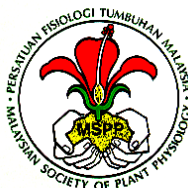


TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 24

Advances in Plant Science and Technology

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Malaysian Society of Plant Physiology

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Chapter 1

Plant Growth and Development

Impact of Natural Farming on Soil, Normalized Difference Vegetation Index and Chlorophyll of Black Pepper

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Introduction

Dubbed as black and white gold in Sarawak, *Piper nigrum* or better known as ‘lada hitam’ is a valued commodity in Malaysia. In addition, Sarawak pepper in particular is held in high regards by international chefs worldwide due to its distinctively refined flavour. Due to its soaring high demand, planting pepper has proven to be a lucrative investment for farmers especially from the rural regions of Sarawak. With the help of the government through Malaysian Pepper Board, the pepper industry has improved the livelihood of tens of thousands of farmers.

Current agriculture practices which include continuous application of farming inputs such as inorganic chemical fertilizers have led to infertile soil over time in most pepper vineyards. Gradually, the infertile soil will reduce the yield and life expectancy of pepper vines. Developing Natural Farming (NF) through the action of beneficial and effective microorganisms in the soil is of fundamental importance for an ecosystem to function by determining the nutrient cycling and organic matter decomposition (Doran and Zeiss, 2000). These microorganisms can help to improve and sustain soil properties thus contributing to healthier pepper growth besides conserving the environment.

Monitoring the impact of using NF method on black pepper via non-destructive sensors will be able to provide information on plant nutrient status and growth response. Non-destructive sensors have been developed to measure the reflectance of incident light at various wavelengths that are related to plant growth. The normalized difference vegetation index (NDVI) for example has gained wide acceptance based on its ease of use, only requiring two wavelengths to evaluate the status of plant nitrogen (N) status and chlorophyll content in real time. Therefore, this study was conducted to compare selected properties of soil as well as *P. nigrum* NDVI and foliar chlorophyll content (SPAD) after amendment with different NF liquid fertilizers. This study was also done to determine the relationship between soil N with both NDVI and foliar chlorophyll content.

Materials and Methods

The soil series at the study site was Miri Series of the Miri Family which is a sandy, isohyperthermic, strongly cemented Typic Haplorthods. Drainage to the top of the spodic horizon is good to excessive. Water can stagnate during the rainy seasons but the soil can be dry in the dry season. Due to their sandy textures and very poor fertility status, soils of the Miri Series are not suitable for agriculture and are best left under their natural vegetation (Paramanathan, 2000). The crop involved in this study was *P. nigrum*. The study area was a black pepper vineyard located in Kampung Serayan Hilir, Lundu, Sematan, Sarawak, Malaysia. The study plot was approximately 0.01 hectare. Duration of the study was from December 2014 to December 2015.

Experimental design and treatments

The experiment was 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 NF liquid fertilizers were applied to the plant and soil every two weeks.

Selected soil properties determination

At the end of study period, soil was analysed for its bulk density (BD) and volumetric water content (VWC). The soil BD was determined using a soil sample ring kit (Model C, Eijkelkamp, Holland) whereas soil VWC was measured by using a soil moisture meter (SMEC 300, WaterScout, USA). To further understand the nitrogen status of the soil, samples were collected at a depth of 0–25 cm and analyzed for total nitrogen (N) and soil organic matter (SOM) according to the method by Tan (1995).

*Selected *Piper nigrum* physiological characteristic*

For every three months from the onset of the treatments, measurements for the normalized difference vegetative index (NDVI) and foliar chlorophyll content (SPAD) were obtained. The NDVI was recorded by using an NDVI meter (CM1000, Spectrum, FieldScout, USA). Foliar chlorophyll content of *Piper nigrum* leaves was determined by using a chlorophyll meter (SPAD-502, Minolta, Japan). Readings were recorded when young fully expanded leaves with the same orientation and the same layer in the crown (middle bottom) were still attached to the tree.

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, NDVI and foliar chlorophyll content (SPAD). The relationships between soil total N with NDVI and foliar chlorophyll content (SPAD) were tested by regression analysis of best fit.

Results and Discussion

Soil properties

The selected soil properties after amendment of different NF liquid fertilizers are presented in Table 1. Except for soil BD, the control plants recorded lower soil VWC and total N value when compared to that of the NF liquid fertilizers treated plants. The soil BD of F0 through F4 suggested that NF liquid fertilizers application did not affect hardness of the soil within the time frame of this study, a result that was because of the time necessary for the full establishment of the microorganisms (Paschoal et al., 1995). Bulk densities were ranged from 1.11 to 1.27 g/cm³ and were typical of the Miri series. In a study by Cai et al. (2002) reported that bulk density of mineral soil that ranges from 1.1 to 1.5 g/cm³ in surface horizons are generally ideal for plant growth. The result also 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 concurs with a study done 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 NF treated soils.

The result in Table 1 shows that soils under NF incorporation showed a significantly ($p < 0.05$) higher VWC than that of the control. Lee et al. (2008) was of the opinion that soil moisture content is related with the changes in water holding capacities. The presence of soil organic matter increases soil water holding capacities and therefore increases the soil VWC as well. Table 1 also shows that total N for treatments applied with NF method increased when compared to that of control. A study by Park and DuPont (2008) suggests that effective microorganism (EM) activities might accelerate

decomposition of organic matter that led to additional N content in the soil. According to Higa (1987), farms treated with EM through NF will increased the nitrogen cycling function of the soil.

Piper nigrum NDVI and chlorophyll content

Comparison of NDVI between the control and *P. nigrum* under the NF method are shown in Table 2. The treatment mean for FFJ was the highest with an NDVI value of 0.94 followed by FPJ, IMO, LABS and control with NDVI values of 0.91, 0.90, 0.89 and 0.79 respectively. The results revealed that the NDVI in pepper grown under NF method were significantly ($p < 0.05$) increased compared to that of the control (Table 2). This result could be attributed to the additional presence of N in the leaf which is an important contributing factor to proper plant NDVI development (Penueles and Inoue, 1999; Richardson and Berlyn, 2002). Multiple studies have also been done describing the increment in plant growth and productivity associated with high levels of N availability (Eck, 1984; Jacobs and Pearson, 1991). The NDVI values are represented as a ratio ranging in value from -1 to 1 but in practice, negative values represent water, values around zero represent bare soil and values over 6 represents dense green vegetation (Rouse, 1973).

Table 1: Effect of different natural farming (NF) liquid fertilizers on soil bulk density (BD), soil organic matter (SOM), volumetric water content (VWC) and total nitrogen (N).

Treatment	BD (g/cm ³)	SOM	VWC (%)	Total N (%)
F0	1.11 ± 0.34 ^a	2.06 ± 0.79 ^a	27.74 ± 0.85 ^a	0.04 ± 0.42 ^a
F1	1.25 ± 0.48 ^a	2.93 ± 0.67 ^b	35.94 ± 0.39 ^b	0.11 ± 0.17 ^b
F2	1.22 ± 0.26 ^a	3.10 ± 0.41 ^b	34.21 ± 0.52 ^b	0.10 ± 0.89 ^b
F3	1.27 ± 0.43 ^a	2.93 ± 0.38 ^b	35.69 ± 0.58 ^b	0.09 ± 0.31 ^b
F4	1.13 ± 0.66 ^a	2.85 ± