need to delay the ripening of banana in order to maintain the quality at the maximum level. Currently, potassium permanganate ($KMnO_4$), zeolite, activated charcoal, and 1 methyl-cyclopropane are widely used in delaying ripening of banana by inhibiting or slowing down the action of ethylene to react with the ethylene receptor, thus block the response of basal level of ethylene in fruit (Sajib et al., 2015) and therefore, prolong the marketable life of banana. However, these chemicals are very expensive, thus a cheaper, light and easy to handle materials such as Palm Kernel Shell (PKS) charcoal as a replacement substance to delay the banana ripening and maintain its quality is proposed.

Currently, there is little information available on the effects of charcoal derived from different substrates in delaying banana ripening and prolonging its shelf life. Siti Amirah (2016) claimed that charcoal derived from PKS had the ability to delay the ripening of Berangan banana, however, the amount of PKS charcoal (25 g) was not enough to delay the ripening of the fruit entirely. Therefore, further investigation is required to determine the exact amount of PKS in delaying the banana ripening.

Materials and Methods

Berangan banana fruits at maturity stage 2 were purchased from a local supplier in Kuala Terengganu. Fruits that free from defects and decay, uniform in size and weight were immediately transferred to the Postharvest Technology Laboratory, School of Food Science and Technology, Universiti Malaysia Terengganu, for further analyses. The PKS charcoal was obtained from the Malaysian Palm Oil Board (MPOB), Bangi, Selangor. Berangan banana containing 13 fingers were washed using 100-200 mg/L sodium hypochlorite to remove dirt and debris. Then, the hands were subjected to dry at room temperature (26 ± 2^{o}C). After that the dried hands of bananas were immersed in 400 mg/L ethephon solution to trigger ripening process. Each hand of banana was placed in polyvinylchloride (PVC) container with respective amounts of PKS charcoal and silica gel that function as moisture absorber. The PVC containers containing bananas were stored at ambient environment (26 ± 2^{o}C) for three weeks. The experiment was laid out in the Complete Randomized Design (CRD) with four treatments, i) control (without PKS), ii) 25 g PKS, iii) 50 g PKS and iv) 75 g PKS. The amounts of PKS were

based on the reports of Siti Amirah (2016) and Wan Zaliha et al. (2014). Each treatment was replicated three times. The postharvest parameters measured were internal ethylene production, fruit colour indices, firmness, titratable acidity (TA), soluble solids concentration (SSC), weight loss and starch pattern index (SPI). The sample was assessed on a daily basis for ethylene production and every four days interval for other assessment. An Internal ethylene concentration was measured using gas chromatography as according to the method of Wan Zaliha (2009). The ethylene concentration in the gas sample was determined by comparing its retention with authentic ethylene standard. Ethylene was estimated using the software program and was calculated from the integrated areas of the sample and the corresponding standard (Wan Zaliha, 2009). A 2 mL of ethylene gas was taken from the sealed containers with a lock-luer syringe and injected into GC. The ethylene concentration was expressed in µg/mL. While, a starch pattern index was observed by cutting the banana fruit longitudinal into half and immersed them in iodine solution for a few minutes. The starch present in the pulp was reacting with iodine causing a dark blue color change. Assessment of starch pattern of each banana was observed by comparing the stain cut surface with the Starch Pattern Chart (Kader, 2002). The starch patterns indicated the relative amounts of starch and sugars. Fruit firmness, SSC and fruit colour were measured as according to the method of Wan Zaliha et al. (2014). Percentage weight loss of banana was recorded on day 0, 4, 8, 12, and 16 by using a weighing balance. Total phenolic content was evaluated by using a modified colorimetric method as described by Singleton and Rossi (1965). Meanwhile, ascorbic acid concentration was based on the method of Association of Official Analytical Chemists (1984).

The data were subjected to one-way analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by Tukey's studentized range test (HSD) for minimum significant difference at $P \leq 0.05$ (SAS Institute Inc., 1991).

Results and Discussion

A 75 g PKS treated-fruit exhibit the occurence of climacteric peak on day 11. Meanwhile, the climacteric peak of 50 g and 25 g treated-fruit was observed on day 9 while on day 6 for control fruit (Figure 1). The climacteric peak of ethylene production in various climacteric fruits is closely related to the ripening process. The earlier the occurrence of climacteric peak, the more rapid the deterioration process. As reported by Zagory (1995), ethylene can be absorbed and adsorbed by a number of substances including activated charcoal where the effectiveness of the adsorption was depends on the type of agricultural wastes and the porosity structure of the charcoal. Wan Daud et al. (2004) claimed that PKS has a higher porosity thus provide more surface area for reaction to occur. In this study, a 75 g of PKS has the ability to delay the climacteric peak as compared to other treatments. The possible reason might be associated to its higher surface area per volume, thus more reactions were able to take place. The non-polar ethylene was adsorbing by the porosity structure of the PKS thus bind to the charcoal surface by van der waals force. As a result, the occurrence of ethylene climacteric peak was delayed.

As the ripening proceeds, the most striking postharvest chemical changes in banana are the hydrolysis of starch and the accumulation of sugar (Dadzie and Orchard, 1997) which is responsible for the sweetening of the fruit. The starch test indicates that the green bananas have an ethylene production rate in the lower half and that the yellow bananas have an ethylene production near the maximum (Kader 1999). The softening of banana fruit during ripening was associated with the conversion of starch to sugar, breakdown of pectin substances and the movement of water from the rind of the banana to pulp during ripening (Thompson et al., 1996).

In addition, Beatriz and Ranco (1995) reported that the appearance of starch reserve during banana ripening is very fast in which the drops average is from 25% in the pre-climacteric phase to less than 1% during climacteric period. The conversion of starch was pronounced in control fruit followed by 25

g of PKS, 50 g PKS and 75 g PKS treated fruits (Figure 2). The application of 75g PKS delayed the conversion process which in agreement with the result claimed by Wan Zaliha et al. (2014). Control and 25 g PKS treated-fruit were fully ripen on day 12 (score 7) indicated that the starch was fully degraded into sugar (Table 1). Similarly, Ding (2008) claimed that as ripening progressed, the starch hydrolysed into sugar.

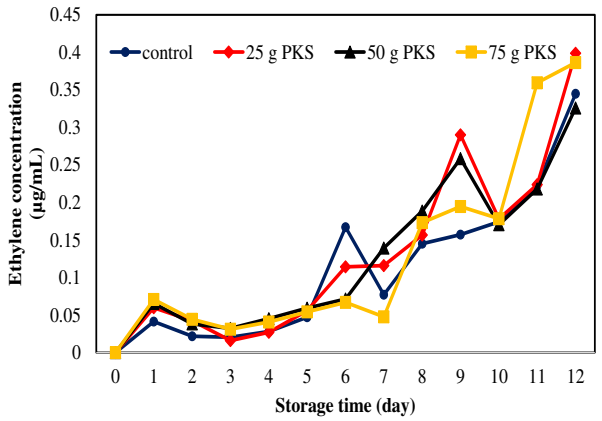

Figure 1: Effects of different rates of palm kernel shell (PKS) charcoal on ethylene concentration of Berangan banana.

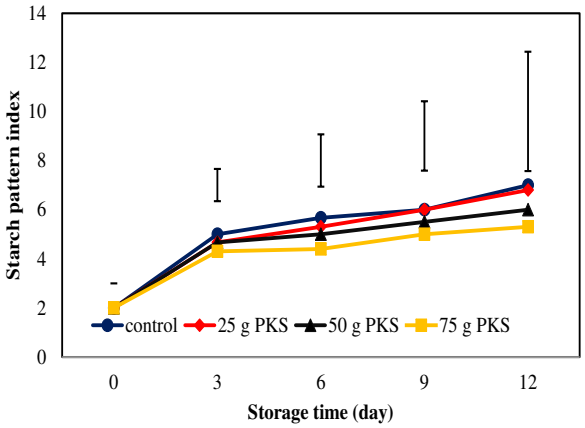

Figure 2: Effects of different rates of palm kernel shell (PKS) charcoal on starch pattern index (SPI) of Berangan banana flesh. Vertical bars represent $HSD_{0.05}$.

Table 1: Effects of different amounts of palm kernel shell (PKS) charcoal on the starch pattern index of Berangan banana (Musa AAA Berangan). Number in the small box indicates SPI score.

Treatment / Day	0	3	6	9	12
Control (0 g PKS)	 2	 5	 6	 6	 7
25 g PKS	 2	 5	 5	 6	 7
50 g PKS	 2	 5	 5	 6	 6
75 g PKS	 2	 4	 4	 5	 6

Starch hydrolysis also is closely related to the fruit firmness, SSC and TA of the fruits. As fruits matured, the conversion of starch to sugar leads to lower starch pattern index (the amount of starch decrease), lower TA and higher SSC due to the degradation of compounds of the banana and accumulation of soluble solids. The firmness of banana was not distinctly differentiated by the application of different amounts of PKS (Figure 3). The fruit firmness decreased as the storage increased. The loss of the banana hardness is often inversely related to ripening; indicate that as ripening progressed, the banana firmness is declined (Smith et al., 1989). Three associated processes that can define this event are the breakdown of starch into sugar, breakdown of the cell walls or reduction in the middle lamella cohesion due to solubilisation of pectic substances in which pectin and hemicellulose are the components of cell wall and their concentration during ripening follows a trend similar to that of starch, dropping from 7 to 8% of the fresh pulp in the green fruit to about 1% at ripeness (Hailu et al., 2013).

Regardless of PKS application, the SSC increased as storage period extended (Figure 4). The increase of sugar content is another characteristic of banana ripening (Blankenship et al., 1993). According to Marriot et al. (1981) sugar content increased from 7.8% at stage one to 18.6% at stage six (full ripe) in which most of the soluble solids content is sugar. During ripening, the starch of banana is fully converted into sugar and the SSC value increased. The increment of SSC is an important trait of hydrolysis of starch into soluble sugars such as glucose, sucrose and fructose (Soltani et al., 2010). In addition, Beatriz and Ranco (1995) stated that fully ripe banana has gradual change of starch content from 25% to less than 1% while soluble solids content increase significantly.

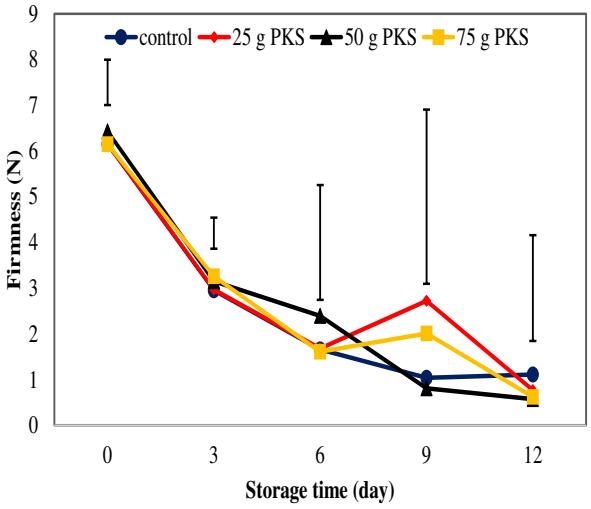

Figure 3: Effects of different rates of palm kernel shell (PKS) charcoal on fruit firmness of Berangan banana. Vertical bars represent $HSD_{0.05}$.

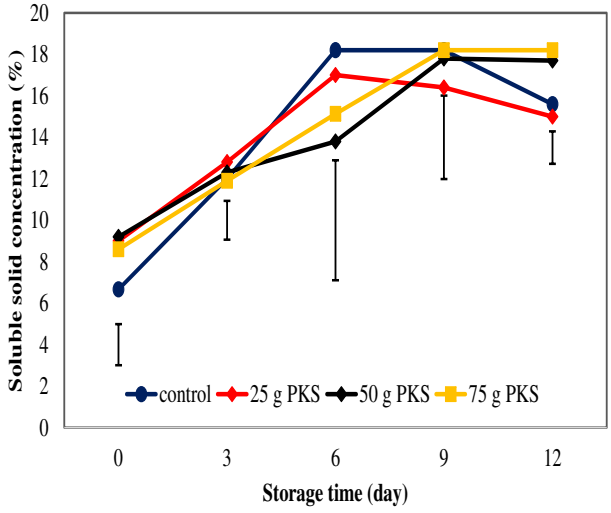

Figure 4: Effects of different rates of palm kernel shell (PKS) charcoal on SSC of Berangan banana. Vertical bars represent $HSD_{0.05}$.

The tiratable acidity (TA) which represents the percentage of malic acid in Berangan banana showed a fluctuation trend during the storage period (Figure 5). As the fruit ripened, these acids were reduced and, the taste changed to a sweet taste, mainly from the hydrolyzed sugar from the starch degradation. Madamba et al. (1977) found a marked increase in malic acid during the earlier of banana ripening and tend to fall during ripening stages. Hailu et al. (2013) reported the same occurrence level of organic acids in fruits where the acidity increased during ripening and as ripening advances the acidity declined due to the utilization as respiratory substrate. However, the reason of fluctuation of TA values is still unknown.

On the other hand, ethylene stimulates chlorophylls losses and resulted yellow in colour of banana fruit and promotes the ripening of the pulp (Saltveit, 1999). In addition, the changes in fruit colour might be ascribed to the degradation of chlorophylls to phytol which catalyzed by chlorophylase enzyme (Table 2). The most important compounds responsible for the change in peel colour are chlorophylls and carotenoids. As reported by Gross and Flugel (1982), chlorophylls decreased rapidly and being absent in ripe fruit. Saltveit (1999) claimed that, removal of ethylene or inhibition of its action can delay colour changes in storage and prolong the shelf life of selected commodities. Moreover, the peel colour of banana changed from green to yellow closely related to the conversion starch into sugar.

The ascorbic acid content was fluctuated throughout the storage as shown in Figure 6. Ripening process of fruit is associated with gradual or rapid depletion of ascorbic acid (Rouse and Aulin, 1977). Songul and Turgut, (2003) reported that ascorbic acid is very sensitive and it is destroyed over time when exposed to atmospheric oxygen. Meanwhile the TPC increased in the beginning of storage, and then start to decrease to the end of the storage (Figure 7). The TPC are important fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals (Maisuthisakul et al., 2007). Fatemah et al. (2012) claimed that the TPC was generally higher in peel than in the pulp and the green had a higher TPC than the ripe components. However, the PKS maintain the changes occur in banana fruit as no profound effect was observed. In addition, further study is highly required to be conducted in banana fruit without soaking ethephon at the early stages of the experiment as this might affect the overall quality assessed.

Table 2: Effects of different amounts of palm kernel shell (PKS) charcoal on fruit skin colour of Berangan banana (Musa AAA Berangan).

Treatment/Day	0	3	6	9	12
Lightness (L*)					
Control (0g PKS)	55.37^{a}	71.35^{a}	72.54^{a}	67.66^{a}	68.34^{a}
25 g PKS	48.82^{a}	67.34^{a}	74.21^{a}	65.23^{a}	67.19^{a}
50 g PKS	54.47^{a}	67.72^{a}	67.97^{a}	66.64^{a}	70.12^{a}
75 g PKS	48.13^{a}	68.40^{a}	73.56^{a}	67.12^{a}	67.88^{a}
$HSD_{0.05}$	11.93^{ns}	10.04^{ns}	14.71^{ns}	13.08^{ns}	6.67^{ns}
Chromaticity a*					
Control (0g PKS)	-13.72^{a}	-0.38	5.99^{a}	4.16^{a}	3.95^{a}
25 g PKS	-11.42^{a}	-3.59	4.77^{a}	3.03^{a}	3.57^{a}
50 g PKS	-13.93^{a}	-1.44	1.62^{a}	4.01^{a}	2.42^{a}
75 g PKS	-11.74^{a}	-5.50	4.52^{a}	6.19^{a}	4.41^{a}
$HSD_{0.05}$	6.41^{ns}	9.37^{ns}	13.64^{ns}	16.22^{ns}	4.27^{ns}
Chromaticity b*					
Control (0g PKS)	27.32^{a}	49.84^{a}	47.43^{a}	49.81^{a}	49.54^{a}
25 g PKS	24.68^{a}	47.55^{a}	49.35^{a}	44.27^{a}	49.33^{a}
50 g PKS	27.45^{a}	46.11^{a}	42.95^{a}	49.13^{a}	49.26^{a}
75 g PKS	25.30^{a}	46.59^{a}	48.67^{a}	47.71^{a}	50.17^{a}
$HSD_{0.05}$	4.90^{ns}	10.60^{ns}	12.29^{ns}	9.88^{ns}	8.37^{ns}
Hue angle (h°)					
Control (0g PKS)	116.63^{a}	90.42^{a}	82.80^{a}	85.23^{a}	85.45^{a}
25 g PKS	114.25^{a}	94.68^{a}	84.48^{a}	94.67^{a}	85.81^{a}
50 g PKS	116.88^{a}	91.86^{a}	92.81^{a}	85.32^{a}	87.19^{a}
75 g PKS	114.74^{a}	97.02^{a}	83.54^{a}	94.38^{a}	84.96^{a}
$HSD_{0.05}$	9.03^{ns}	11.97^{ns}	20.85^{ns}	21.70^{ns}	5.28^{ns}

*ns= not significant ($p>0.05$), **= very significant ($p<0.05$) and *** = highly significant ($p<0.01$). Means with the same letter(s) are not significantly different at $P>0.05$.*

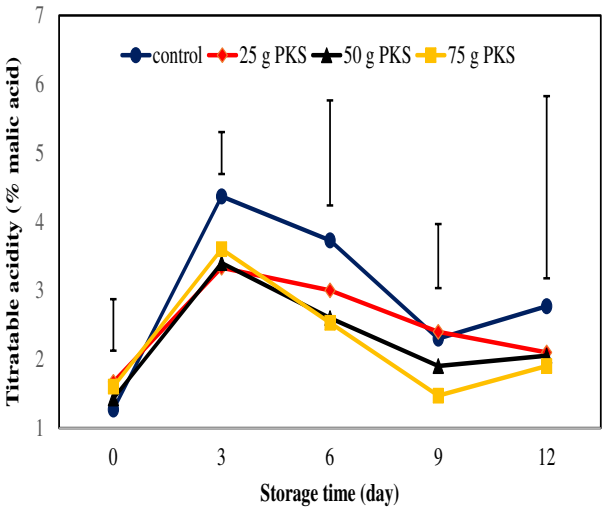

Figure 5: Effects of different rates of palm kernel shell (PKS) charcoal on titratable acidity of Berangan banana. Vertical bars represent $HSD_{0.05}$.

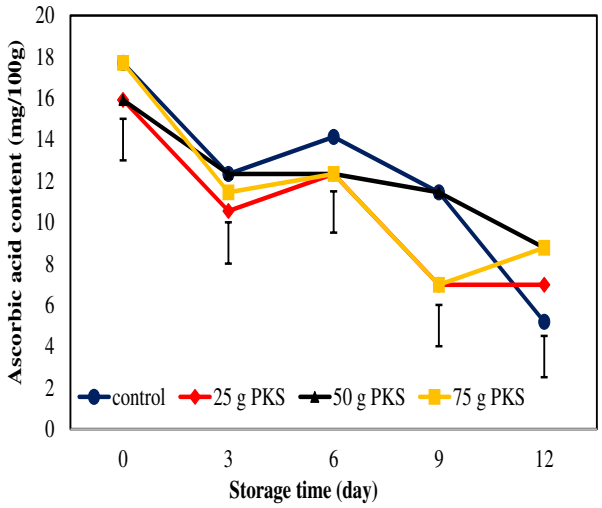

Figure 6: Effects of different rates of palm kernel shell (PKS) charcoal on ascorbic acid content of Berangan banana. Vertical bars represent $HSD_{0.05}$.

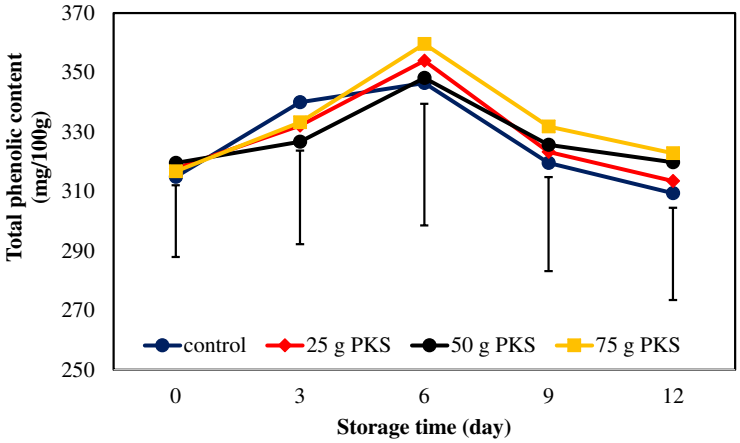

Figure 7: Effects of different rates of palm kernel shell (PKS) charcoal on total phenolic content of Berangan banana. Vertical bars represent $HSD_{0.05}$.

Conclusions

The application of 75 g PKS has the ability to delay the occurrence of ethylene climacteric peak, retain the lowest score of SPI, without retarding the changes of skin colour, firmness and soluble solids concentration. In addition, the storage shelf life of PKS treated fruits can be extended up to 12 days. For the future research, the amount of PKS should be increased as it shows a positive effect in reducing the ethylene production of Berangan banana. Other than that, a further research can be done on various types of agricultural wastes as an ethylene absorbent such as coconut shell, coconut pulp, sugarcane bagasse can also be conducted in order to reduce the ethylene production on climacteric fruits and maintaining its postharvest shelf life.

Acknowledgement

The authors wish to thank the Universiti Malaysia Terengganu for the financial support.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Chapter 5

Pest and Disease Management

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Evaluation of Neem Oil Pesticide against Scale Insects (Hemiptera: Diaspididae) of *Piper nigrum* L.

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Introduction

Infestation by insect pests is one of the major problems that are responsible for the reduction in productivity in black pepper producing countries in the world. In Malaysia, black pepper is infested by 13 species of insects which have been documented in X-crop II, an interactive system developed by the Sarawak Department of Agriculture (DOA) in 2006 (Paulus et al., 2006). These insects feed on various parts of pepper vines by feeding on roots, stem, shoots, leaves, spikes and berries. In addition, they can also reduce the plant vigor by removing plant sap, secreting toxic enzymes or transmitting plant diseases (Khoo, 1974). One of the most common insect pests of black pepper in Malaysia is the scale insects that suck the sap of black pepper leaves and branches. These insects are very small, flattened on plants surface and are hard to spot. Some species like *Pinnaspis* sp. are protected by a fungus that mutually exists with the insect. *Pinnaspis* sp. is plant parasite that associates with fungus *Septobasidium* sp. and causes the velvet blight disease in pepper (Wong, 2009). After the scale insects mature, the fungi develop hypha surrounding the maturing insects while they live and reproduce within the hyphal mass (Hudson, 1992). The fungi also provide protection to the insect from predators and parasites and the scale insect provides the fungi with nutrients, in return. The movement of the young scale insects from one plant to another results in the dissemination of the spores and thus, the fungus (Hudson, 1992). Heavily infected plant parts will dry up and break at the nodes and infected leaves will turn brown and eventually dropped (Wong, 2009). Scale insects belong to the order Hemiptera, and superfamily Coccoidea, which consists of about 8,000 species around the world. Scale insects normally feed on plants by inserting their mouthparts into plant tissues to suck out the sap. Scale insects are difficult to control with conventional insecticides (Schread, 1970; Hamlen, 1977) as they are protected from sprays by their sedentary habits, sheltered feeding locations (under leaves, at plant nodes, or on roots within the soil) and the water-repellent waxes that cover their bodies (Donahue and Brewer, 1998).

Neem tree (*Azadirachta indica*. A. Juss) is a tropical evergreen tree from the Meliaceae mahogany family. It is native to India and Burma, but widely grown in Southeast Asia and Africa. Neem oil extracts had been used for the insecticidal properties of the bioactive tetranortriterpenoid compounds (e.g. Azadirachtin A, B, H). They include growth regulation, oviposition, repellence, fecundity suppression and sterilization (Barceloux, 2008). Neem products' mode of actions are different from the conventional pesticide because they alter the insect's behavior in subtle ways. For example, Azadirachtin can prevent the formation of new insect generation by disrupting the metamorphosis of insect larvae that keeps the larvae from developing into pupae and to adults (Orwa et al., 2009). Since early days of pepper cultivation in Malaysia, chemical measures such as deltamethrin pesticides and white oil have been recommended as control measure for scale insects on black pepper. The *Septobasidium* sp. fungal mat has to be scrapped off before the scale insects can be terminated (Wong, 2009). Another control measure includes spraying copper fungicide to kill the fungus before applying insecticide. However, health and environmental concerns associated with the use of chemicals have resulted in an increased interest in alternative control strategies such as biological control and botanical-based pesticides. Hence, this study was conducted to gather information regarding the scale insect that attacks black pepper (*Pinnaspis* sp.) and to evaluate the potential of Neem oil pesticides

against its infestation and its symbiont, *Septobasidium* sp. so as to evaluate alternative measures to deltamethrin pesticides.

Materials and Methods

Sampling of scale insects and velvet blight fungus from infected black pepper vines was conducted at various pepper farms in Padawan, Sarawak. Laboratory identification was also carried out under a stereomicroscope for morphological identification of scale insect species. Identification of velvet blight fungus by using molecular technique was also conducted by growing the fungus on potato dextrose agar (PDA) (Strobel et al., 2005) and transferring the pure cultures to potato dextrose broth after seven days and left for incubation at 24^{o}C under 100 rpm using an orbital shaker. After seven days, the fungal cultures were harvested for genomic DNA isolation. Fungal genomic DNA isolation was conducted according to modified CTAB method (Doyle and Doyle, 1990). The polymerase chain reaction (PCR) was performed using universal primers, 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGCA-3'. Subsequently, purified DNA for the seven isolates was sent to First Base Malaysia for sequencing and the sequence results were analyzed with the Basic Local Alignment Search Tool (Altschul et al., 1997) (BLAST; from http://www.ncbi. nlm.nih.gov/BLAST/).

Field trial was carried out at two pepper farms located in Padawan areas in Sarawak for a period of 12 weeks. These two farms were heavily infected by velvet blight disease and scale insects. Trials were conducted according to randomized complete block design (RCBD) by comparing four different treatments to evaluate the effectiveness of the treatments against scale insect populations and also velvet blight disease. The pepper vines were tagged and mapped according to four treatments, Neem oil pesticide, coarse salt, commercial pesticide (Dimethoate) and water (control). Insect populations were calculated and velvet blight fungus mat were measured before and after treatments were applied. Data was statistically analyzed using two-way ANOVA and the significant differences between treatments were determined by using Tukey's range test (HSD).

Results and Discussion

Identification of scale insects from infested pepper vines was conducted using stereomicroscope before counting the population of insects based on three different stages which were eggs, adult and dead insects. Morphological observation confirmed that the scale insects collected were *Pinnaspis* sp. DNA sequencing ran using Nucleotide BLAST indicated that the isolated fungus had 91% similarity to a Basidiomycete or Basidiomycota (Martin et al., 2015). Due to its nature, which is obligate parasite, *Septobasidium* fungus was difficult to isolate and grow under in vitro conditions.

Experimental plots for field trial had been established at Kampung Garung and Kampung Begu in Padawan area, Sarawak. The plot design was based on RCBD and four treatments were applied, which were water (control), Neem oil pesticide, coarse salt and commercial systemic pesticide (Dimethoate). Treatments were applied six times in a period of 12 weeks and data for insect population were collected before and after treatments. Beside scale insect population, the occurrence of velvet blight disease at the trial plots was also determined by measuring the fungus mat on the branches of the pepper vines. The concentration of each treatment was determined according to the recommended concentrations for black pepper and from the previous research study. Concentrations for Neem oil pesticide, coarse salt and Dimethoate pesticide used in this study were applied according to the products' labels which are 0.0563%, 1.56% and 0.045%, respectively.

Results showed that the overall populations of scale insects treated with Neem oil pesticide were significantly reduced up to 77.71%, which was the second highest after Dimethoate pesticide (90.37%). Meanwhile, treatment using coarse salt reduced the population of scale insects by 76.89%

and control treatment resulted in the lowest population reduction (57.65%). Figures 1 and 2 show the populations of scale insects before and after treatments.

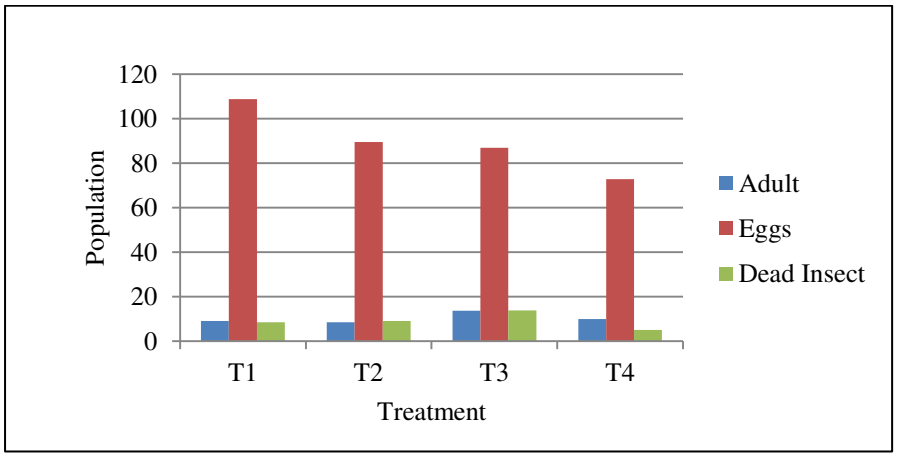

Figure 1: Scale insect populations on each pepper vines before treatments.

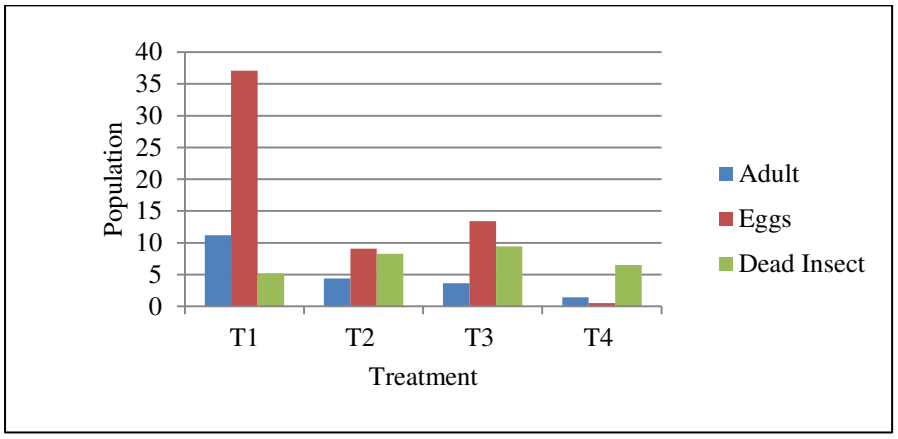

Figure 2: Scale insect populations on each pepper vines after treatments.

Neem oil pesticide showed effectiveness by reducing 48.17% population of adult scale insects, 89.86% of scale insect eggs and 9.61% of dead scale insects. The high percentage of reduction on the eggs calculation showed that Neem oil pesticide was able to deter scale insect populations on pepper vines by preventing oviposition or the ability to lay eggs. Statistical analysis using two-way ANOVA also revealed that there was a significant difference in the effect of treatments on the populations of scale insects (p-value < 0.05).

The effectiveness of Neem oil pesticide against velvet blight fungus was also conducted and the results are shown in Figure 3. The chart shows the growth comparison for four treatments (T1, T2, T3 and T4) on two plots (P1 and P2). Fungal growth was measured before and after the treatments were applied for the period of 12 weeks. T4 (Dimethoate pesticide) showed minimum growth after treatment which means that it performed the highest inhibition, followed by T2 (Neem oil pesticide), T3 (Coarse salt) and T1 (Control).

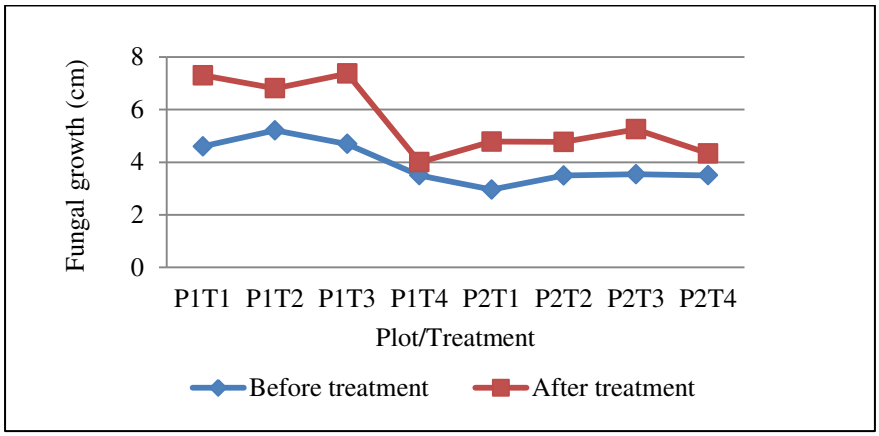

Figure 3: Growth comparison of velvet blight fungus (*Septobasidium* sp.) on black pepper vines before and after treatments.

Table 1 shows the percentage of fungal growth inhibition for four different treatments, respectively. The results indicated that the Dimethoate pesticide showed the highest percentage of inhibition (76.29%), followed by Neem oil pesticide (66.90%) and coarse salt (53.53%).

Table 1: Percentage of velvet blight fungal growth inhibition on pepper vines after treatments.

Treatment	Treatment type	Fungal growth inhibition (%)
T1@ Control	Water	40.22
T2	Neem oil pesticide	66.90
T3	Coarse	46.47
T4	Dimethoate Pesticide	76.29

Analysis of variance (ANOVA) on the results showed that there was no significant difference between the effects of treatments on velvet blight disease. This indicated that the treatments were less effective in inhibiting the fungal growth, compared to the scale insect populations.

Conclusions

As a result, this study has found out that Neem oil pesticide can significantly inhibit scale insect populations at 77.71% on pepper vines and 66.90% of velvet blight fungal growth. This effectiveness is considered high compared to Dimethoate pesticide, a systemic chemical insecticide. Neem oil pesticide can reduce the population of scale insects on pepper vines by deterring the oviposition of the insects, which also has been proven in previous studies on other pests. Although Neem oil pesticide does not possess significant effect on the *Septobasidium* sp. or velvet blight growth inhibition, it can inhibit the occurrence of velvet blight disease in pepper farms, because scale insects and *Septobasidum* sp. exist through symbiosis. Hence, Neem oil pesticide is recommended for control of velvet blight disease in black pepper farms.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Isolation and Identification of the Causative Pathogen of Buds Rot *Dendrobium*

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Introduction

Severe flower buds rot of *Dendrobium* has adversely affected orchid cultivation in Malaysia. Infection can be observed in young flower buds where the buds showed water soaked lesion and progress to permanent wilting and the buds will ultimately shrivel and become dark brown in color. This unknown disease has huge impact on *Dendrobium* orchids by decreasing marketable flowers. Prior to previous report in Sri Lanka, flower bud wilting of *Dendrobium* was associated with *Fusarium proliferatum* (Dissanayake, 2015). The disease reported were similar to those observed in the nursery. However in Malaysia, only stem and root rot of *Dendrobium* orchids associated with *Fusarium* were reported (Latifah et al., 2008; Latiffah et al., 2009). Due to limited knowledge of the disease, effective control measure has yet to be established. Therefore, the objectives of this study were to isolate and identify the causal pathogen of buds rot on *Dendrobium* orchid.

Materials and Methods

Isolation and identification of causal agent

Buds showing water soaked lesion and wilting were collected from MARDI Headquarters orchid nursery. The samples were surface sterilized with 10% (v/v) sodium hypochlorite and rinsed in sterile distilled water. The samples were then plated onto Potato Dextrose Agar (PDA) and incubated at 27 ± 1°C for 7 days or until visible sign of mycelial growth from the sample. The isolates were then purified and identified using morphological and ribosomal internal transcribed spacer (ITS) region (Qiagen GmbH, Germany). The ITS nucleotide sequences for each isolate were then compared to those in the public domain databases NCBI (National Center for Biotechnology information; www.ncbi.nih.gov) using Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN)

Pathogenicity test

Healthy *Dendrobium* orchids at flower bud initiation stage were sprayed with 20 mL conidial suspension at 1 x 10^6 conidia/mL of the representative isolate and then incubated in green house at 25°C-30°C in humid condition. The whole plants were washed and surface sterilized before inoculation. The control plants were sprayed with 20 mL of sterile distilled water. The experiment was conducted in a Completely Randomized Design (CRD) with three replicate until disease symptoms appeared. The infected plant parts were plated onto PDA for reisolation of the pathogen as described by Latiffah et al. (2009) with slight modification.

Results and Discussion

Identification of the causal pathogen

F. fujikuroi were isolated from infected flower buds collected from MARDI Headquarters orchid nursery (Table 1) showing water soaked lesion, shrivel and dark brown in color (Figures 1a-b). *F. fujikuroi* grown on PDA produced white mycelia and developed to whitish purple while aging (Figure

1c). All isolates of this species produced abundant microconidia in cylindrical, slightly tapered and no septate (Figure 1d). Macroconidia were oval shaped with a flattened base with no septate (Figure 1e). In BLAST analysis, isolate had a 100% sequence identity with sequence of *F. fujikuroi* (KJ000434.1) at 546 bp. Based on these morphological characteristics and rDNA spacer sequence, isolates were identified as *F. fujikuroi*.

Table 1: Isolation of *F. fujikuroi* from buds rot of *Dendrobium* orchid.

Dendrobium spp.	Isolated plant part	Number of isolates
Dendrobium Abdullah Badawi	Bud	1
Dendrobium Tunku Fauziah	Bud	5
Dendrobium Dato' Sharif	Bud	5

Pathogenicity test

Pathogenicity of the fungus was confirmed under glasshouse condition by artificial inoculation. Symptoms were reproduced on orchids inoculated with the isolates. Wilted flower buds showed after 2 days post inoculation (Figure 1f). The disease symptoms observed corresponding to the symptoms observed in the nursery. There were no symptoms on control plants. The inoculated fungi were consistently isolated from the infected plants, but not from control plants. Thus, the Koch's postulates were fulfilled.

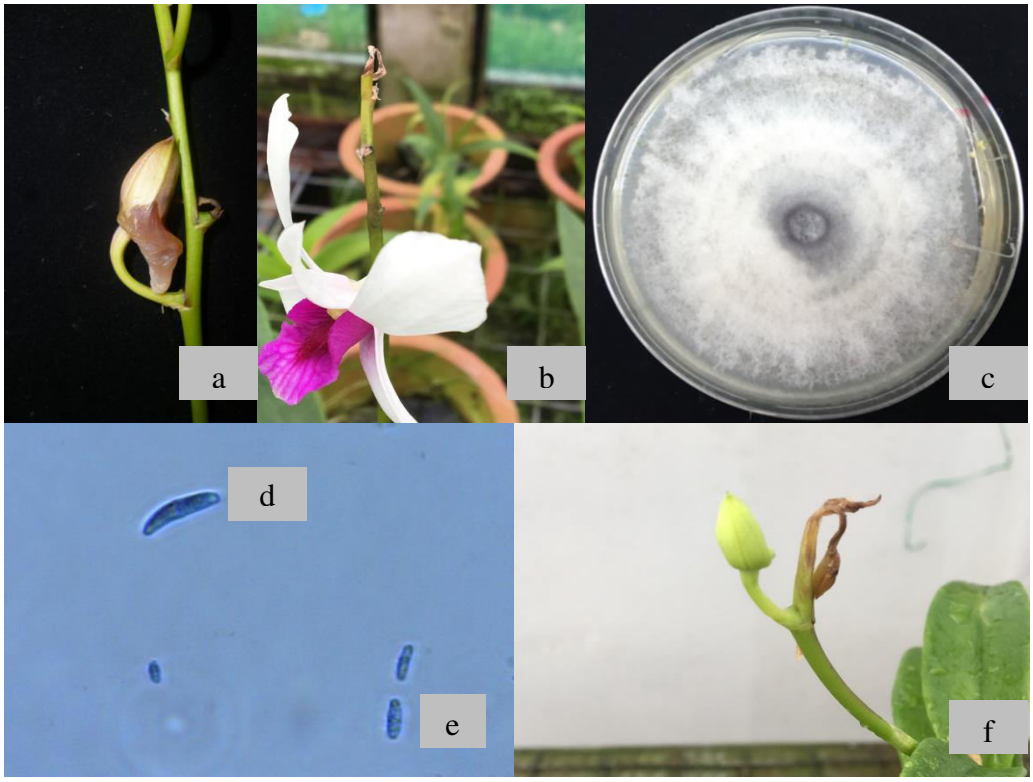

Figure 1: Symptoms of buds rot on *Dendrobium* orchids. a) Flower buds with water soaked lesion, b) permanent wilting of flower buds, c) morphology of *F. fujikuroi* on PDA, d) Microconidia e) Macroconidia. f) Buds rot symptom after 2 days inoculation.

Conclusion

The present study concludes that *F. fujikuroi* is the causal pathogen of buds rot on *Dendrobium* orchids in Malaysia. This is the first record of flower buds rot disease on *Dendrobium* orchid in Malaysia.

Acknowledgements

This work was funded by MARDI Development Fund, P21003004140001-2017-A. The author would like to express gratitude to Maizatul Ilyana Rosli, Najah Yahya, Norhasbulloh Abdullah, Nur Syahirah Fazli and Mohd Asyraf Ikhsan for their assistance.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Chapter 6

Biotechnology

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Micro RNAs and Sesquiterpene Biosynthesis in *Arabidopsis thaliana*

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Introduction

Sesquiterpenes (SQTs) are secondary metabolites, which are synthesized by the assembly of three isoprene units (C5 unit). They are volatile in nature and play important role in plant growth and development. They are produced in plants under stress conditions and serve as defense agents or pheromones. They belong to terpene group and are the most widely spread volatile plant secondary metabolites in nature constituting up to 28% of overall plant terpenes (Helmig et al., 1999).

SQTs are well known due to their broad role in agriculture, food, cosmetics, chemical and pharmaceutical industry. In agriculture industry, SQTs have important role in plant defense against various herbivores (Moreno-Osorio et al., 2008), fungi, microbes and bacteria (Hartmann and Meisel, 2007). Polygodial which is a SQT compound, is a well known deterrent against a number of herbivorous insects (Moreno-Osorio et al., 2008). Polygodial 40 from *Polygonum hydropiper* was reported to exhibit antifungal and fungicidal activities against a broad range of fungi. Another SQT, (E)-β-farnesene (*Eβf*) has been shown to act as alarm pheromone against *Lipaphis erysimi* (L.) which is an aphid (Verma et al., 2015). In maize (*Zea mays* L.), lepidopteran attack has resulted in the emission of volatile blend which consisted mainly of SQTs. The volatile blend attracted natural enemies of invading lepidopteran herbivores, paving the way to biological control of herbivores. In pharmaceutical industry, SQTs are also well known for a number of benefits to mankind such as β-caryophyllene which helps to decrease anxiety and depression (Bahi et al., 2014). Humulene, a SQT from *Cordia verbenacea* exhibited profound anti-inflammatory effects very similar to dexamethasone (Passos et al., 2007). SQT lactones extracted from *Eupatorium semialatum* were found to exhibit antimalarial activity (Lang et al., 2002).

Micro RNAs (miRNAs) are small (19~24 nucleotides) non-coding RNAs, which are important components of gene regulation. They negatively regulate the expression of a wide range of mRNAs involved in many important biological processes such as plant defense pathways and mediating plant response to stress (Sunkar and Zhu, 2004). Intensive research is being carried out in understanding the role of miRNAs in normal growth and development, but very little is known about the role of miRNAs in regulating the secondary metabolite production, in particular SQTs.

Some miRNAs are known to be involved in regulating the biosynthesis of various secondary metabolites through targeting the genes and transcription factors of the secondary metabolite biosynthesis pathways. In *A. thaliana*, a number of miRNAs have been investigated with their target transcripts involved in the secondary metabolite biosynthesis pathways. An indirect involvement of miR156 has also been investigated in the biosynthesis of (E)-β–caryophyllene, where miRNA156 had targeted SQUAMOSA Promoter-Binding-Like (SPL) transcription factor, which influenced (E)-β-caryophyllene biosynthesis (Yu et al., 2015). miR156 has also been shown to target SPL transcription factor and down-regulate anthocyanin biosynthesis (Yu et al.,

2015). In another study, the overexpression of miR393 in *A. thaliana* through influencing auxin biosynthesis pathway had changed the level of glucosinolate and camalexin.

Due to the importance of SQTs in different sectors ranging from agriculture to pharmaceutical, it was very important to explore their role in the biosynthesis of such important biomolecules.

Materials and Methods

Prediction of miRNAs involved in SQT biosynthesis

A. thaliana miRNA sequences were retrieved from miRBase database (http://www.mirbase.org/). Current release, miRBase 21, is linked with the latest versison of *Arabidopsis* database (TAIR 10) and comprises 427 mature miRNAs. Transcripts or genes involved in the biosynthesis of SQTs were obtained from various literature searches and the sequences were subsequently retrieved from The *Arabidopsis* Information Resource (TAIR) and The National Centre for Biotechnology Information (NCBI).

For prediction of miRNAs targets (mRNAs) involved in SQT biosynthesis, a well-established plant small RNA target analysis server (psRNATarget) was used. This server is linked with up to date miRBase release, miRBase 21. In addition to psRNATarget, TAPIR was also used for plant miRNAs target prediction. To avoid false positive prediction, a stringent cut-off threshold (0-3.0) was selected. The maximum allowed energy to unpair the target site (UPE) was selected (25.0), and for miRNAs showing multi targets, a target with least UPE was selected. Range of central mismatch was selected from 9 to 11.

Development of amiRNAs over-expression constructs

For functional analysis and confirmation of amiRNA targets (mRNA), the amiRNA was required to be over-expressed in plants. A plant amiRNA over-expression vector (pMDC32b-AtMIR390a b/c) was received from Carrington lab, Donald Danforth Plant Science Center, USA, as a gift. This vector carries *A. thaliana* MIR390a precursor (AtMIR390a) for the expression of amiRNA. The development of amiRNA over-expression involved designing amiRNA oligonucleotide (http://p- sams.carringtonlab.org) (Figure 1). The insert (amiRNA) was synthesized by annealing two partially complementary and overlapping 75-base oligonucleotides, which covered the amiRNA/*AtMIR390a*-distal-loop/amiRNA* sequence following the protocol adapted from Carbonell et al. (2014). The ligation mixture was transformed into *Escherichia coli* Top 10, lacking *ccd*B resistance using heat shock method. Agar plates with 50 µg/µL kanamycin antibiotic were used for overnight inoculation of transformation mixture. Five colonies per construct were randomly selected for colony PCR using attB1 and attB2 primers. Positive colonies were then grown in LB media using Kanamycin antibiotics (50 µg/mL) and plasmids were purified using Qiaprep mini Plasmid extraction kit (Qiagen, USA). The extracted plasmids were then confirmed through PCR. The PCR positive constructs were then confirmed through sequencing.

Developing transgenic A. thaliana plants

The constructs were used in *Agrobacterium* (GV3101) transformation using floral dip method into *A. thaliana* plants at anthesis stage and grown at 28^{o}C for 2 days. The plants were then dipped in the liquid agrobacterium culture (Clough and Bent, 1998). The transgenic plants were screened using hygromycin. T_0 seeds were plated onto MS gelrite plates using 50 µg/µL hygromycin antibiotic. After 15 days, the plants which survived were transferred to soil pot as single plant.

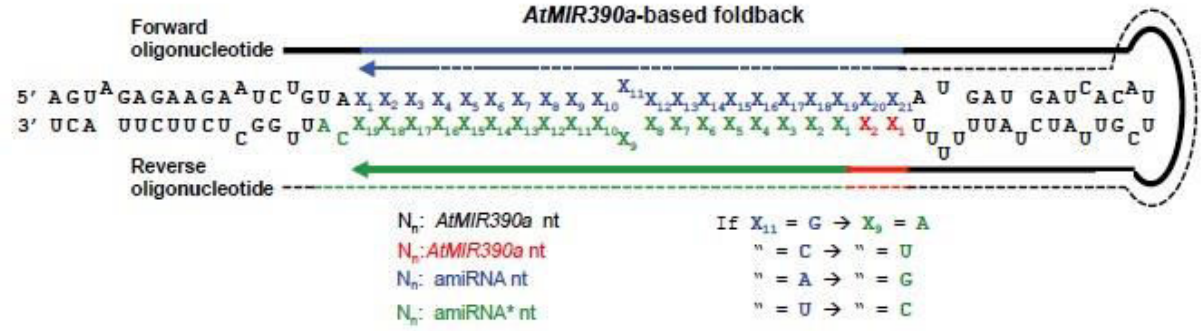

Figure 1: Design of overlapping oligonucleotides for direct amiRNA cloning. Sequences covered by the forward and the reverse oligonucleotides are represented with continuous or dotted lines, respectively. Nucleotides of *AtMIR390a* foldback, amiRNA guide strand and amiRNA* strand are in black, blue and green, respectively.

Results and Discussion

For the *in silico* prediction of miRNA and their targets involved in terpene biosynthesis pathway, over 1000 transcripts were retrieved from TAIR and NCBI. Then, 19 miRNAs were predicted with their targets involved in SQT biosynthesis using psRNATarget and TAPIR tools/databases. For prediction of miRNA targets, stringent selection criteria were adopted to avoid any false positive selection The 19 predicted miRNA targets were further narrowed down to 6 miRNAs (Table 1) with their targets in the terpene biosynthesis pathways.

Table 1: List of miRNAs with their predicted targets (mRNAs).

Sr. No.	miRNAs	Target transcript (mRNA)
1	miR826b	1-deoxy-D-xylulose-5-phosphate synthase
2	miR835	alpha-humulene/(-)-(E)-beta-caryophyllene synthase
3	miR399a	pentacyclic triterpene synthase 6 Mrna
4	miR863-5p	ATTPS12 , Terpnnoid synthase 12, TPS12
5	miR414	terpenoid synthase 26
6	miR2937	geranylgeranyl pyrophosphate synthase

The expression analysis of these amiRNAs will shed light to their role in regulating the synthesis of important terpene biomolecules. The above selected miRNAs have key positions in the terpenoid biosynthesis pathway.

The amiRNAs were ligated into plant amiRNA over-expression vector (pMDC32b-AtMIR390a b/c). The constructs were transfromed into *E. coli* Top10 and well grown colonies were selected and positive plasmids were confirmed through PCR (Figure 2).

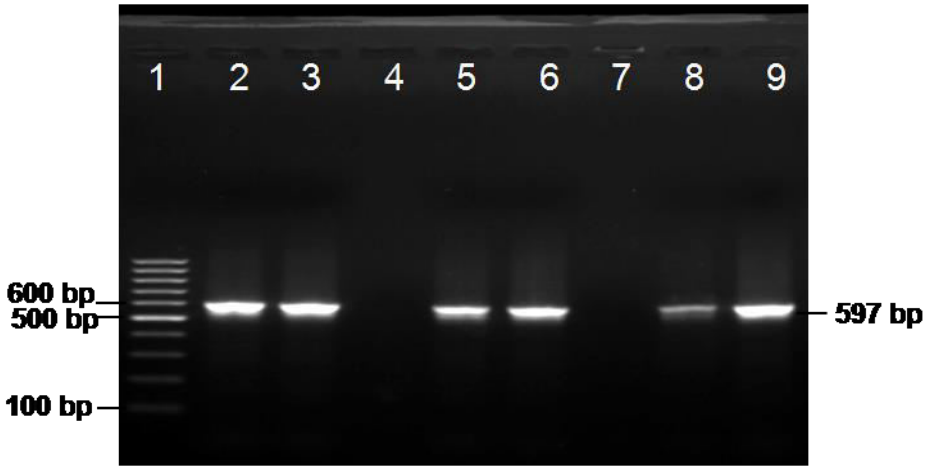

Figure 2: PCR of amiRNA constructs. Lane 1 is 100 bp ladder, lane 2 miR826b, lane 3 miR835, lane 5 miR399a, lane 6 miR863-5p, lane 8 miR414 and lane 9 miR2937.

The positive amiRNA constructs were then transferred to agrobacterium cells using heat shock transformation and *A. thaliana* plants were transformed using floral dip method. The transformed seeds were then harvested and screened on MS gelrite media with Hygromycin antibiotic 50 µg/mL (Figure 3).

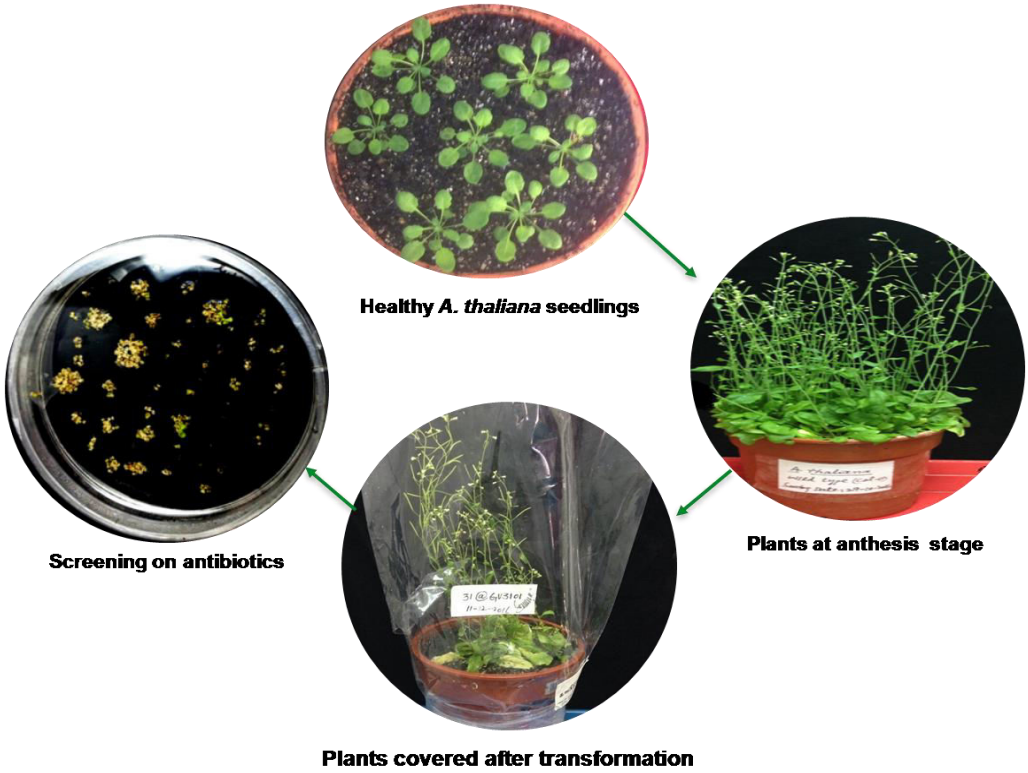

Figure 3: Floral dip transformation and screening of transgenic *A. thaliana* seeds.

Once the T_2 generation of transgenic plants are obtained, the validation of predicted miRNAs and their targets will be carried out using qRT-PCR. Further analysis of the transgenics will shed light on the role of miRNA in the production of these important biomolecules.

Conclusions

miRNAs and their target transcripts (mRNAs) in the SQTs biosynthesis pathway in *A. thaliana* have been predicted. All six miRNAs have been over-expressed in *A. thaliana* using floral dip method and the screening of transgenic seed is in progress. Analysis of the transgenics plants will contribute towards manipulation of the production of the desired SQTs biomolecules according to the need of particular sector.

Acknowledgement

The authors would like to thank and show our appreciation to Universiti Kebangsaan Malaysia for financing this project through Research University Grant DIP-2015-018.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Effects of Drought Yield QTLs to Morpho-physiological Traits of Malaysia's New Rice (*Oryza sativa* L.) Lines

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Introduction

Rice (*Oryza sativa* L.) is the most widely cultivated crop and act as the staple food for nearly 31.7 million people of Malaysia. Current rice self-sufficiency level is at 71% and the remaining 29% dependent on imported rice from neighbouring countries. Rice production level must be increased or at least maintained at current level to ensure adequate food supply but most high yielding rice cultivars in Malaysia are susceptible to drought. Drought had caused adverse effects to rice production and caused high loss to farmers especially in non-granary areas where their fields were not perfectly equipped with proper irrigation system. Apart from providing irrigation system in rice fields which is costly, it is also vital to maintain rice production by providing rice with the ability to tolerate water scarcity in the occurrence of drought (Swamy and Kumar, 2012). The efforts to breed rice adaptable to drought prone environments were slow due to several reasons such as low heritability of grain yield under drought stress and insufficient criteria for selection of traits related to drought tolerance (Ouk et al., 2006). Venuprasad et al. (2009) also highlighted that failure to detect large effect quantitative trait loci (QTL) is another reason for this progress to slow down.

Extensive research by International Rice Research Institute had found several QTL for drought yield (*qDTYs*) with consistent and large effect on grain yield under reproductive drought stress (RS) such as $qDTY_{3.1}$, $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{4.1}$, $qDTY_{9.1}$, and $qDTY_{10.1}$ (Swamy and Kumar, 2012) and $qDTY_{12.1}$ (Bernier et al., 2007). These *qDTYs* were introgressed and pyramided into mega cultivars around the world and were released to farmers such as Sahbhagi dhan in India, Sahod Ulan in Philippines and BRRI dhan56 in Bangladesh (Swamy and Kumar 2012). Therefore, to ensure sustainable rice productivity in Malaysia despite bad climate, research on drought tolerance have been done including the pyramiding of $qDTY_{2.2}$, $qDTY_{3.1}$ and $qDTY_{12.1}$ into two Malaysia's cultivars; MR219 and MRQ74 (Shamsudin et al., 2016). The results had shown that *qDTYs* help to improve grain yield (GY) of pyramided lines (PLs) under reproductive drought stress (RS) (Shamsudin et al., 2016). However, previous study only recorded limited traits such as plant height (PH), days to 50% flowering (DTF), number of panicles (NP) and grain yield (GY). Every plants including rice will try to adjust physiological processes for them to cope with water stress. Therefore, this study aims to evaluate how the presence of *qDTYs* will improve morpho-physiological traits of PLs under RS and non-stress conditions (NS).

Materials and Methods

Field trial was done during October 2015 to February 2016 using 39 MRQ74 pyramided lines (PLs), three *qDTY* donors (IR 84984-83-15-18-B, IR 77298-14-1-2-10 and IR 81896-B-B-195), recipient parent (MRQ74) and five checks (Vandana, Siraj, Basmati 370, MR219-4 and Nagina 22). Experimental design used was alpha-lattice with two replications for each condition, NS and RS. RS was established 30 days after transplanting of 21 days old seedling into the field. For NS plot, 5 cm standing water was maintained and drained before harvest. Each seedling was planted at the distance of 25 cm between row and hills. Abiotic parameters such as humidity, temperature and soil water content throughout the trial were recorded. For measurement of soil water content, two methods were used; self-made peiziometer and gravimetric method (Torres et al., 2012). Fertilizers (N:P:K

17.5:15.5:10) and Urea 46% N and other additional fertilizers were applied as suggested by Department of Agriculture of Malaysia.

Morpho-physiological traits recorded were days to flowering (DTF), plant height (PH), number of panicles (NP), chlorophyll content (CC), root length (RL), root dry weight (RDW) and leaf rolling (RL). Yield related traits such as 1000 seed weight (TSW) and seed length (SL) apart from the most important trait, grain yield (GY) were also recorded. For all the traits observed and recorded, Standard Evaluation Sytem (IRRI, 2013) was used as the main reference. Collected data were analysed using PB Tools v1.1.0 (International Rice Research Institute) and Minitab 17 (Minitab Pty Ltd).

DNA extraction of each genotype was done using CTAB protocol (Murray and Thompson, 1980). DNA quantity and quality were checked (Nanodrop ND-1000 UV-Vis Spectrophotometer, Nano Drop Technologies Llc, DE, USA) and diluted to 25 ng/µL to be used in polymerase chain reaction (PCR). PCR using 20 primers related to $qDTY_{2.2}$, $qDTY_{3.1}$ and $qDTY_{12.1}$ was done to each genotype to validate the presence of those *qDTYs*.

Results and Discussion

Presence of *qDTYs* showed positive effects to performance of PLs compared to MRQ74. Even though RS caused some traits to decrease, the percentage of reductions were lower for PLs compared to MRQ74. Some selected high yielding PLs under RS had greater RL, RDW, CC, PH, NP and lower LR score than MRQ74 (Table 1). DTF of selected PLs were delayed compared to MRQ74 where in this case, MRQ74 showed drought escape mechanism which was not in favour because early DTF will cause GY reduction. Most PLs flowered later than MRQ74 and GY were higher (r=0.4111). However, this result contrasted what Shamsudin et al. (2016) obtained where PLs flowered earlier than MRQ74. Environmental factors such as temperature, soil and climate might play important role in determining flowering days.

Some PLs also have longer root compared to MRQ74 which is vital as better root system categorised as drought escape mechanism (Lestari et al., 2006). RL and RW were positively correlated with GY (Table 2) which further validate the importance of these traits in selection of drought tolerant lines. Even though drought causing premature leaf senescence (Wahid and Rasul, 2005), PLs and MRQ74 were not affected as both have high CC under RS where 'stay green' trait during RS period is important for drought recovery mechanism (Noraziyah, 2014). PLs were also taller than MRQ74 under RS which means that PLs were less affected by RS and able to maintain good plant water status. According to Fischer and Fukai, (2003), plant water status will be the first trait to be affected in rice during RS and will halt development of leaves and stem elongation.

MRQ74 have higher LR score than selected PLs under RS. LR is important for plant to maintain plant water status (Ha, 2014), reduce transpiration and dehydration (Kodiaglu and Terzi, 2007) by decreasing leaf surface area exposed to sun. However, we found that high LR score is a sign of low leaf relative water content which makes the leaves wrinkled and wilted after losing water in the cell's sap. Therefore, PLs with lower LR score were able to maintain good leaf relative water content and maintain cells turgidity to stay sturdy and erect.

IR 98010-134-4-1-2-1, IR 98008-103-77-1-4-1 and IR 98010-126-77-1-5-1 were the top three PLs with GY under RS with yield advantage of 1254.88 to 1751.63 kg ha^{-1} compared to MRQ74. Under NS, selected PLs (IR 98010-126-77-1-5-1, IR 98010-126-18-4-1-1 and IR 98010-134-4-1-1-1) also had better performance of morpho-physiological traits than MRQ74 and produced higher GY (yield advantage of 631.33 to 1160.08 kg ha^{-1}).

Table 1: Means comparison of selected pyramided lines (PLs) and MRQ74 in RS.

Genotypes	*qDTYs*	DTF	HT	NP	CC	RL	RW	LR	TSW	GL	GY
IR 98008-103-77-1-4-1	$qDTY_{12.1}$,$qDTY_{3.1}$	92.50^{a}	66.17^{b}	11.00	39.79	14.03^{ab}	2.53^{ab}	1.50*	21.13^{a*}	0.97*	3053.71^{ad*}
IR 98010-126-18-4-1-1	$qDTY_{12.1}$,$qDTY_{2.2}$	95.50^{a}	75.50^{ab*}	15.00	42.17	16.38^{b}	2.91^{b}	2.00	21.77^{a*}	0.89	1826.23^{ab}
IR 98010-126-77-1-5-1	$qDTY_{12.1}$,$qDTY_{3.1}$,$qDTY_{2.2}$	90.00^{a}	60.00^{ab}	20.50	40.92	12.68^{ab}	2.36^{ab}	1.50*	16.85^{b}	0.84	2872.94^{ad*}
IR 98010-126-840-1-7-1	$qDTY_{12.1}$,$qDTY_{2.2}$	113.00^{a*}	75.00^{b*}	13.00	41.30	7.08^{a*}	1.33^{a*}	3.00	18.52^{bde}	0.97	1891.74^{ad}
IR 98010-126-846-1-2-1	$qDTY_{12.1}$,$qDTY_{3.1}$,$qDTY_{2.2}$	89.00^{a}	64.42^{ab}	13.50	40.50	11.38^{ab}	2.20^{b*}	1.50*	23.11^{c*}	0.97*	2290.53^{ad}
IR 98010-134-4-1-2-1	$qDTY_{2.2}$	96.50^{a}	65.84^{ab}	16.00	42.75	12.05^{ab}	2.10^{ab*}	1.50*	18.80^{d*}	0.82	3369.69^{d*}
MRQ74	No *qDTY*	89.00^{a}	58.17^{a}	14.50	42.60	13.88^{ab}	3.66^{b}	3.00	17.21^{e}	0.89	1618.06^{bc}

**The value differed from control (MRQ74) by Dunnett's test ($p<0.05$); Values with the same small letter in each column are not significantly different by Tukey test ($p>0.05$); DTF: days to flowering, PH: plant height (cm), NP: number of panicle, CC: chlorophyll content, RL: root length (cm), RW: root weight (g), LR: leaf rolling, TSW: 1000 seed weight (g), GL: grain length (cm), GY: grain yield (kg ha^{-1}), PL: pyramided line.*

Table 2: Correlation between traits recorded in RS.

Traits	DTF	PH	NP	CC	RL	RW	LR	100GW	GL
PH	0.4071*								
NP	0.4583*	0.5101*							
CC	0.0887	0.1473	0.4029*						
RL	0.3143*	0.5536*	0.9291*	0.4935*					
RW	0.3788*	0.5647*	1.0000*	0.3464*	0.7515*				
LR	-0.0567	-0.2392	0.0048	0.1888	0.0491	0.2221			
TGW	0.2244	0.2339	-0.0013	0.4437*	0.3296*	0.0722	-0.0569		
GL	0.3954*	0.3158*	0.6238*	0.7210*	0.5011*	0.7263*	-0.3036*	0.6606*	
GY	0.4111*	0.4600*	0.8903*	-0.0832	0.4216*	0.5828*	-0.1306	-0.0016	0.1452

**Significant correlation ($p>0.05$), DTF: days to flowering, PH: plant height (cm), NP: number of panicle, CC: chlorophyll content, RL: root length (cm), RW: root weight (g), LR: leaf rolling, TGW: 1000 seed weight (g), GL: grain length (cm), GY: grain yield (kg ha^{-1}).*

Conclusions

Under RS, some PLs showed better morpho-physiological traits than MRQ74. These results confirmed the positive effects of these *qDTYs* in improving morpho-physiological traits and GY under RS in MRQ74 PLs. High yielding PLs could be released as new cultivars for drought prone areas in Malaysia to increase productivity of Malaysia's rice fields.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Purification of Polyclonal Antibody against Banana Blood Disease Bacteria

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Introduction

In Malaysia, banana is an important fruits cultivated in the country and has been listed in the non-seasonal tropical fruits in the National Key Economic Area (NKEA) Agriculture program under EPP7. The area cultivated with fruits stands at 250,000 hectare and banana covers 27,500 hectare which constitute 11% of total fruit area in the country. The area is expected to expand in the future due to increasing local and foreign demand and newly implemented Economic Transformation Programme (ETP) by the government (Tengku et al., 2011). However, banana production in Malaysia has decreased due to an emerging serious disease known as Banana Blood Disease which was first observed to be attacking banana in Johore in 2007 (Moktaruddin and Robert, 2011; Jaafar and Rozeita, 2013). The disease is caused by Blood Disease Bacterium (BDB). The severity and disease spreading are even higher than Panama disease which is caused by the fungus, *Fusarium oxysporum cubense (Foc)* and another major disease in the banana production industry.

Banana blood disease symptoms start with colour changing of young leaves from green to yellow or brown and finally withered. Other common symptom is the presence of bacterial slime which is smelly, white greyish to brown reddish in colour that comes out from a few parts of the banana plant such as fruit, hump, fruit stalk, rod cluster and stem (Latupeirissa et al., 2014). During severe attack, the yield loss can be up to 100% (Subandiyah et al., 2013). Banana blood disease is difficult to control, particularly because the pathogen can survive and adapt to its ecosystem. BDB is present not only within the plant but also within the soil in which the plant is growing. It can be spread through infested soil on hands, tools, shoes, machinery, animals and water run-off.

Early disease diagnoses and early detection of the bacterium are important for a successful disease control (Machmud and Yadi, 2008). These could facilitate elimination and certainly reduce the risk of crop loss. Once Moko disease is detected, all infected plants should be removed and destroyed immediately including plants surrounding the infected mat within 5 to 10 meter in diameter.

Antibodies have frequently been selected for a wide variety of applications including immunodiagnostics. Polyclonal antibodies are typically raised in rabbits, goats or sheep and usually used as bioreceptor molecule in immunosensor-based assays for pathogen detection. In this study, polyclonal antibody was produced against Blood Disease Bacterium and the purification process of the antibody was described.

Materials and Methods

Immunization schedule and immunogen preparation

Each immunizing consisted of bacterial cells in 0.5 mL PBS. The bacterial cells were mixed with 0.5 mL of Freund's complete adjuvant until an emulsion was formed, and then injected subcutaneously into

New Zealand white rabbits. For the next three weeks, the rabbits were injected with the same immunogen suspended in 0.5 mL of Freund's incomplete adjuvant. First bleed sample was taken one month after the injection. Blood was obtained by bleeding the ear vein of the rabbit. A booster injection was given two weeks after the bleed. These booster injection and bleed were repeated in two weeks intervals until the sixth bleed.

Antibody purification

The collected blood sample was allowed to coagulate for a few hours. The serum was then separated by centrifugation at 839 x g for 15 min. Antiserum against blood disease bacterium was diluted with distilled water (1:10) and then precipitated with saturated ammonium sulphate with continuous slowly stirring to precipitate the serum protein. The serum mixture was then centrifuged at 839 x g for 30 minutes at 4°C. The pellet was resuspended in PBS and dialyzed in 0.01M PBS buffer to remove ammonium salt. Buffer was changed three times.

Partially purified antibody was then run through a protein A affinity column (nProtein A Sepharose^{TM}) using AKTA prime Plus protein purifier instrument. Phosphate buffer (0.01 M, pH 7) was used as the binding buffer and glycine buffer (0.1 M, pH 3) was used as the elution buffer. The column was equilibrated with 5 column volumes before applying the samples. Then, 3 mL of partially purified antibody was injected into the column and run with the flow rate of 2.0 mL/min and 0.3 MPa pressure limit. Fractions with the highest absorbance reading at 280 nm were collected. One molar of Tris HCl pH 9 was used as neutralized buffer and added to the eluted samples. Purified antibody was run through dialysis again to remove salt.

SDS-PAGE analysis

SDS-PAGE using 12% polyacrylamide gel was performed according to the method of Laemmli (1970). Broad range protein ladder (Bio-Rad, USA) was used as protein ladder. The electrophoresis was done using the Mini PROTEAN 3 Cell Apparatus (Bio-Rad, USA) according to manufacturer's instructions.

Results and Discussion

Bacterial cells of BDB were injected into white New Zealand rabbit with the addition of Freund's Adjuvant. It is an inexpensive strategy for polyclonal production. Freund's adjuvant which is paraffin oil based has been used for stimulation of the immune system by *Mycobacterium* in Complete Freund's Adjuvant to generate high antibody titers (Trott et al., 2008). Not only does this adjuvant activate the immune system, it also retains the antigen so that it is released slowly in the injection site (Bollen et al., 1996).

Serum obtained from each bleed was purified using two main step of antibody purification involving ammonium sulphate precipitation and column chromatography. Affinity chromatography is one of the best methods for purifying polyclonal antibody which utilizes Protein A as specific IgG-binding ligand (Moser and Hage, 2010). Figure 1 shows the chromatogram of antibody fraction elution from a Protein A affinity chromatography column. There were two protein elution peaks where the first peak (peak 1) eluted with binding buffer represented protein contaminants in the serum sample loaded into the column under conditions that allowed maximum binding onto the affinity ligand. The antibody binds to the affinity ligand through non-covalent forces such as hydrogen, ionic and hydrophobic bindings. The second peak (peak 2) which showed the highest absorbance was obtained after the desorption step took place where the elution buffer caused a weakening to the non-covalent forces holding the antibody to the affinity ligand, subsequently dislodging the antibody from the affinity ligand. These also proved that the binding and elution buffer used in this experiment were suitable for purification of the antibody.

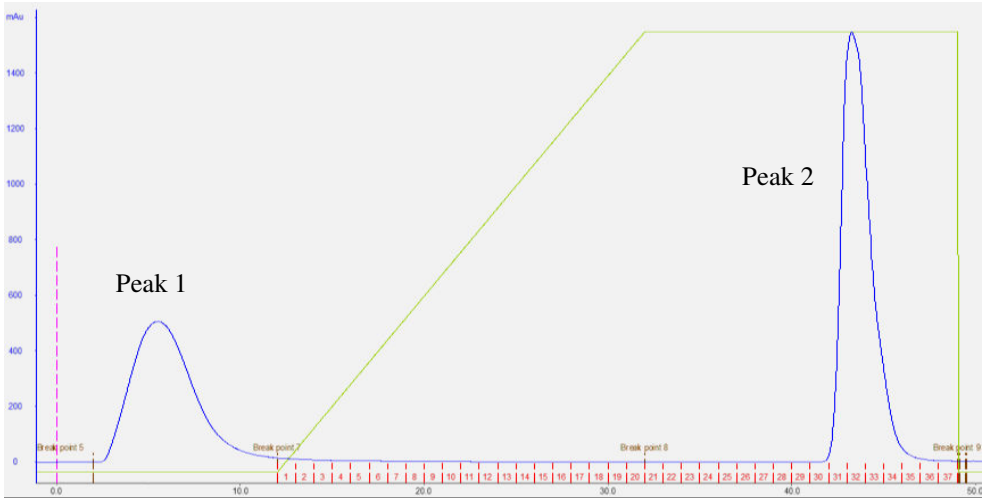

Figure 1: Chromatogram of antibody elution from Protein A affinity column using AKTAprime chromatography system.

To confirm the successful and the purity of the antibody purification, SDS-PAGE analysis was performed which showed two bands of heavy and light chains of antibody subunit (Figure 2). The molecular mass of the heavy chain was approximately 47 kDa whereas the molecular mass of the light chain was approximately 22kDa.

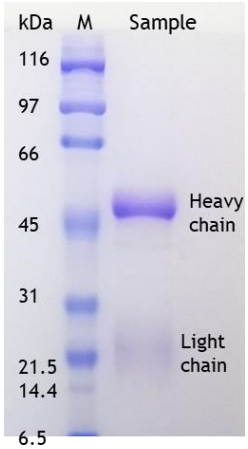

Figure 2: SDS-PAGE analysis of the purified antibody.

Conclusion

Polyclonal antibody against Banana Blood Disease pathogen has been purified successfully using ammonium sulphate precipitation and affinity column chromatography. Pure antibody is important to avoid interference since the antibody will be used as bioreceptor molecule in the development of immunosensor for Banana Blood Disease detection.

Acknowledgement

This work was financially supported by MARDI RMK11 Development Fund (P-RM405).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

A Simple and Rapid Molecular Diagnostic Method for the Detection of Bacterial Leaf Blight Disease (BLB)

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Introduction

Simple and rapid DNA-based point-of-care (POC) diagnostic methods that can be performed in field without sophisticated equipment and at low cost are in high demand (Yager et al., 2006). Classical techniques and methods for detecting diseases include the polymerase chain reaction (PCR) and ligase chain reaction (LCR) (Barany, 1991). However, these methods require a thermal cycler to achieve rapid DNA detection and hence are not suitable for field or on-site applications. Nonetheless, recent developments in isothermal DNA amplification methods may potentially overcome this limitation (Craw and Balachandran, 2012). For instance, isothermal amplification methods combined with lateral flow strips and portable fluorometers have been developed to enable POC detection of pathogenic DNA (Vincent et al., 2004; Piepenburg et al., 2006). Such readout methods whilst convenient are still dependent on the use of relatively sophisticated equipment and may still present financial and technical obstacles for worldwide adoption. A field-ready comprehensive assay incorporating the complete process from on-site sample preparation to results display is still elusive.

Agriculture is one area that can benefit from the use of low cost on-site assays. While being a major contributor to the world economy, crop diseases are still a major concern in agriculturally reliant economies (Agrios, 2005). In Malaysia, paddy is one of the important crops and yet it faces problem of high crop losses every year. The paddy fields are frequently infected by several fungal and bacterial diseases. Bacterial leaf blight (BLB) caused *Xanthomonas oryzae* pv. *oryzae (Xoo)* is one of the major disease of rice worldwide. This disease can be spread through several factors such as wind and rain (Nino-liu et al., 2006). Traditionally, the detection of BLB was based on an experienced plant pathologist identifies the disease by a subjective visual examination of disease symptoms (Horsfall and Cowling, 1977). To address this, more analytical diagnostic methods have since been developed (Price et al., 2010; Dai et al., 2013). However, these detection methods require expensive and sophisticated equipment and can only be performed in specialized laboratories by well-trained technicians. Although As early detection is the ideal method to for controlling BLB outbreaks, the current lack of on-site detection protocols delays deployment of BLB control strategies thus resulting in huge crop losses.

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification method developed in 2000 (Notomi et al., 2000). It has been widely used due to its high efficiency, specificity, simplicity and quickness. LAMP has three major advantages. Firstly, it can be carried out at a constant temperature with a short reaction time. This rapid isothermal process makes it ideal for point-of-care detection of plant pathogens in the field (Fukuta et al., 2014) and has been used to detect the plum pox virus in 2.5 hrs (Hadersdorfer et al., 2011). Secondly, it has very high amplification efficiency and sensitivity as it generates large amounts of PCR product with low amounts of input DNA (Tomlinson et al., 2010; Bhat et al., 2013). Finally, this method is relatively cost effective as it requires simple equipment to perform the assay. Although LAMP has been used for point-of-care (POC) application in plant diseases, but to the best of our knowledge, detection of rice diseases using LAMP assay incorporated with flocculation of nano materials as readout method is still new.

Bridging flocculation is a well-known phenomenon in colloid chemistry and is used for a wide variety of colloidal separation processes (e.g., waste water treatment). The phenomenon was first described in the

1910's by Ruehrwein, R.A (Ruehrwein and Ward, 1952) and explained in the 1960's by La Mer and Healy (Healy and La Mer, 1964) to be the result of the surface adsorption of polymers which are long enough to cross-link multiple particles together and thus flocculate out of solution. To make use of this phenomenon, we have developed a POC diagnostic method using LAMP incorporated with bridging flocculation of magnetic particles for binary (Yes/No) detection of BLB. Successful DNA amplification in positive sample from LAMP with long DNA is expected to form the flocculation phenomenon which can be a naked eye assay. We believe that this assay can make a significant contribution in rice industries as enabler tools for diagnosing important rice diseases.

Materials and Methods

Bacterial strain and culture condition

Xanthomonas oryzae pv. *oryzae* was obtained from the National Collection of Plant Pathogenic Bacteria (NCPPB). The bacteria was cultured on PDA media (1000 mL media consists of 300 g fresh potato, 2 g $Na_2HPO_4.2H_2O$, 0.5 g $Ca(NH_3)_2.4H_2O$, 5 g Bacto-peptone and 20 g sucrose). The plate was incubated at 30°C for 3 days.

DNA extraction

The GenExTM Cell kit (GeneAll) was used for genomic DNA extraction of bacteria *Xoo* as recommended by the manufacturer with minor modification (http://geneall.com). A single loop of bacteria culture was obtained from plate culture and added into 300 µL of Complete lysis (CL) buffer. The DNA extraction method was then continued with the protocol provided by the manufacturer.

LAMP reaction

A LAMP Primer Mix was prepared with all 6 primers (Table 1) which containing 40 µM FIP, 40 µM BIP, 5 µM F3, 5 µM B3, 10 µM LoopF, 10 µM LoopB. Briefly 25 µL reactions (1X ThermoPol Buffer, 6 mM $MgSO_4$, 1.4 mM each dNTP, 8U Bst DNA polymerase, 3 µL of primer mix) were performed at 65°C for 40 min using 1 µL of the extracted nucleic acid (concentration of gDNA was depended on the experimental design). Following amplification, 3 µL of the LAMP reaction was verified by gel electrophoresis.

Table 1: Oligonucleotide sequences used in this study.

Primer name	Sequence (5´ to 3´)
FIP	AAGCCTTCCGCTGAGGTTGTAAGTAGTGCGGTCAGGAA
BIP	GAGGCTGCTTCTATGGGAACCAGTTCTGGCGAATCTATTAGC
F3	GCTGACGAAGAGCAACTT
B3	CAATAACTGCTGGCAATGATC
LoopF	ATACAACACGATGCTGGCA
LoopB	GAGCTTCGGCCCACTTAG

Bridging flocculation assay

A volume of 5 µL of the LAMP product was used in the flocculation assay by adding 1.5-1.8 volumes of Solid Phase Reversible Immobilization (SPRI) bead solution and mixed well. After bead separation with a magnet, 30 µL of flocculation buffer (100 mM sodium acetate, pH 4.4, 1% (v/v) Tween 20) was added to the beads and gently agitated.

Results and Discussion

This diagnostic method was conceived to be a comprehensive integrated solution for on-site sensitive and rapid detection of *Xoo* with minimal equipment. Figure 1 briefly describes the diagnostic concept which consists of three main phases. The first phase is DNA extraction; the second phase is the sensitive, rapid isothermal amplification of *Xoo* nucleic acids using LAMP; the third and final phase is the specific capture of amplified DNA using SPRI followed by a naked-eye visual inspection of the same SPRI particles in a flocculation inducing buffer.

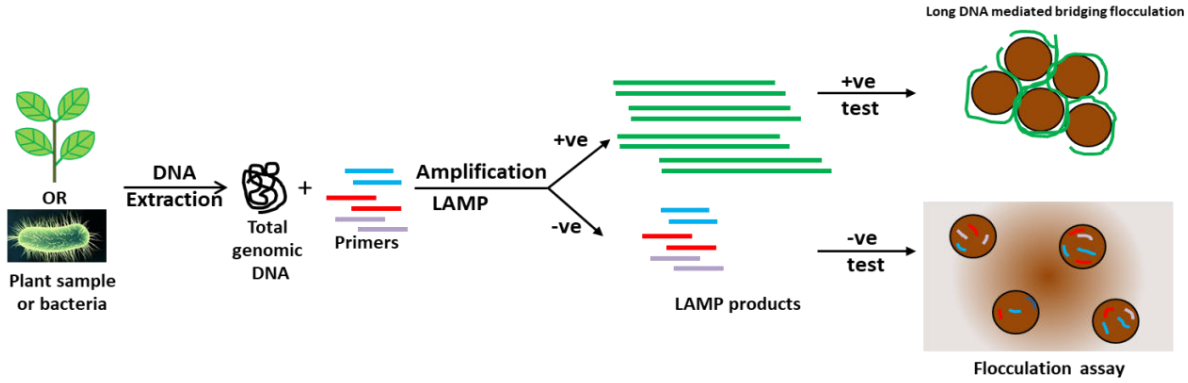

Figure 1: Schematic diagram of the flocculation assay. DNA from *Xoo* was extracted, amplified and visualized by a naked-eye, equipment-free display.

The specificity of the assay was assessed by detecting *Xoo* and *Xoc* (Figure 2). Genomic DNA was extracted from pure bacteria cultures (*Xoo* strain 3951; *Xoc* strain 1585 and 2921). Replicate assays containing different amounts of purified *Xoo* and *Xoc* genomic DNA were performed with LAMP and followed by flocculation assay. As expected, only gDNA from *Xoo* (BLB 3951) was amplified successfully and the flocculated SPRI beads showing clear solution. This indicated that the primers are very specific on *Xoo* but not *Xoc*.

To investigate the sensitivity of the assay (Figure 3), gDNA ranging from 0.0001 ng to 1 ng were used to perform LAMP. From the pictures of gel electrophoresis and flocculation assay, successful amplified DNA was observed in gDNA sample 0.0005 ng and higher DNA concentration samples. No bands were observed when the amount of input gDNA was decreased below 0.0005 ng which was same as the control reaction containing no genomic DNA.

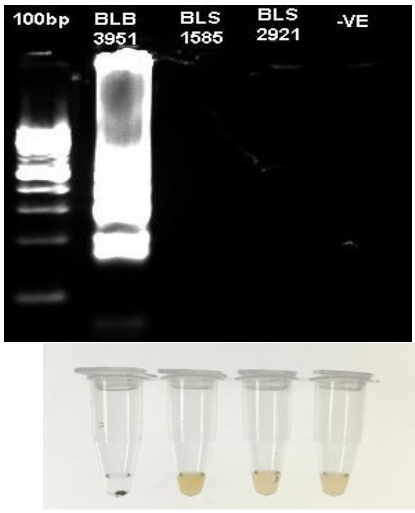

Figure 2: Specificity test of the assay**.** Top row: gel electrophoresis images of corresponding LAMP reactions performed on different sample (Xoo = BLB 3951, Xoc = BLS 1585, BLS 2921). Bottom row: photographs of the flocculation assay corresponding to the LAMP reactions. Each figure is a representative of at least 3 experimental replicates.

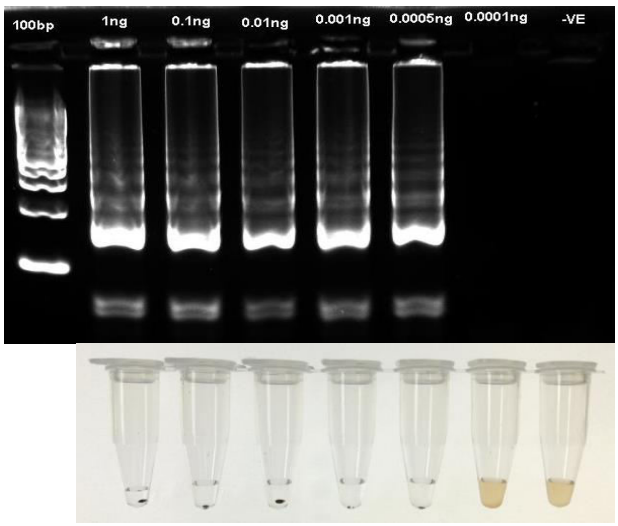

Figure 3: Sensitivity test of the assay**.** Top row: gel electrophoresis images of corresponding LAMP reactions performed on different concentration of Xoo gDNA sample (5 ng, 0.1 ng, 0.01 ng, 0.00 1ng, 0.0005 ng, 0.0001 ng). Bottom row: photographs of the flocculation assay corresponding to the LAMP reactions. Each figure is a representative of at least 3 experimental replicates.

Conclusion

In conclusion, the LAMP primers are specific for *Xoo* detection. Flocculation assay is a potential diagnostic method for on-site application in the future because of it offers rapid, sensitive, requires minimal equipment and less skilled labours.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Sensitivity and Specificity Test of the Polyclonal Antibody Produced against *Xanthomonas oryzae*

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Introduction

Rice diseases are among the most significant limiting factors that affect rice production, causing annual yield losses conservatively estimated from 5 to 30%. More than 70 diseases caused by bacteria, fungi, viruses or nematodes have been reported on rice. One of the most destructive rice diseases is bacterial leaf streak (BLS) causes by *Xanthomonas oryzae* pv. *oryzicola* (Xoc). The BLS bacterium is Gram negative, rod-shaped and motile by a single flagellum (Sullivan et al., 2011).

This disease has a wide geographical distribution particularly in the tropical and subtropical Asian region, including Malaysia, India, China, Thailand, Vietnam, the Philippines and Indonesia. BLS bacterium can be found in a field area that is equipped with sprinkler irrigation in temperate climates and subtropical highlands with high capacity of rainfall where humidity is high (Forster et al., 1986). This bacterium tolerates a wide range of temperatures between 15 to 30°C (Duveiller et al., 1991), and some studies have shown that this wide range of temperature is the major reason for the wide location of this disease (Duveiller and Maraite, 1995). However, low temperatures retard the multiplication of the bacterium and also the progress of the disease.

The BLS disease may transmit from infected plants to healthy plants in nearby fields by prevailing wind, rainstorms and also dew. The bacterium enters rice leaves through the stomata or wound sites and colonizes intercellular spaces in the mesophyll, resulting in water-soaked interveinal lesions that develop into translucent streaks. The bacterial streak may develop during any development stage of the rice plant. Initial symptoms include the formation of interveinal, water-soaked leaf streaks or spots. The streaks eventually turn yellow to orange. Individual plants also may develop a pronounced yellowing of the tips of new leaves if infection occurs early in the season. In its advanced stages, the disease is difficult to be distinguished from bacterial leaf blight (BLS) which is caused by *X. oryzae* pv. *oryzae* (Xoo) (David et al., 2006).

Biochemical test and fatty acid and metabolic profiling could be used as methods to distinguish both strains, however, these methods are relatively slow. Therefore, the development of an inexpensive and accurate enzyme immunoassay screening kit for detecting BLS disease in a paddy field is highly desirable in deciding upon appropriate control for this disease. In this work, we report on the production of polyclonal antibody against *X. oryzae* pv. *oryzicola* using bacterial suspension as an antigen for immunization in rabbits for the production of polyclonal antibody. The sensitivity and specificity of the polyclonal antibody produced were evaluated using indirect ELISA method. In future, these study output can be used for a development of immuno-lateral flow strip test kit or integrated with a sensor system (biosensor) for in-field screening of early warning detection of BLS disease.

Materials and Methods

Bacteria

The strains of *X. oryzae* pv. *oryzicola* (NCPPB 1585 and 2921) were obtained from the culture collection of National Collection of Plant Pathogenic Bacteria. Both of the strains were cultured on potato sucrose agar (PSA) at 30°C for 48 hours. Large quantities of the mass-produced bacterium on PSA agar were collected by washing the colony surface on the agar plate with 1 mL of ultra-pure water. Cells were harvested by centrifugation at 5,000 × g for 15 min at 4°C on a benchtop centrifuge. The pelleted cells were then washed with Phosphate Buffered Saline (PBS) and the procedures were repeated three times.

The bacterial cells were then re-suspended in PBS and the bacterial suspensions were adjusted to optical densities (OD) at 600 nm of between 1.0 and 1.3 to obtain bacterial concentrations between 1×10^9 and 1×10^{10} CFU mL^{-1} on a UV/VIS spectrophotometer. The bacterial concentrations were confirmed by a spread plate method. Bacterial suspensions were then diluted to the desired target concentrations ranging between 1×10^1 and 1×10^9 CFU mL^{-1} in PBS. The cells were used in the preparation of standard curve plotting for *X. oryzae* pv. *oryzicola.*

Sensitivity test

The specificity test of the rabbit polyclonal antibody against *X. oryzae* pv. *oryzicola* was carried out using the indirect enzyme-linked immunosorbent assay (ELISA) method. The microtiter plate was coated with 100 µL per well with different concentrations of *X. oryzae* pv. *oryzicola* ranging from 1×10^1 to 1×10^7 CFU mL^{-1} (prepared in carbonate buffer, pH 9.6), and was incubated overnight at 4°C. The control for this experiment was carbonate buffer (pH 9.6). The next day, the plate was emptied and washed three times with PBS-T and the uncoated site of the well surface was blocked with a 200 µL per well of 1% (w/v) BSA at 37°C for one h. After a further washing of three times with PBS-T, well plate was incubated at 37°C for another 2 h with 100 µL per well of primary antibody (anti- *X. oryzae* pv. *oryzicola)* at the concentration of 0.001 mg mL^{-1}. The plate was washed a further three times with PBS-T again and then was incubated at 37°C for 30 min with secondary antibody (Goat anti-rabbit IgG-AKP, 1:10 000). After final washing for three times, 100 µL of the AKP substrate solution, *p*-NPP was added per well and incubated in the dark for 15 min. When AKP and p-NPP were reacted, a yellow water-soluble reaction product is formed. The absorbance of the resulting yellowish solution was measured at 405 nm using an ELISA reader. The entire experiment was carried out in triplicates.

Specificity test

The specificity test of the rabbit polyclonal antibody against *X. oryzae* pv. *oryzicola* was carried out with other plant pathogens including *X. oryzae* pv. *oryzae* (P0.0), *X. oryzae* pv. *oryzae* (P7.3), *X. oryzae* pv. *oryzae* (NCPPB 3951), *Xanthomonas* spp (SS14), *Xanthomonas* spp (NCPPB 3949), *Pantoea* sp (TS3), *Pantoea* sp (DB2), *Pantoea amanatis* (TS5), *Pseudomonas* sp (DB3) and negative control (carbonate bicarbonate buffer). Aliquots of all plant pathogens at the concentrations of 1×10^7 CFU mL^{-1} were dissolved in carbonate coating buffer, pH 9.6, and were dispensed about 100 µL per well. The subsequent steps were the same as described in the above method. The entire experiment was carried out in triplicates.

Limit of detection (LOD) calculation for ELISA method

Calibration curves were fitted with a non-linear regression using four-parameter logistic equations (Karpinski, 1990) as showed below:

$$y = \frac{a-d}{1+\left(\frac{x}{c}\right)^{b}} + d$$

where y is the absorbance obtained (nm), *a* and *d* are the maximum and minimum response (nm) of calibration curve, respectively, *c* is the concentration of bacterial cells (log CFU mL^{-1}) that produced a 50% signal response (EC50) value, *x* is bacterial cell concentration (log CFU mL^{-1}), and *b* is the slope-like parameter (Hill coefficient). The limit of detection (LOD) was calculated as the mean value of absorbance at a blank concentration of bacteria at three standard deviations (SD). LOD and regression analysis were calculated using four-parameter logistics model available from PRISM non-linear regression analysis software from www.graphpad.com.

Results and Discussion

The sensitivity test of the produced polyclonal antibody against *X. oryzae* pv. *oryzicola* was evaluated through the construction of standard curve with different concentrations of *X. oryzae* pv. *oryzicola* ranging from 1×10^{1}-1×10^{7} CFU mL^{-1} using an indirect ELISA method. The results obtained in Figure 1 shows that a typical sigmoid curve based on the four-parameter logistic equation. There was no increase in binding response observed at *X. oryzae* pv. *oryzicola* concentrations in between 1×10^{1} to 1×10^{2} CFU mL^{-1}. However, there was a slight increase in the biding response observed at *X. oryzae* pv. *oryzicola* concentration in between 1×10^{2} to 1×10^{3} CFU mL^{-1} with an absorbance response of 0.213. The calculated LOD value was $3.51\text{x}10^{2}$ CFU mL^{-1} with good correlation coefficient value of 0.998.

The use of the indirect ELISA assay offers several advantages such as; (i) an increased in sensitivity due to the use of more than one labelled antibody per primary antibody, (ii) the assay offers flexibility since a multitude of different primary detection antibodies and secondary labelled antibody can be used, and (iii) economy, as fewer labelled antibodies will need to be prepared.

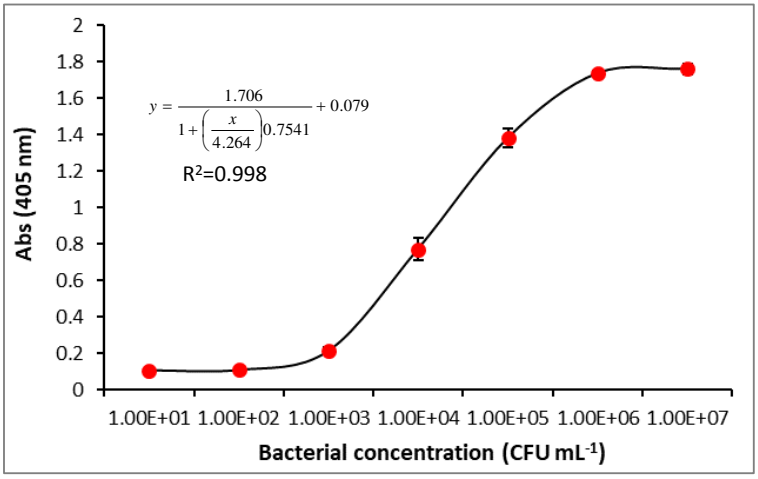

Figure 1: The sensitivity test of the polyclonal antibody against *X. oryzae* pv. *oryzicola.*

The specificity test of the produced polyclonal antibody against *X. oryzae* pv. *oryzicola* was tested with other plant pathogens. The results in Figure 2 shows that the produced polyclonal antibody overwhelmingly detected *X. oryzae* pv. *oryzicola* (both NCPPB 1585 and 2921) with a good signal giving an absorbance value of about 1.812 and 1.814, respectively. When translated as a percentage of response compare to *X. oryzae pv. oryzicola* (NCPPB 1585), the response obtained for other related of the *Xanthomonas* spp. were relatively low (<50%) (Table 1). These include *X. oryzae* pv. *oryzae* (P0.0),

X. oryzae pv. *oryzae* (P7.3), *X. oryzae* pv. *oryzae* (NCPPB 3951), *Xanthomonas* spp (SS14), *Xanthomonas* spp (NCPPB 3949). Generally, a polyclonal antibody is raised against many epitopes-some of which are shared in bacteria resulting in non-specific interaction. There is a linear relationship between amino acid substitutions and the cross-reactivity of polyclonal because antigen on the surface of a protein appears to show sets of epitopes that are nearly continuous and overlapping (Hochel et al., 2004).

The results also show that the produced polyclonal antibody did not react with the other plant pathogens with the percentage of cross reaction below than 10%. These include plant pathogens such as *Pantoea* spp (TS3), *Pantoea* spp (DB2), *Pantoea amanatis* (TS5) and *Pseudomonas* spp (DB3) indicating the developed polyclonal antibody is very specific.

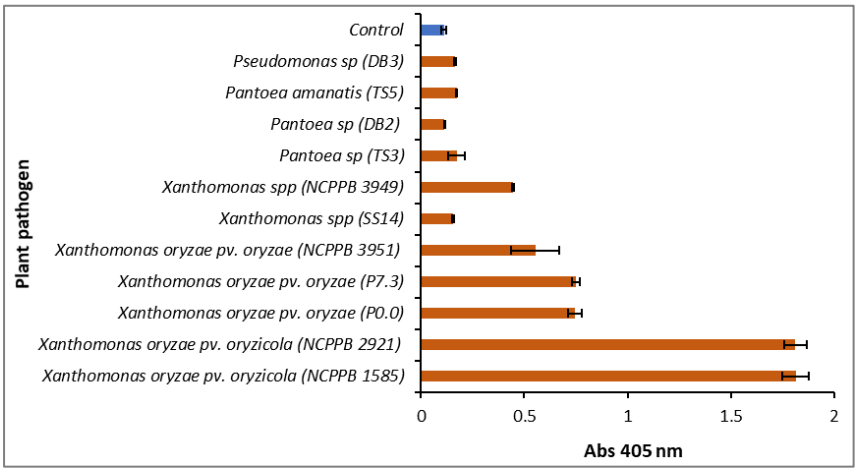

Figure 2: Cross-reaction against different plant bacteria at the concentration of 1×10^7 CFU mL^{-1} and control using indirect ELISA. Error bars represent the average ± standard deviation of triplicates.

Table 1: Relative response of polyclonal antibody binding against different other plant pathogens in percentage calculation.

Plant pathogen	Percentage of response (%)
Xanthomonas oryzae pv. *oryzicola* (NCPPB 1585)	100.0
Xanthomonas oryzae pv. *oryzicola* (NCPPB 2921)	99.9
Xanthomonas oryzae pv. *oryzae* (P0.0)	41.0
Xanthomonas oryzae pv. *oryzae* (P7.3)	41.3
Xanthomonas oryzae pv. *oryzae* (NCPPB 3951)	30.5
Xanthomonas spp (SS14)	8.5
Xanthomonas spp (NCPPB 3949)	24.5
Pantoea spp (TS3)	9.5
Pantoea spp (DB2)	6.3
Pantoea amanatis (TS5)	9.5
Pseudomonas spp (DB3)	9.1
Control	6.2

Conclusions

In conclusion, a polyclonal antibody against *X. oryzae* pv. *oryzicola* has been successfully developed with the calculated LOD of $3.51\text{x}10^2$ CFU mL^{-1}. Specificity test through cross reaction studies showed

that the developed polyclonal antibody shows very low percentage of cross reaction to other genus of plant pathogens. The development of an early detection kit for this bacterium could save thousands of tonnes of rice lost yearly and the results obtained in this work is a promising start in realising this goal.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Specificity of PCR Primers for Identification of *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* with Ten Plant Bacterial Pathogens

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Introduction

Bacterial leaf blight (BLB) and bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* respectively, are prevalent and destructive rice diseases that have resulted annual yield losses up to 50% to 70% in severely infected fields (Lu et al., 2014). Severe disease epidemics often occur following typhoons, fierce winds, wind-blown rain and hail by which wounded the rice plant and disperse bacteria (Niño-Liu et al., 2006). These bacteria are difficult to be identified by standard culturing method due to lack of selective agar to eliminate unwanted microbes. Moreover, bacterial infected plants showed almost similar symptoms with nutrient deficiency by naked-eye visualization. Previously, we have reported the evaluation of PCR primers which capable to distinguish both bacterial pathogens (Mohd Afendy et al., 2016). Here, we report the progress on the specificity test of the previously established multiplex polymerase chain reaction (mPCR) primers to accurately identify the intended targets by using actual samples consisting of 10 plant bacterial pathogens (plus with one additional strain).

Materials and Methods

Bacterial strains and culture condition

All bacteria strains (Table 1) in this study were cultured in peptone sucrose agar (PSA) for 48 h at 30^{o}C. Subsequently, 10 mL of phosphate buffered saline (PBS) was transferred to the petri dish containing the colonies, and then the colonies were scraped and transferred to 50 mL falcon tubes. The bacterial suspension were centrifuged and rinsed three times with PBS.

Crude DNA extraction

Crude DNA extraction was performed on bacterial suspension of each bacterial strain 1 mL portion of each broth culture was centrifuged at 15,000 g for 4 min. The pellet was resuspended in 500 µL sterile distilled water and vortexed vigorously. The cell suspension was boiled for 10 min, immediately chilled on ice for 10 min and centrifuged again at 15,000 g for 4 min. The supernatant containing crude DNA was transferred into a new tube and 5 µL was used as DNA template in PCR.

Primers and PCR amplification

Primer sequences from Lu et al. (2014) were chosen to be used in this study after evaluation using *in silico* PCR program (http://insilico.ehu.es) against all genome database of *Xantohomonas* genus. The primers sequence used in this study is shown in Table 2. A reaction volume of 25 µL of PCR mixture using 1 X PCR master mix (2 mM $MgCl_2$, 0.025U/µL *Taq* DNA polymerase and 0.2 mM of each dNTP), 0.5 µM of each ENT primer, 5 µL of crude DNA extract, and nuclease-free water adjusted to a

total volume of 25 µL. PCR reaction was performed out in a thermocycler (DNA Dyad, BioRad). The thermocyler was programmed by preheated 2 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 58°C, 30 s at 72°C, and final extension for 4 min at 72°C. 5 µL of PCR product was analysed by electrophoresis on 2% agarose gel and stained with ethidium bromide to visualize the amplicons under UV light.

Table 1: List of plant bacterial strains for mPCR identification.

No. / lane	Plant bacterial strains
1	*Pseudomonas* sp. (DB3)
2	*Pantoea amanatis* (TS5)
3	*Pantoea* sp. (DB2)
4	*Pantoea* sp. (TS3)
5	*Xanthomonas* spp. (NCPPB 3949)
6	*Xanthomonas* spp. (SS14)
7	*Xanthomonas oryzae* pv. *oryzae* (NCPPB 3951)
8	*Xanthomonas oryzae* pv. *oryzae* (P7.3)
9	*Xanthomonas oryzae* pv. *oryzae* (P0.0)
10	*Xanthomonas oryzae* pv. *oryzicola* (NCPPB 2921)
11	*Xanthomonas oryzae* pv. *oryzicola* (NCPPB 1585) *(additional)*

Table 2: List of primer for identification of *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*.

Primer name	Sequences (5'- 3')	Target gene	Amplicons size
JLXocF	CAAGACAGACATTGCTGGCA	*AvrRxo* (Xoc)	112 bp
JLXocR	GGTCTGGAATTTGTACTCCG		
JLXooF	CCTCTATGAGTCGGGAGCTG	putative glycosyltransferase (Xoo)	230 bp
JLXooR	ACACCGTGATGCAATGAAGA		

Results and Discussion

In silico PCR results showed that the primers amplified PCR product with *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* by generating a theoretical product of 230 bp and 112 bp, respectively. This was in agreement with the results obtained using actual bacterial strains samples when the primers were tested in the multiplex PCR reaction (Figure 1).

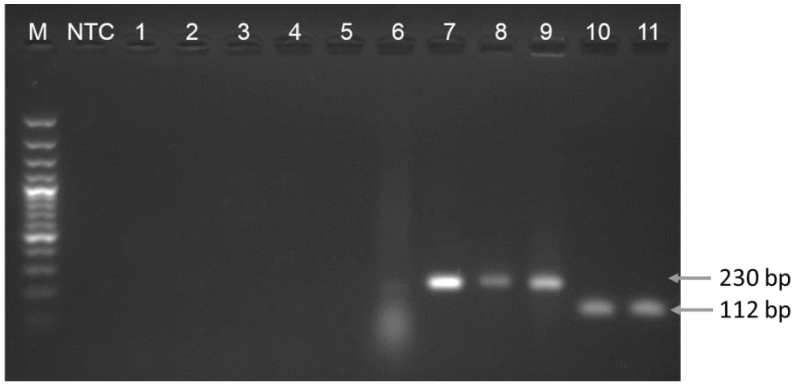

Figure 1: Multiplex PCR to identify *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* among tested 11 plant bacterial strains. Lane M: 100 bp DNA marker; Lane NTC: No template control; Lane 1-11, plant bacterial strains (as in the Table 1).

Figure 1 shows that the primers sequences, JLXooF/R and JLXocF/R amplified the targeted gene yielded 230 bp of *X. oryzae* pv. *oryzae* (lane 7 to 9) and 112 bp of *X. oryzae* pv. *oryzicola* (lane 10 and 11) genes respectively and no amplification for other non-targeted bacterial pathogens (lane 1-6). Although faint amplicon was observed for *Xanthomonas* spp. (lane 6), the size was slightly smaller than the amplicons for Xoo, thus the result is inferred as negative. However, retesting will be carried out to confirm whether the *Xanthomonas* spp. (SS14) is actually a Xoo species or not. Evaluation of the primers specificity by mPCR proved that the respective primers used were specific to the intended targets to identify *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola.*

A study by Jung, et al. (2008) revealed that their single set of primers can only amplify single target, *X. oryzae* pv. *oryzicola* with amplicon size of 488 bp. In this study, both *X. oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryzae* can be detected in a single reaction with the use of another specific primer. The primers sequences, JLXocF/R and JLXooF/R specifically amplified the targeted gene and yielded 112 bp and 230 bp amplicon for *X. oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryza*, respectively, without any amplifications detected for other non-targeted bacterial pathogens.

Conclusions

The multiplex PCR developed was proved to be very useful as a method for faster, easier and simultaneous identification of *X. oryzae* pv. *oryzae* (BLB) and *X. oryzae* pv. *oryzicola* (BLS) in a single reaction. It is hoped that the multiplex PCR reaction can be extended for the diagnosis of paddy plant/rice seeds samples that has been infected with BLS and BLB disease to address laborious conventional culturing method. For future study, the established multiplex PCR will be used to identify the presence of both pathogens in parallel with other technique such as enzyme-linked immunosorbent assay (ELISA) and biosensor for comparison study.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Bacteria Leaf Blight Disease of Rice: Characterisation of Pathogen and Polyclonal Antibody Production of *Xanthomonas oryzae* pv. *oryzae*

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Introduction

Bacterial Leaf Blight (BLB) disease caused by *Xanthomonas oryzae pv. oryzae* (Xoo) causes wilting of paddy seedlings and yellowing and drying of the leaves (Afolabi et al., 2016). This Gram-negative bacterium was first detected in Japan in 1884 (Tagami and Mizukami, 1962) and has now spread to many rice-growing regions worldwide (Mew, 1987). BLB was initially located in the paddy fields of Peninsular Malaysia during the early 80s with limited widespread (Sankaran et al., 2010). There have not been BLB cases in Malaysia for almost 10 years, but it has re-emerged recently. BLB is considered as one of the most serious diseases of paddy plants. In brief, the earlier the disease happens, the higher the yield losses. In serious cases, BLB is capable to affect as much as 50% to 70% of the amount of yield product (Kamarudin et al., 2013). The losses caused by this disease could jeopardize global food security and therefore, it is important to discover the solution to this problem.

The conventional methods used to detect Xoo in rice plants and rice seeds include field observations, biochemical tests (Cruz et al., 1984), serological assays (Benedict et al., 1989) and fatty acid analysis (Chase et al., 1992). However, these methods are labour intensive, time consuming, lack sensitivity and specificity. Although polymerase chain reaction (PCR) technology (Song et al., 2014), the electrochemiluminescence polymerase chain reaction (ECL-PCR) method (Wei and Wu, 2009) and real-time Bio-PCR (Zhao et al., 2007) have been reported for the highly sensitive detection of Xoo, these methods require specialized instruments and trained operators. Moreover, they have not been successful in the detection of this pathogen from artificially inoculated, symptomatic and symptomless tissues or naturally infected rice (Sakthivel et al., 2001). Thus, studies aimed at the development of a simple, sensitive, rapid and cheap method for detecting Xoo remain necessary.

Immunosensor is a simple, specific and is a powerful way to detect pathogenic bacteria. Low sample consumption and good possibility for miniaturization are the main reasons for extensive development of immunosensors (Ricci et al., 2012). In an immunosensor set up, plant-pathogen specific antibodies are mainly based on injection of whole pathogens, surface fragments or soluble surface components into a suitable animal-host for polyclonal antibody (PAb) (Werrers and Steffens, 1994). Obtaining pathogen-specific antibodies is a challenging task and antibodies should preferably be directed against a single target molecule that is surface exposed and constitutively expressed in the target species only (Leonard et al., 2003). The intent of the project described here was to characterise Xoo and develop Xoo polyclonal antibodies that will be utilized in the development of an immunosensor for BLB.

Materials and Methods

Microorganism

For this study, three pathotypes of Xoo were used namely P 0.0, P 1.0 and P 7.3. These pathotypes were chosen for their dominance and prevalence in BLB outbreaks in Malaysia. Collection and maintenance of Xoo isolates were conducted according to Saad and Habibuddin (2010) with slight

modifications. Rice leaves with typical BLB lesions were collected from rice fields. Infected leaves were cut into small pieces and washed with distilled water. The leaf samples were then placed on moist tissue in petri dishes and kept at 4°C for 24 h. The developed bacterial ooze was then streaked on LB agar (LBA). All pure culture isolates were maintained on LBA and stored at 4°C. The inoculum of each isolate was prepared by scraping the bacteria in sterile distilled water.The suspensions were then centrifuged at 5000 rpm for 20 min. The bacterial pellet was re-suspended in sterile distilled water before the absorbance was read. The absorbance value (λ = 600nm) was adjusted to 1 to give a bacterial suspension with a concentration of approximately 1 x 10^8 cfu/mL.

Gram staining

Bacteria were heat fixed on a glass slide treated with crystal violet for 1 min then washed with tap water. Then treated with iodine solution for 1 min, washed again and decolorized with (95%) ethanol for 30 seconds, washed again and counter-stained with safranin for 1 min. Magnifications of X-10, X-40 and X-100 were used for microscopic observation. Gram negative bacteria stained red whereas Gram positive bacteria retained the color of crystal violet.

KOH (Potassium hydroxide) test

One bacterial colony was vigorously stirred in a drop of 3% KOH solution for 10 seconds. The thread-like slime formation will be indicated by the presence of Gram negative bacterium.

Polyclonal antibody production

The polyclonal antibodies were produced by immunizing New Zealand white rabbits with an emulsion consisting the mixture of Xoo pathotypes in phosphate buffered saline (PBS) and complete Freund's adjuvant via subcutaneous injection. The rabbit was bled from the main artery of the ear of the rabbit. Antibody purification of blood samples were carried out using a two-step procedure involving ammonium sulphate precipitation and column chromatography using protein A. GE Healthcare's Protein A resin was used for all chromatography experiments. Chromatography experiments were carried out using an AKTA Prime Plus instrument (GE Healthcare, Uppsala, Sweden) controlled by PrimeView 5.0 software. All columns were packed in the lab according to the manufacturer's recommendations. Equilibration of the resin was carried out using phosphate buffer at pH 7.0 before eluting the polyclonal antibody using glycine-HCl buffer at pH < 4.0.

Results and Discussion

Isolation, gram staining and KOH test

LBA was used for isolation and screening of the pathogens. After 72-96 hours of incubation at 30°C**,**the bacteria appeared on LBA as light yellow, circular dome shaped colonies (1 to 2 mm in diameter) (Figure 1). The yellow colour and mucoid colonies is cultural characteristics of *Xanthomonas* and was due to the production of extracellular polysaccharides slime in media containing sugar (Jabeen et al., 2012). LBA contained both peptone and yeast extract as one of its key ingredients. Peptone and sucrose agar medium were formed as best sources for the bacterial growth (Tsuchiya et al., 1982).Thimmegowda (2006) studied that among the five isolates tested, yeast extract glucose agar and potato sucrose agar supported the excellent growth of Xoo. Thus, LBA is found to be suitable for Xoo growth as it is known that Xoo is a slow growing bacterium. For Gram staining study, all pathotypes were stained as Gram negative with rod shape (Figure 2) and showed positive reaction in 3% KOH test by forming the thread-like slime (Figure 3). The Gram stain reaction is not always truly indicative of the organism's true cell wall structure and KOH test was performed to support the results of Gram staining. According to Gregersen (1978), only Gram type negative bacteria are lysed

when cells are mixed with 3% KOH. Potassium hydroxide is a strong alkali by which when bacteria are mixed with KOH, it softens. If the cells lyses, the liberated cellular DNA makes the mixture viscous or "stringy." The positive string test indicates a Gram-negative organism. All three pathotypes consistently gave similar results for Gram staining and KOH test, confirming that they are gram negative bacterium.

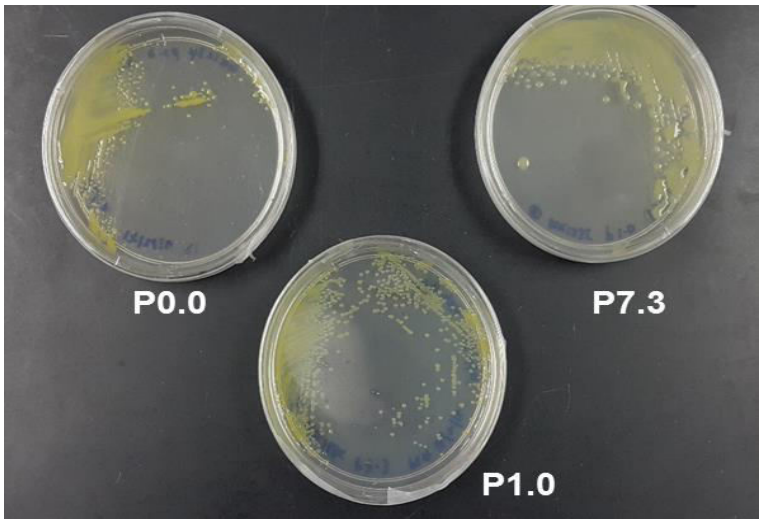

Figure 1: Isolation of Xoo bacterium on LB agar.

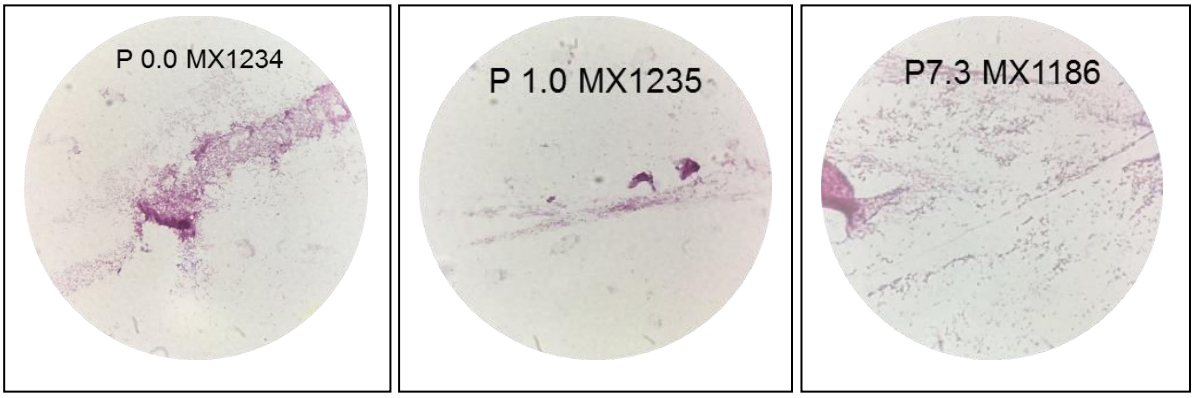

Figure 2: Gram negative of Xoo bacterium shows red stain, rod-shaped bacteria under X-100 magnifications.

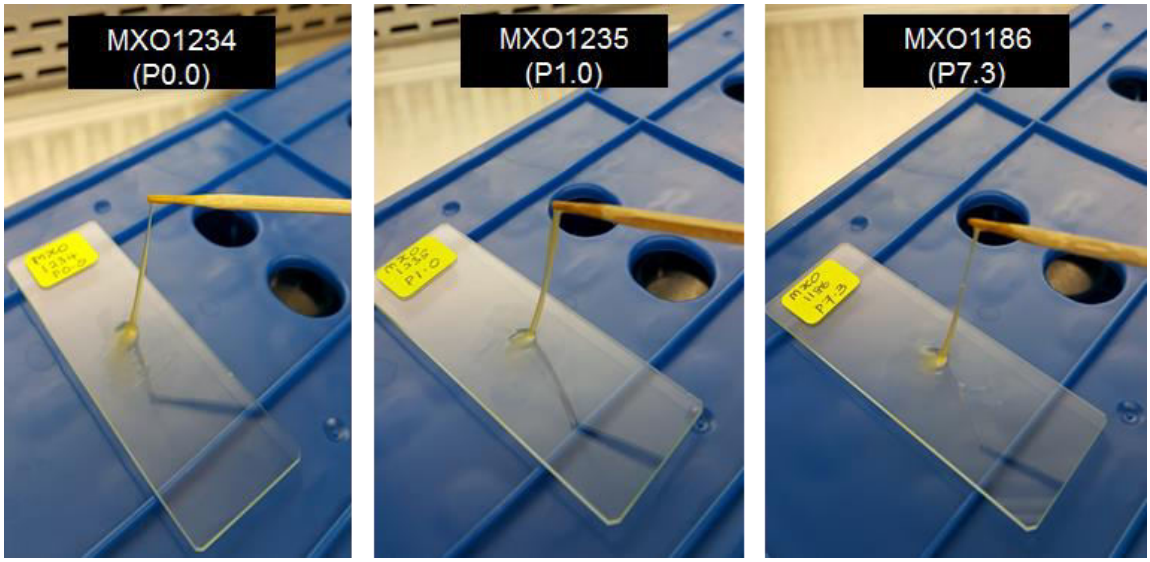

Figure 3: Thread-like slime formation of Xoo bacterium.

Antibody production and purification

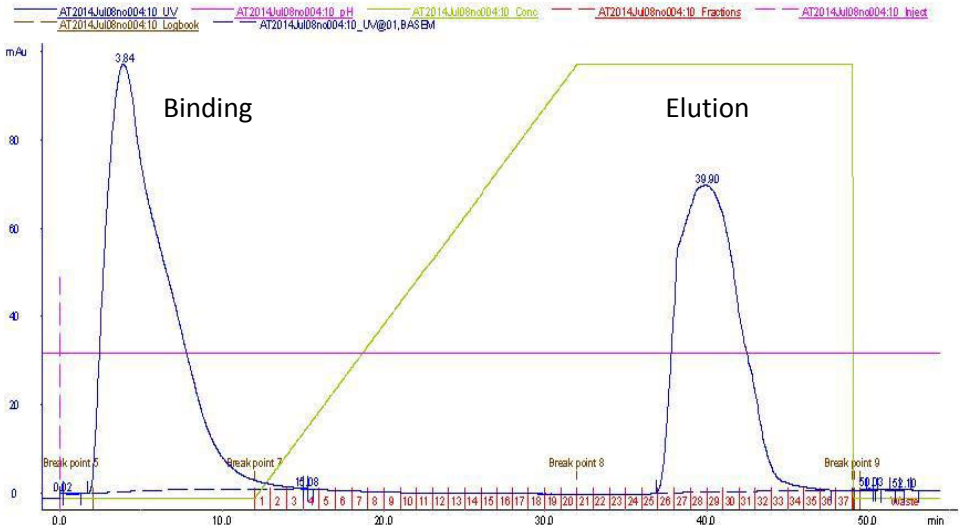

Figure 4: Peaks obtained during antibody purification process.

The first peak was obtained during the binding process with phosphate buffer whereas the second peak was acquired after the elution buffer (glycine-HCl) was run through the column (Figure 4). The purification of antibodies presents several practical complications, especially for polyclonal antibody production (Verdoliva et al., 2000). Separation and recovery of proteins from column chromatography are affected by factors such as buffer type and pH, length of gradient, flow rate of the mobile phase and characteristic of the proteins. The selection of ideal conditions for protein purification involves changing some or all parameters (Tishchenko et al., 1998). Protein A is a bacterial protein whose antibody-binding properties have been well characterized. During binding phase (Figure 4), rabbit serum was injected to the column and allowed to slowly pass through Protein A resin. Any IgG (the targeted antibody class) will bind to the immobilized ligand. Phosphate buffer was added to wash away non-bound serum components. The acidic elution buffer, glycine-HCl disassociates the antibody from Protein A, and the IgG is recovered in its purified state (Elution phase). From the graph, the presence of the peak during elution phase indicated that the purification process was successful.

Conclusions

Three pathotypes of Xoo have been isolated and characterized based on Gram stain and KOH test. Immunization of rabbits with Xoo were completed and the polyclonal antibodies were successfully purified.

Acknowledgements

We thank Mrs. Kogeethavani Ramachandran and Mrs. Siti Norsuha Misman of Paddy and Rice Research Centre, MARDI Seberang Perai for supplying Xoo cultures and for their advice and helpful discussion; Mrs. Nurul Hidayah Hussin of Biotechnology and Nanotechnology Research Centre, MARDI Serdang, Selangor for her technical assistance. This work was supported by Duit Pembangunan MARDI project code P-R1401.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Single Nucleotide Polymorphism (SNP) Mining of *Xa21* Gene: A Gene Controlling Bacterial Leaf Blight (BLB) Disease Resistance in Rice

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Introduction

Bacterial leaf blight (BLB), caused by the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious rice diseases in the world (Rafi et al., 2013). This disease is especially common in the areas that have stubbles of infected plants and weeds. The disease tends to develop in both the tropical and temperate regions, particularly in the rainy lowland and irrigated areas, which leads to wilting seedlings, and yellowing or drying leaves. The earlier the disease attacks the fields, the higher it will be in terms of the yield loss. Yield loss owing to BLB can be up to 50%, when susceptible varieties are being cultivated in favourable environments for the disease (Gnanamanickam et al., 1999). When plants are infected at the beginning of the reproductive phase, the disease did not significantly affect the yield, but will result in poor quality grains and a high proportion of broken kernels. The most reliable, efficient and cost effective approach to control the disease is, by cultivating the resistant varieties. Most of Malaysian cultivars are susceptible to this disease, hence requiring the introgression of resistance genes to enhance the resistance level, and increase the resilience towards the disease. To date, more than 40 BLB resistance genes have been identified in rice, including the *Xa21* gene (Habarurema et al., 2012). *Xa21* gene is a resistance gene (*R* gene) that encodes a protein bearing nucleotide binding site (NBS) and leucine-rich repeats (LRR). *Xa21* was reported to be resistant to many *Xoo* strains across the world (Wang et al., 1996).

Recent advancement in the molecular marker technologies provides the best way to introgress desired genes into the Malaysian rice cultivar, through the marker-assisted selection (MAS) breeding programme. Traditionally, plants were selected by breeders based on their visible or measurable traits, called the phenotype. This process requires a lot of time and cost, inconsistent and unreliable, as phenotypic measurements can be influenced by the environmental factors (Collard and Mackill, 2008). To overcome this problem, a method of selecting desirable individuals in a breeding scheme, based on the DNA marker that linked to a particular trait has been introduced. MAS allows breeding selection based on the genotype of plants, rather than only assessing its phenotype. The efficiency of MAS technology highly depends on the marker selection. When markers are closely linked to a trait of interest, they can be used to indirectly select the traits, save time, money and labour (Iyer-Pascuzzi and McCouch, 2007).

Indirect selection can be inefficient if recombination occurs between the target genes or if additional unwanted alleles show linkage to the marker, in particular the germplasm accessions, which may lead to a linkage drag (Varshney et al., 2005). Commonly, the recombination will occur at an unacceptable rate, if the distance between the marker and the target gene is larger than 1-2 centiMorgan (cM) (Iyer-Pascuzzi and McCouch, 2007). To overcome this problem, functional markers (FM) which are highly predictive of phenotype/trait of interest, will be beneficial as they target the functional polymorphism within a desired gene. Thus, they should be greatly useful to overcome the recombination problem. In this study, we aimed to develop a functional marker for Xa21 gene based on single nucleotide polymorphism (SNP), which will be greatly useful to increase the efficiency and accuracy in the MAS activities.

Materials and Methods

Plant material and DNA extraction

A seed of BLB resistance variety harbouring the *Xa21* gene (IRBB66) and two susceptible rice varieties (MR263 and MR84), were germinated for three weeks prior to the DNA extraction. A young fresh leaf of each variety was extracted using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) based on the manufacturer's protocol. The quality of the DNA was determined using 0.8% (w/v) agarose gel electrophoresis for evaluation of DNA purity and integrity, NanoDrop® 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) measurement for DNA purity by assessing the OD_{260}/OD_{280} ratio, and Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA) quantitation for accurate measurement of the DNA concentration. Only the samples that passed the NGS requirements were forwarded for library preparation. The high quality samples were sent to Novogene (Beijing, China) for library preparation and paired-end sequencing, using the HiSeq platform (Illumina®, San Diego, CA).

Bioinformatics analysis for NGS data

The raw sequencing data were filtered and cleaned to eliminate any adapter contaminations, uncertain nucleotides that constitute more than 10% of either paired reads, or reads with low quality nucleotides. The clean reads were mapped to *Oryza sativa japonica (Nipponbarre)* genome (http://rapdb.dna.affrc.go.jp/download/archive/irgsp1/IRGSP-1.0_genome.fasta.gz). The SNPs were mined and identified using the GATK software, and ANNOVA was used to annotate the detected SNPs.

Comparative sequence analysis

Xa21 was physically mapped from 21,274,696 bp to 21,277,443 bp, on chromosome 11 in the japonica rice genome. The targeted region sequence was compared between the resistance variety harbouring the *Xa21* gene (IRBB66) and the two susceptible rice varieties namely MR84 and MR263.

Results and Discussion

A total of 1,366,786 SNPs have been successfully identified from the IRBB66, 1,387,694 from MR263 and 1,421,170 from MR84, when assembled and mapped using the *O. sativa japonica (Nipponbarre)* genome. The numbers of SNPs were narrowed down to the targeted location, from position 21,274,696 bp to 21,277,443 bp on chromosome 11. A total of 46 SNPs have been identified from the targeted location, which composed of 29 nonsynonymous, 16 synonymous and one stopgain SNPs (Table 1). The nonsynonymous SNP refers to the mutation of a single nucleotide, which leads to the alteration of amino acid, making it highly possible as a putative functional marker. A previous study on the *Xa5* gene by Iyer and McCouch (2004) revealed two adjacent nonsynonymous SNPs, which lead to the substitution of amino acid from valine (susceptible) to glutamic acid (resistant). The utilisation of functional marker in MAS will significantly increase the accuracy of selection, compared to the implementation of linked marker to the gene of interest and conventional phenotypic selection.

The annotation of targeted region showed that the region contains a protein coding gene namely OS11T0569733 (Figure 1) which encodes the *Xa21* resistance gene. Gene ontology analysis also showed that the gene is a component of integral membrane (cellular component), is responsible for protein phosphorylation in defence response (biological process), and is involved in the protein kinase activity (molecular function).

Table 1: Characterization of 46 SNP markers identified in the *Xa21* gene region.

No	SNP ID	Chromosome	SNP Position	Nucleotide MR263	Nucleotide IRBB66	Type of SNP
1	SNP_Xa21_1	11	21274757	C	A	Nonsynonymous
2	SNP_Xa21_2	11	21274840	C	G	Synonymous
3	SNP_Xa21_3	11	21274881	C	T	Nonsynonymous
4	SNP_Xa21_4	11	21274929	A	G	Synonymous
5	SNP_Xa21_5	11	21274933	A	G	Synonymous
6	SNP_Xa21_6	11	21274994	G	T	Nonsynonymous
7	SNP_Xa21_7	11	21275036	C	T	Nonsynonymous
8	SNP_Xa21_8	11	21275173	T	C	Synonymous
9	SNP_Xa21_9	11	21275275	A	G	Synonymous
10	SNP_Xa21_10	11	21275426	A	G	Nonsynonymous
11	SNP_Xa21_11	11	21275467	A	G	Synonymous
12	SNP_Xa21_12	11	21275558	T	G	Nonsynonymous
13	SNP_Xa21_13	11	21275592	T	C	Nonsynonymous
14	SNP_Xa21_14	11	21275763	A	G	Nonsynonymous
15	SNP_Xa21_15	11	21276085	T	C	Synonymous
16	SNP_Xa21_16	11	21276120	C	G	Nonsynonymous
17	SNP_Xa21_17	11	21276137	C	T	Nonsynonymous
18	SNP_Xa21_18	11	21276229	T	C	Synonymous
19	SNP_Xa21_19	11	21276256	A	T	Nonsynonymous
20	SNP_Xa21_20	11	21276258	C	G	Nonsynonymous
21	SNP_Xa21_21	11	21276339	C	T	Nonsynonymous
22	SNP_Xa21_22	11	21276391	T	A	Nonsynonymous
23	SNP_Xa21_23	11	21276434	G	T	Nonsynonymous
24	SNP_Xa21_24	11	21276435	T	C	Nonsynonymous
25	SNP_Xa21_25	11	21276495	C	A	Nonsynonymous
26	SNP_Xa21_26	11	21276504	G	T	Nonsynonymous
27	SNP_Xa21_27	11	21276506	T	A	Nonsynonymous
28	SNP_Xa21_28	11	21276507	G	C	Nonsynonymous
29	SNP_Xa21_29	11	21276513	G	A	Synonymous
30	SNP_Xa21_30	11	21276573	C	A	Nonsynonymous
31	SNP_Xa21_31	11	21276640	G	T	Nonsynonymous
32	SNP_Xa21_32	11	21276648	T	A	Nonsynonymous
33	SNP_Xa21_33	11	21276714	G	C	Nonsynonymous
34	SNP_Xa21_34	11	21276751	T	C	Synonymous
35	SNP_Xa21_35	11	21276754	C	T	Synonymous
36	SNP_Xa21_36	11	21277004	C	T	Nonsynonymous
37	SNP_Xa21_37	11	21277041	T	A	Nonsynonymous
38	SNP_Xa21_38	11	21277068	G	A	Nonsynonymous
39	SNP_Xa21_39	11	21277080	T	C	Nonsynonymous
40	SNP_Xa21_40	11	21277114	C	G	Synonymous
41	SNP_Xa21_41	11	21277147	C	G	Synonymous
42	SNP_Xa21_42	11	21277265	A	T	Stopgain
43	SNP_Xa21_43	11	21277266	A	G	Synonymous
44	SNP_Xa21_44	11	21277278	G	A	Nonsynonymous
45	SNP_Xa21_45	11	21276163	T	A	Synonymous
46	SNP_Xa21_46	11	21276847	C	T	Synonymous

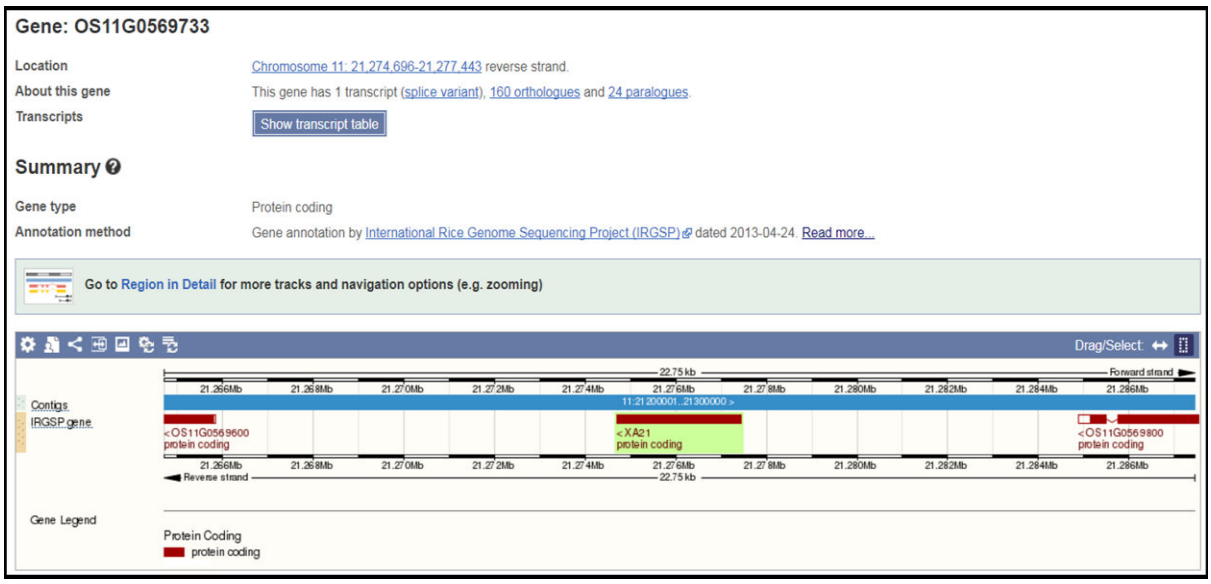

Figure 1: Information on the *Xa21* gene (OS11G0569733).

Conclusion

The utilization of a marker linked to the target gene for indirect selection, may lead to false positive selection, if recombination occurs between the marker and the target gene. These functional markers targeting the *Xa21* gene, have the potential to be able to significantly increase the accuracy and efficiency of the selection process.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Multiple Shoot Induction from Shoot Tip Explants of Meyer Lemon (*Citrus* x *meyeri*)

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Introduction

Citrus plants are accounted as one of the integral fruit crops all around the world which is contributed by their remarkable nutritional value, results in the citrus industry becoming a sought after fruit crop in the market (Ali and Mirza, 2006). Up to now, citrus plants are predicted to consist of approximately 150 genera and 15,000 species that are extensively grown in tropical and temperate climates (Hussain et al., 2016). One lemon cultivar that gains much popularity and recognition intercontinentally for the past recent years is the Meyer lemon, or scientifically known as *Citrus* x *meyeri*. Meyer lemon is the product from natural hybridization between true lemon (*Citrus limon*) and sweet orange (*Citrus sinensis*). With reference to historical perspectives, Meyer lemon is native and indigenous from Beijing, China and has been consumed continuously all around the globe for many years (Miyake et al., 2012). Astonishingly, this cultivar is considered a lemon, yet the outer appearances of this lemon hybrid are seen much more to resemble oranges whereby the peel and pulp is orange-yellow in colour and the skin is much thinner as compared to the typical lemon. Apart from that, Meyer lemon possess of much sweeter flavor and less acidic in comparison to the regular lemons (Uckoo et al., 2015). Miyake et al. (2012) emphasized that although Meyer lemon morphologically resembles the regular lemon particularly its shape and size, yet it is still distinct in qualities such as lower citric acid level, quality of essential oil and the peel colour that is orange-yellow. It is known to grow extremely well in warm climates and has enormous juice (Uckoo et al., 2015).

Most of citrus species acknowledged to have a significant spot in industrial market as they are known to be raw materials for cosmetics and pharmaceutical products as well (Khan and Kender, 2007). This was supported by Carimi and Pasquale (2003) as they claimed that even though the most well-known products of citrus are fresh fruits and juices, yet citrus as essential oils, pectin, marmalade and processed foods (candy and dried rinds) have also high commercial values in the current market. According to Miyake et al. (2012), the level of dihydrocarveol and thymol volatile components from the essential oil of Meyer lemon was significantly higher as compared to other lemon varieties making it being much utilized as food flavouring agent.

Most lemon varieties are scientifically proven to possess elevated levels of vitamin C as well as several health-promoting macromolecules such as amino acids, organic acids, sugars, and along with sufficient amount of minerals (magnesium and calcium, particularly) (Hussain et al., 2016). Lemons are also excellent dietary source of health-beneficial compound such as flavonoids which consist of narirutin, hesperidin and didymin that are likely to be found in lemon juices where hesperidin is mainly present (Uckoo et al., 2015). They also had reported that the Meyer lemon fruit contains high amounts of both organic acids and health-promoting phytochemicals such as amines, flavonoids and limonoids. These advantageous elements contribute highly to the human's health, acting as antioxidant, anti-proliferative and anti-inflammatory agents that aids in the prevention of cardiovascular disease. Miyake et al. (2012) had corroborated by stating that several polyphenolic compounds were discovered abundantly in the Meyer lemons as compared to other citrus plants gaining much attention and recognition particularly due to the flavonoids such as hesperidin and diosmin, linked improvement of vascular tone inflammatory disorders. Besides, they also proved that

lemon fruits comprise of amines compounds such as octopamine, synephrine and tyramine that can be used in the production of weight-reducing dietary supplements because of their apparent lipophytic and thermogenic effects. Moreover, amines also play a crucial role in plant defense mechanisms for repelling insects, pests and reactive oxygen species from plants as well (Miyake et al., 2012).

In Malaysia, lemons are imported at a high price from abroad and local growers lack the knowledge and exposure in lemon farming. Meyer lemon has been found to thrive in the local soils and is currently being propagated through grafting and cutting by a few local growers. However, the conventional methods are not effective in producing large numbers of lemon plants for commercial scale planting. Plant tissue culture particularly micropropagation is an efficient alternative method in propagating large number of plants that are disease free and true-to-type within shorted period of time. This allows consistent supply of lemon plants for the industry which indirectly leads to consistent production of lemon fruits that can soon reach the consumers. The current study aims to investigate the efficiency of shoot tip explant in inducing multiple shoots and also to identify plant growth regulators that improve multiple shoot induction.

Materials and Methods

Plant materials

Shoot tips with the length of 3 cm long were collected from the Meyer lemon plants grown at the School of Biological Sciences, Universiti Sains Malaysia. The two-weeks-old of shoot tips were harvested and thorns were removed prior to surface sterilization.

Surface sterilization

The shoot tips were immersed in tap water containing 5% (v/v) of liquid detergent (Sunlight©) for 10-15 min with gentle brushing prior to washing under running tap water for 60 min. The cleaned shoot tip explants were sterilized by agitation in 70% (v/v) of ethanol for 1 min, followed by 5% (v/v) of commercial bleaching solution (Clorox® Regular-Bleach) for 6 min and rinsed 7-8 times with sterile, distilled water. Sterile explants were blot dry on filter paper and placed in media for shoot regeneration.

Inoculation for multiple shoot induction

The shoot tips were excised into 1.5 cm length and inoculated on full strength MS basal media (Murashige and Skoog, 1962) containing sucrose (30 g/L) and supplemented with different concentrations of plant growth regulators such as Benzylaminopurine (BAP) and Kinetin (Kn). Two explants were placed for each culture jars (80 mm x 90 mm) containing 50 mL of above mentioned MS media. Subcultures were carried out for every four weeks on the same fresh media. The explants were maintained in culture room at 25 ± 2 °C for 16 hours photoperiod and 8 hours dark regime and 50% of relative humidity.

Parameters and statistical analysis

After 8 weeks of inoculation, the percentage of shoot induction, average shoot number, average shoot length and increment in height were evaluated. Each experiment consisted of three replicates, with each replicate containing 10 explants. All data were subjected to One-Way Analysis of Variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) at the significance level of $p \leq 0.05$.

Results and Discussion

Micropropagation has been described as an effective approach to overcome the constrictions of conventional breeding methods including very limited multiplication rate in Citrus plants for mass production and high risks towards disease-prone new plantlets production (Goswami et al., 2013). Pati et al. (2011) specifically mentioned that beyond to improvise the existing cultivars, this modern approach is also capable to reproduce the generation of novel plants within much shorter time span as compared to vegetative propagation. After 8 weeks of inoculation, it was observed that all explants from BAP supplemented media exhibited extremely high percentage of shoot induction whereby all the BAP treatments had shown 100% of shoot induction except for 2.0 mg/L which displayed 91.7% (Figure 1a). In agreement to the percentage of shoot induction, 1.0 mg/L BAP treatment indicated the significance outcomes of parameters such as average shoot number, average shoot length and increment in height with the values of 2.56 ± 0.38, 0.83 ± 0.16 and 0.80 ± 0.18 respectively after 8 weeks of culture. Figure (Figure 1b, c and d) shows that this treatment also performed better than other treatments in comparison to the other plant growth regulator.

In contrary, the explants from Kinetin-supplemented media exhibited reduction on the percentage of shoot induction as the concentration of Kinetin increased. The highest percentage of shoot induction was acquired from 0.5 mg/L Kinetin (88.9%) while the lowest value from 1.0 mg/L Kinetin (Figure 1a). However, this experiment highlighted that single treatments of Kinetin produced no significant difference on interested variables such as average shoot number, average shoot length and increment in height for the shoot tips of Meyer lemon after 8 weeks in culture. The morphological observation on multiple shoot induction from both BAP and Kinetin treatments on single shoot tip explant displayed as below (Figure 2). No callus and root formations were observed from single treatments of both cytokinins throughout this study.

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

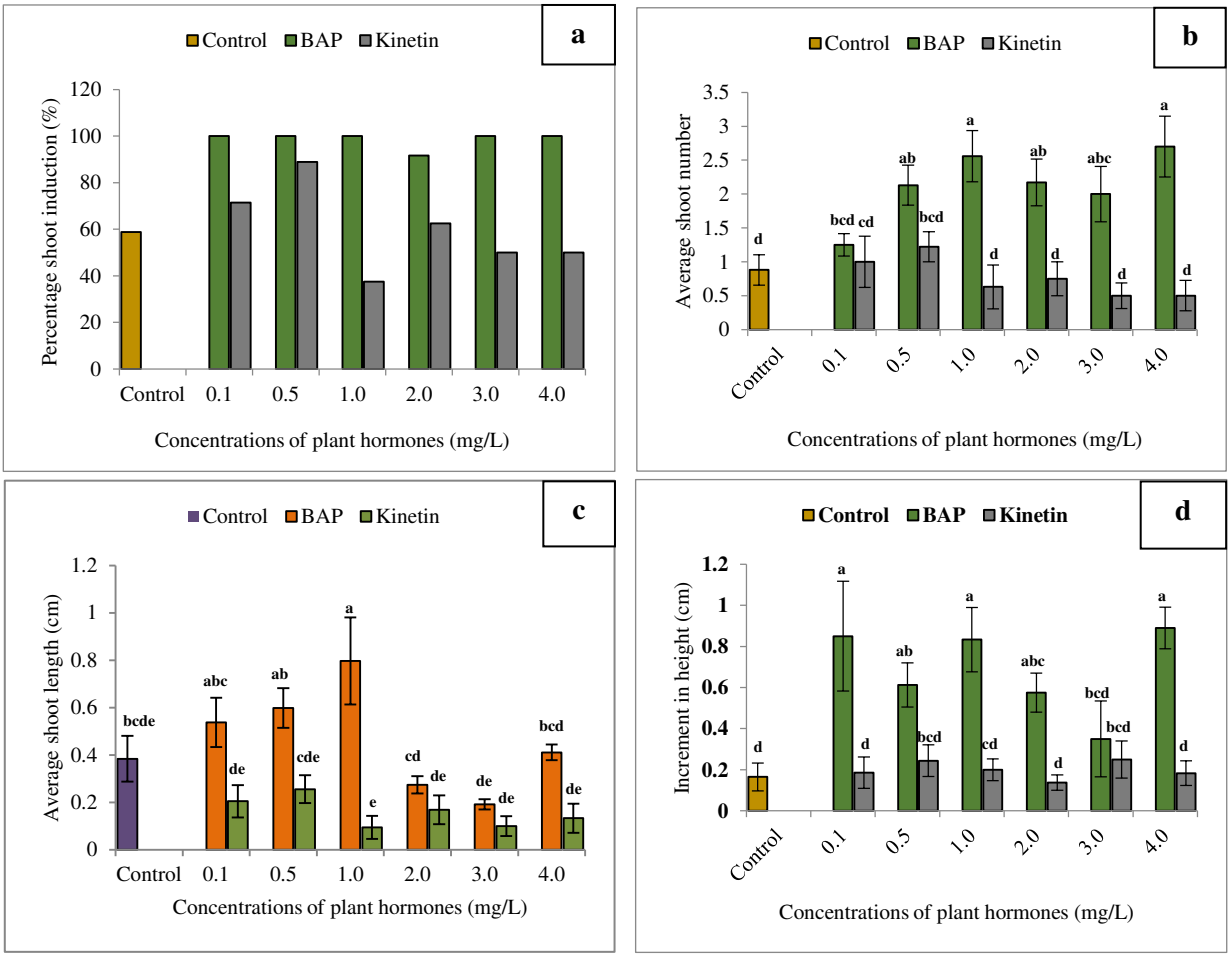

Figure 1: Comparison among Control, BAP and Kinetin in parameters (a) Percentage of shoot induction (b) Average shoot number (c) Average shoot length (d) Increment in height of shoot tips of Meyer lemon after 8 weeks of culture.

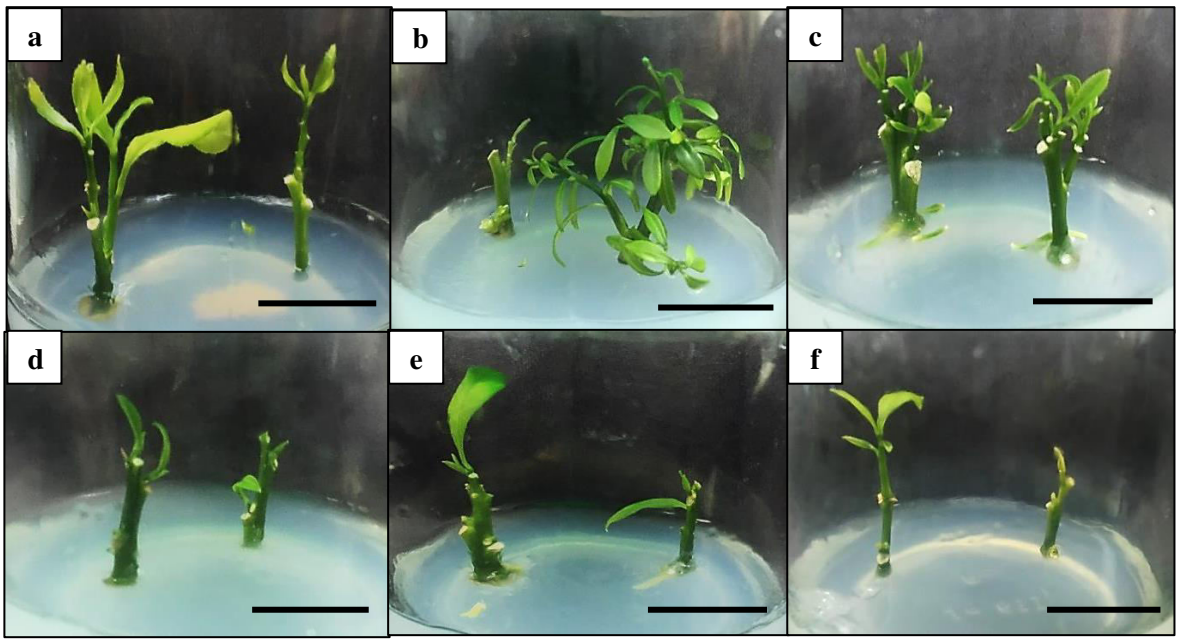

Figure 2: Multiple shoot induced on different concentrations of (a) 0.5 mg/L BAP, (b) 1.0 mg/L BAP, (c) 4.0 mg/L BAP, (d) 0.5 mg/L Kinetin, (e) 1.0 mg/L Kinetin, (f) 4.0 mg/L Kinetin of Meyer lemon after 8 weeks of culture (Scale bars represent 1cm).

The results obtained are exactly in parallel to the study performed by Mukhtar et al. (2005) as different concentrations of BAP displayed the most optimal outcomes on different parameters. The concentration of 1.0 mg/L BAP produced the highest percentage of shoot induction (84%), while 0.5 mg/L BAP for highest shoot number per explant from both shoot tip and nodal segments of *Citrus reticulata* Blanco. They also emphasized the crucial role of plant growth regulators concentrations in obtaining significant frequency of shoot initiation. Apart from that, Al-Bahrany (2002) also was proved that 1.0 mg/L BAP optimal to encourage maximum shoot elongation of *Citrus aurantifolia* (Christm.) Swing and BAP concentrations higher than that hindered shoot elongation.

The utilization of BAP as remarkable cytokinin to gain optimal values of parameters is in accordance with several previous findings on various varieties of *Citrus* plants such as combination of 2.0 mg/L BA and 2.0 mg/L GA exhibited the most optimal productivity and proliferation rate for shoot tips and nodal segments of six cultivars of *Citrus limon* (Tornero et al., 2010); the maximum shoots buds and axillary shoot numbers were obtained from the shoot tips of *Citrus megaloxycarpa* in media of 0.25 mg/L BA and 0.5 mg/L NAA combination (Haripyaree et al., 2011); and other Citrus *in-vitro* studies such as Al-Khayri and Al-Bahrany (2001); Mukhtar et al. (2005); Marques et al. (2011) that were proved the presence of BAP is vital for multiple shoot initiation and multiplication at optimal rate, either singly or in combination with other plant hormones as compared to other cytokinins. Carimi and Pasquale (2003) supported this fact and mentioned that 6-Benzyladenine (BA) has been the most typically utilized plant hormone at several different concentrations for high multiplication rate of Citrus shoot parts.

According to Kleyn et al. (2013), generally cytokinins such as BAP, Kinetin and 2ip are commonly used and featured in media composition for optimizing cell division, shoot multiplication and axillary bud proliferation. They also emphasized that BAP was more typically utilized and more likely favoured to encourage axillary bud growth, particularly. The importance of cytokinin involves not only for cell division in micropropagation method, but also to regulate protein synthesis responsible in the formation and function of mitotic spindle (George et al., 2007).

The apical meristem is defined as new, undifferentiated tissue located at the microscopic shoot tip which is usually free from virus infection in diseased crops as the meristematic cells are not assimilated to the vascular system of plant. Hence, plants could grow and reproduce healthily (Kleyn et al., 2013). The utilization of shoot tip as an explant had been implemented numerous times previously and there was no exception among Citrus varieties. Similar starting material of micropropagation method demonstrated to be an efficient explant for mass production of *Citrus aurantifolia in vitro* clonal (Al-Khayri and Al-Bahrany, 2001). In addition, a study carried out by Mukhtar et al. (2005) highlighted the comparison of shoot formation frequency of both BAP and Kinetin media between shoot tips and nodal segments of *Citrus reticulata* Blanco. They were significantly proved that shoot tip explants were better in obtaining the high frequency of shoot initiation (80-84%), and also induced a high shoot number per explant (7.99) as compared to the nodal explants. The selection of explants is critical to determine a successful rate of micropropagation as the utilization highly responsive starting material such as young shoot tips would influence the shoot regeneration processes. Moreover, mature tissues were proven to reduce the regeneration potential for new plantlet production (Marques et al., 2011). With reference to the work of Goswami et al. (2003), high multiplication rate of new axillary buds have been extensively performed in *in vitro* Citrus industry to assure maximum genetic consistency of the resulting offspring or plantlets.

Conclusions

The results from data above proved that supplementation of BAP is important for optimal multiple shoot induction from the shoot tips explants of Meyer lemon with the most optimal concentration is 1.0 mg/L. A combination treatment of plant growth regulators is currently being carried out and observed in order to achieve the aims of this study.

Acknowledgements

The author would like to acknowledge Universiti Sains Malaysia for funding this project under the Research University Grant (1001/PBIOLOGI/8070004) and Fig Direct Sdn. Bhd. for the supply of lemon plants in this research.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Tissue Culture Establishment and Callus Induction of *Vernonia amygdalina* using Young Leaves as Explants

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Introduction

Vernonia amygdalina is a multipurpose and rapid regenerating soft wooded shrub of 2 to 10 m height with petiolate leaves of around 6 mm in diameter (Yeap et al., 2010). More than 500 species of these *Vernonia* plants are distributed in Africa and Asia, approximately 300 in Mexico, Central and South America and around 16 can be found in the United States (Yeap et al., 2010). The leaf of *V. amygdalina* has been used as traditional medicine to treat fever, malaria, dysentery, hepatitis, diarrhoea as well as cough. Also, the leaves are used to treat headache, stomach ache, scabies, malaria and gastrointestinal disorders (Fomum, 2004). Secondary metabolites or phytochemicals possess in *V. amygdalina* are the ones that are responsible for pharmacologic activities such as hypoglycaemic, hypolipidemic, antimalarial, antioxidant and anti-inflammatory activities (Audu et al., 2012). The *V. amygdalina* produces a variety of flavonoids and bitter sesquiterpene lactones which contribute to the bioactivities of this plant (Nangedo et al., 2002; Favi et al., 2008).

V. amygdalina can grow into a tree, but in cultivation it is mostly pruned to a shrub or hedge. Propagation is possible by seed, but most farmers use stem cuttings. Cuttings grow faster than seedlings. Studies has also shown that *in vitro* micropropagation played a key role in clonal propagation of elite genotypes of *V. amygdalina* for the production of secondary metabolites that are valuable by pharmaceutical means (Khalafalla et al., 2007). *In vitro* technologies have been effective tools for both studying and producing plant secondary metabolites under *in vitro* conditions and for plant improvement (Ramakrishna and Ravishankar, 2011). With *in vitro* micropropagation, the multiplication rate can be greatly increased and also permits the production of pathogen free material (Khalafalla et al., 2007). Opabode and Adebooye (2005) reported that micropropagation of *V. amygdalina* is an urgent need to produce virus free plantlets against leaf curl virus that is threatening the availability of high quality leaves. Thus, the aim of this study is to establish surface sterilization protocol and to investigate the effects of various plant growth regulators on the induction of callus from *V. amygdalina* leaf explants.

Materials and Methods

Plant materials

The leaves of the *V. amygdalina* were collected from shade house in a farm in Mantin, Negeri Sembilan. Third until fifth leaves from the shoot tip were chosen as explants. The leaves were kept in a cooler box and transported to the lab within 30 min for immediate use.

Surface sterilization

The leaves were put in a container with water, 2-3 drops of Tween 20 was added and agitated for 2-3 min. The leaves were thoroughly washed under running tap water for 10 min. Three- step surface sterilization method was used with prior treatment with 1% (v/v) Carbendazim for 30 min and followed by rinsing with distilled water thrice for 5 min per wash. The leaf explants were then disinfected at various duration (0, 10, 20, 30, 40 and 50 min) using 20% (v/v) Clorox® (contain

5.25% of sodium hypochlorite) before rinsing with sterile distilled water for three times. The leave were then treated with 70% (v/v) ethanol for 1 min. The explants were then excised into approximately 1.0 cm^2 and inoculated into half strength basal MS (Murashige and Skoog, 1962) media with 30 g/L sucrose, 7 g/L bacto agar, 0.5 mg/L of 6-Benzylaminopurine (BAP) and α-Naphthalene acetic acid (NAA) at pH 5.8 ± 0.1. Two replicates were prepared for each treatment with 10 explants in each replicate. The explants were placed in constant temperature of 25 ± 2ºC under 16 hours illumination of cool white fluorescent lamps at light intensity of 3000 lux in the plant growth chamber. The humidity of the chamber was maintained at 70-80%.

Optimization of plant growth regulators for callus induction

The best sterilization protocol developed earlier was employed in this experiment. The explants were excised into 1cm x 1cm in size and inoculated into petri dishes containing basal MS medium supplemented with various concentrations of NAA (0, 0.5, 1.0, 2.0 and 4.0 mg/L) or BAP (0, 0.5, 1.0, 2.0, and 4.0 mg/L), 30 g/L sucrose and 7 g/L bacto agar. The pH of the medium was adjusted to 5.8 ± 0.1 before autoclaving at 121ºC for 15 min. The experiment was repeated four times with ten explants per replicate for each treatment. The explants were incubated as described earlier.

Data recording and statistical analysis

The experiments were carried out using randomized complete block design (RCBD) by blocking the replication. Data were recorded at daily basis up to 21 days for surface sterilization. Explants that remained green and free from contamination were considered as clean culture and had survived the treatments. Explants that had changed color to white or transparent were over-sterilized. Data were recorded on a daily basis up to four weeks for optimization of plant growth regulators. The data were analyzed using One-way and Two-way ANOVA and the mean values were compared Using Tukey's test at $p \leq 0.05$ with the aid of GENSTAT Statistics 18.

Results and Discussion

Surface sterilization

The explants were subjected to 20% (v/v) Clorox® at different duration. Explants with no sign of fungus or bacterial contamination and remained green were regarded as clean culture. There were significant differences between different duration according to Table 1. Exposure duration of 20, 40 and 50 min with mean value gave of 90%, 80% and 85% clean culture respectively as compared to control (0 min). The explants produced friable, light green callus once sub-cultured into fresh half strength basal MS media. The explants were contaminated by fungus and bacteria within one week after inoculation. After one week the culture became more stable and there were less contamination for all the duration. The results suggested that sterilization with 20% (v/v) Clorox® for 20 min was the best. Many different materials have been used to surface sterilize the explants, but the most commonly used are 0.5-1% (v/v) sodium hypochlorite (commercial Clorox® contains 5.25% sodium hypochlorite) and 70% (v/v) ethanol. The type of disinfectant used, the concentration, and the amount of exposure time vary depending on the sensitivity of the tissue and how difficult it is to disinfect (Beyl, 2011).

Table 1: The percentage of clean culture, callus formation and over-sterilization after (being) treated with 20% (v/v) Clorox® at different duration after 21 days of culture.

Duration (Minute)	Clean culture (%) ± SD		Callus formation (%) ± SD		Over sterilization (%) ± SD	
0	40±0	b	40±0	b	0±0	a
10	60±0.14	ab	60±0.14	ab	0±0	a
20	90±0.14	a	90±0.14	a	0±0	a
30	70±0	ab	70±0	ab	10±0	a
40	80±0	a	80±0	a	10±0.14	a
50	85±0.07	a	85±0.07	a	10±0	a

Numbers in the same column with the same alphabet(s) are not significantly different at P≤0.05.

Optimization of plant growth regulators

Either auxin or cytokinin alone or at combinations had induced callus formation (Table 2). Good and prolific callus formed for treatment 7, 8, 10, 12, 14, 17, 18 and 22. The percentage of callus formation was ranged from 98 to 100. All of the explants in treatments 7, 8, 10, 12, 14, 17, 18 and 22 formed calluses. However, the leaf explants underwent necrosis within two weeks after inoculation and callus did not form in basal MS media only. MS media for treatmenst 2, 3, 4, and 5 were not supplemented with NAA. Therefore, the treatments 2, 3, 4 and 5 gave the lowest percentage of callus formation which were 50, 48, 50 and 50% respectively. Absence of auxin would have been the reason for low yielding callus formation. According to Nor et al. (2011), inclusion of auxin and cytokinin were necessary for callus growth and somewhat higher auxin concentration might be required for best result of callusing. Treatments 10, 12, 15, 17, 18, 19, 22, 23, and 25 induced 100 percentage calluses but degree of callus formation was ranging from medium to good callus only. However treatments 7 and 8 induced 98% callus which were profuse, friable and pale green calluses. NAA and BAP are known to be very effective and often used for the induction of callus in some species (Polanco et al., 1988; Biswas et al., 2007; Renu and Nidhi, 2011). It was notable in the present study that low concentration of auxin with the combination of slightly high cytokinin had resulted in profuse callus induction.

Friable and pale green callus is the best source for cell suspension culture compared to compact callus. In compact callus, the cells are densely aggregated, whereas in friable callus, the cells are only loosely associated with each other and the callus becomes soft and breaks apart easily. Friable callus provides the inoculum to form cell-suspension cultures. The friability of the callus can be improved by manipulating the medium components or by repeated sub-culturing or by culturing it on semi-solid medium (medium with a low concentration of gelling agent). When friable callus is placed into a liquid medium and then agitated, single cells and/or small clumps of cells are released into the medium. Under the correct conditions, these released cells continue to grow and divide, eventually producing a cell-suspension culture (Adrain et al., 2008). Therefore the best hormone combination for profuse and friable callus induction was treatment 8, 0.5 mg/L NAA + 1.0 mg/L BAP.

Degree of callus formation was comparatively higher in treatments 7 and 8, which the basal MS media supplemented with 0.5 mg/L of NAA + 0.5 mg/L of BAP and 0.5 mg/L of NAA + 1.0 mg/L of BAP respectively. Obute, et al. (2016) reported that the best concentration and combination of growth hormone for callus induction in *V. amygdalina* was 0.7 mg/L BAP + 3.5 mg/L NAA that is in contrast to the current study. The callus proliferated in treatments 7, 8, 12 and 14 were friable and pale green in color (Figure 1). Meanwhile, the callus proliferated in treatments 17, 18 and 22 were compact and white in colour (Figure 2).

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Table 2: The effects of different concentrations of NAA, BAP on callus induction in leaf explants of *V. amygdalina* after four weeks of culture.

Treatment (mg/L)	Growth regulator NAA	BAP	% of explants with callus ± SD		Degree of callus formed	Callus morphology
1	0.0	0.0	0±0	b	-	No callus formed
2	0.0	0.5	50±0.58	ab	++	Compact, white
3	0.0	1.0	48±0.55	ab	++	Compact, white
4	0.0	2.0	50±0.58	ab	++	Friable, green
5	0.0	4.0	50±0.58	ab	++	Compact, white
6	0.5	0.0	95±0.06	a	++	Friable, pale green
7	0.5	0.5	98±0.05	a	++++	Friable, pale green
8	0.5	1.0	98±0.05	a	++++	Friable, pale green
9	0.5	2.0	88±0.15	a	+++	Friable, pale green
10	0.5	4.0	100±0	a	+++	Friable, white
11	1.0	0.0	98±0.05	a	+++	Friable, pale green
12	1.0	0.5	100±0	a	+++	Friable, pale green
13	1.0	1.0	95±0.06	a	++	Compact, white
14	1.0	2.0	98±0.05	a	+++	Friable, pale green
15	1.0	4.0	100±0	a	++	Friable, green
16	2.0	0.0	88±0.15	a	++	Compact, white
17	2.0	0.5	100±0	a	+++	Compact, white
18	2.0	1.0	100±0	a	+++	Compact, white
19	2.0	2.0	100±0	a	++	Compact, white
20	2.0	4.0	95±0.06	a	+	Compact, white
21	4.0	0.0	93±0.15	a	++	Friable, pale green
22	4.0	0.5	100±0	a	+++	Compact, white
23	4.0	1.0	100±0	a	++	Compact, white
24	4.0	2.0	98±0.05	a	++	Compact, white
25	4.0	4.0	100±0	a	++	Compact, white

Degree of callusing: + poor callus, ++ medium callus, +++ good callus, ++++ profuse callus. Numbers in the same column with the same alphabet(s) are not significantly different at P≤0.05.

Figure 1: Formation of friable, pale green callus on MS media supplemented with 0.5 mg/L NAA and 1.0 mg/L BAP.

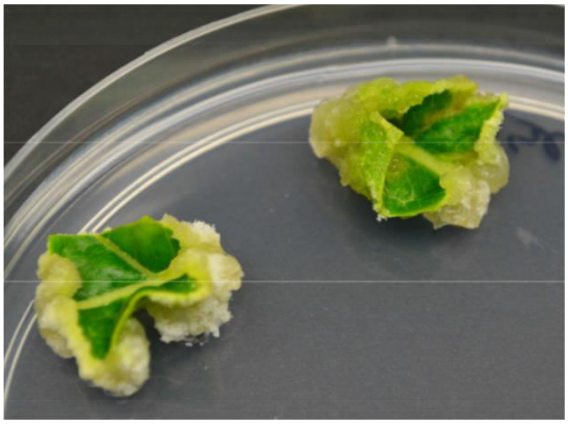

Figure 2: Formation of compact, white callus on MS media supplemented with 2.0 mg/L NAA and 0.5 mg/L BAP.

Plant growth regulator is indispensable for plant tissue induction, growth and organ differentiation (Zhou and Liu, 2009). Auxins and cytokinins are the most crucial for morphogenesis and regulating growth in plant tissue and organ cultures. In plant tissue culture, changes in auxin concentrations could change the type of growth that was, from stimulation of root formation to callus induction (George et al., 2007). This explains the results obtained in the present study. Obute, et al. (2016) has also reported some calli in the culture tubes were observed to possess roots at the fifth week of culture. These roots were observed in media supplemented with 3.5 mg/L BAP + 0.7 mg/L NAA, 0.1 mg/L BAP + 0.5 mg/L NAA and 0.5 mg/L BAP + 2.5 mg/L NAA.

Conclusions

It is concluded, therefore 20% (v/v) of Clorox® for 20 min to be used for surface sterilization of leaf explants of *V. amygdalina.* This present study indicates that callus formation in *V. amygdalina* is feasible in the presence of various concentration and combination of BAP and NAA. Manipulation of the concentration of plant growth regulators will result in different type of callus induction. *V. amygdalina* explants were subjected to 25 treatments in the present study. The best concentration for callus induction is 0.5 mg/L NAA + 1.0 mg/L BAP. An established tissue culture technique plays an important role in propagating callus of *V. amygdalina* that is rich in secondary metabolites of high medicinal properties. Further studies on callus induction of *V. amygdalina* are recommended to determine best technique to proliferate callus for secondary metabolites elicitation studies.

Acknowledgments

The authors wish to thank University of Nottingham Malaysia Campus for the laboratory facilities and the technical staff for their continuous support.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 25
First Published, 2018

Preliminary Study on Tissue Culture Protocol Development for *Tithonia diversifolia* (Insulin) using Shoot Explants

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Introduction

Tithonia diversifolia (Hemsl.) known as Mexican Sunflower or Insulin Tree is a native to the regions of Mexico and Central of America. It is a woody herbaceous plant which is rich in protein. Insulin tree has been introduced to Africa and Asia. Now in Asia has been naturalized and it can be found in a disturbed areas. It also have a several names which are "Mexican Sunflower" common for Mexico, "Paitan tree" local name for Indonesia, "Wild Sunflower" common for Africa, "Insulin tree" local name for Malaysia and it is a member of the Asteraceae family. According to Orwa et al. (2009), this plant typically grows from 1.5 m to 3 m tall. It has a yellow flower that occurs on the mature stem which up to 12 cm diameter. Insulin tree is a fast growing species, it also an ornamental plant and it could be applied as a potential in green manure in industrial agroforestry. Based from the other reports, Philippines claimed that this Insulin tree could be a potential organic foliar fertilizer for rapeseed (Dela Pena et al., 2013). Based on Moronkola et al. (2007) in Nigeria, the used of essential liquid from various part of *T. diversifolia* can be a treatment for sore throat, malaria, menstrual pains and anti-inflammatory. It also widely used as a cover crop because it has a high of nitrogen.

According to Oyerinde et al. (2009), Insulin tree can be propagated from the seed however *in vitro* techniques can be applied as an alternative for multiplication and germplasm maintenance of this species (Iva et al., 2009). These plants are drought tolerant; by the application of fertilizer it can promote a good growth of *T. diversifolia*. A germplasm conservation tools for direct organogenesis has been identified and reported from stem cuttings and also leaf segments (Estrella and Lazart, 1994). The present study describes the best method of surface sterilization in order to establish a clean culture of *T. diversifolia* (Insulin tree).

Materials and Methods

Young shoots of *Tithonia diversifolia* (Insulin tree) were collected from Paya Jaras, Sungai Buloh. This study utilized young shoots and nodal segments as explants for surface sterilization methods. Explants were trimmed and cut in the laboratory (Figure 1).

Figure 1: *Tithonia diversifolia* young shoots.

Surface sterilization method is the most important and fundamental technique to develop a tissue culture protocol. In this procedure, some chemicals were used to clean the surface of explants. The objective of this technique was to obtain clean cultures and reduce or eliminate contamination with fungus and bacteria. This experiment requires used different concentration of Ethanol and Clorox® to remove dirt and debris as well as to avoid contaminants such as fungi, bacteria and other contaminants. However, different species requires different surface sterilization protocol. Thus, for this surface sterilization methods, nodal segments were used and cut into a suitable size. The nodes were washed under running tap water to remove the dirt and debris. This study applied three methods to obtain the best surface sterilization protocol for these explants. In Method 1, explants were washed with Tween 20 and distilled water for 15 minutes. After that, samples were soaked into fungicide plus Tween 20 for 60 minutes then rinsed and washed with distilled water for 10 minutes. Then, explants were rinsed with 50% of Ethanol plus Tween 20 for 10 seconds. Subsequently, the samples were washed with 40% Clorox® plus Tween 20 for 15 minutes (Figure 2). After rinsed with distilled water, the explants were soaked into 10% Clorox® plus Tween 20 for another 15 minutes. Finally, samples were rinsed and cut into a small and proper size and were dried for 2 hours.

Figure 2: Surface sterilization of *Tithonia diversifolia* (Insulin) explants using Clorox®.

On the other hand, Method 2 applied slightly different length of time and concentration of the chemical used. Explants were washed with distilled water and Tween 20 for 20 minutes. Similar to Method 1, samples were soaked in fungicide plus Tween 20 for 60 minutes. Then, samples were rinsed and washed

with distilled water for 20 minutes. After that, the samples were rinsed with 70% Ethanol plus Tween 20 for 10 seconds. Later, the explants were washed with 40% Clorox® plus Tween 20 for 20 minutes. After rinsed with distilled water, the explants were soaked in 20% Clorox® plus Tween 20 for 10 minutes before cut and dried for 2 hours.

Meanwhile, for Method 3; the explants were washed using Tween 20 and distilled for 20 minutes. Like Method 1 and 2, those explants were soaked in fungicide plus Tween 20 for 60 minutes. After that, samples were rinsed and washed using Tween 20 for at least 20 minutes then rinsed with 70% of Ethanol plus Tween 20 for 10 seconds. After that, explants were washed with 50% Clorox® plus Tween 20 for 20 minutes, and then the samples were rinsed with distilled water and washed again with 20% Clorox® plus Tween 20 for 10 minutes. Finally, the explants were rinsed with distilled water and the shoots were cut and dried for 2 hours in laminar air flow (Figure 3). All samples were cultured in Murashige and Skoog, MS (Murashige and Skoog 1962) media supplemented with 0.5 mg/L Benzyl aminopurine (BAP). The observation was done weekly and the percentage of clean cultures and viability were recorded.

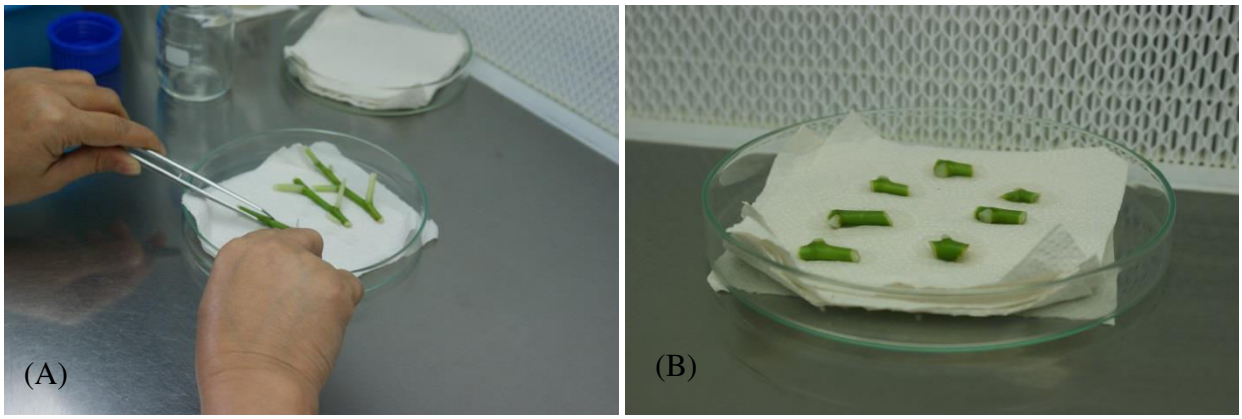

Figure 3 A and B: The nodal segments of *Tithonia diversifolia* (Insulin) were cut and dried.

Results and Discussion

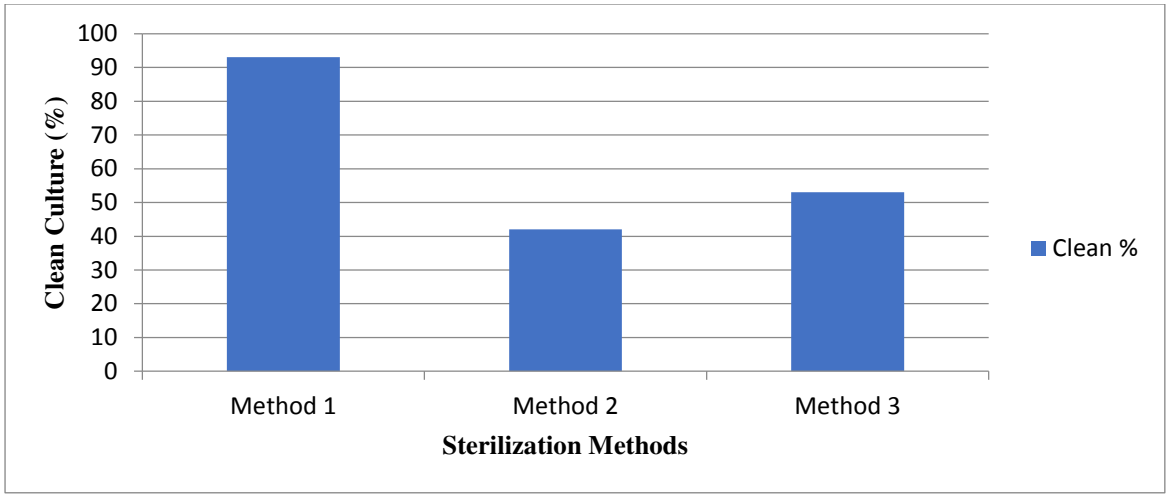

Figure 4: The percentage of *Tithonia diversifolia* (Insulin) clean culture using three different methods of surface sterilization.

Method 1 showed the highest percentage of clean culture (93%) compared to Method 2 (42%) and Method 3 (53%) respectively. In conclusion, Method 1 is the best sterilization method for *Tithonia diversifolia* (Insulin) (Figure 4).

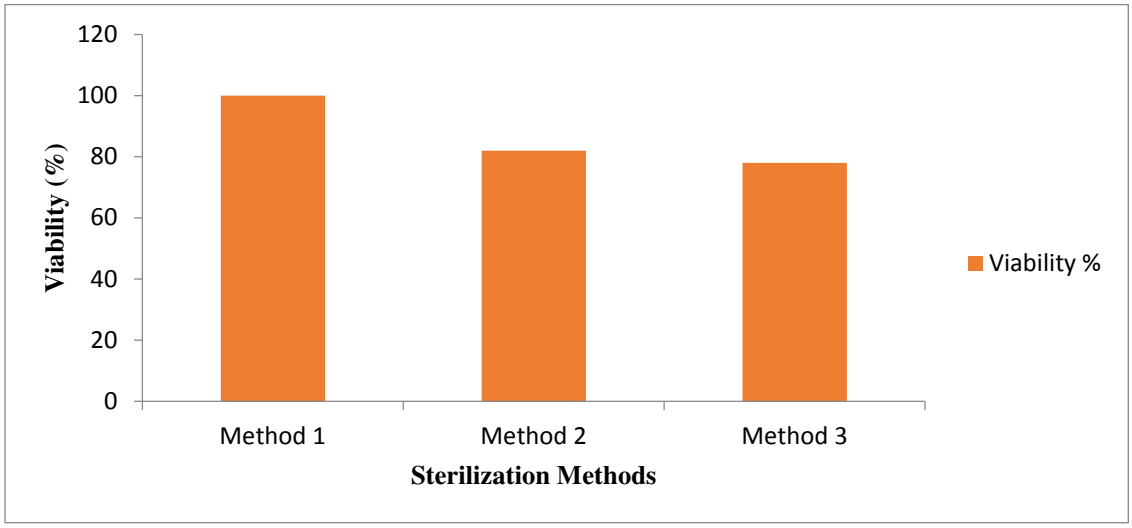

Figure 5: The percentage of *Tithonia diversifolia* (Insulin) viability using three different methods.

Figure 5 showed that Method 1 gave the best result in viability (100%) compared to Method 2 (82%) and Method 3 (78%). New shoot induction from nodal explants were shown in Figure 6.

Figure 6: Shoot induction from *T. diversifolia* explants.

It is a common practice to use commercial bleach or Clorox® in tissue culture surface sterilization techniques. Based on the results as shown in Figure 4 and 5, Method 1 gave the highest viable samples and the lowest contamination rate which is the best protocol for Insulin tree. In fact, the concentration of the chemical used in the experiment played an important role in the percentage of clean culture obtained.

This study obtained high percentage of Insulin tree clean culture because suitable concentration of Clorox® is sufficient to remove the contaminants. On the other hand, Method 2 and Method 3 showed high contamination rate with moderate percentage of viability. Tween 20 was utilized in this experiment to reduce the background staining and enhance the reagent spreading into the explants. The exposure of the explants to the bleach or chemical also could affect the viability of the explants.

Conclusion

The protocol of surface sterilization for *Tithonia diversifolia* (Insulin tree) using young shoot and nodal segments as explants has been established. This study has obtained a high percentage of clean cultures and viability of the shoots. Less concentration of Ethanol and Clorox® applied obtains good results. New shoot induced were transferred to the shoot multiplication media for further study. Thus, producing Insulin tree using tissue culture method is needed to supply a mass planting stock for plantation.

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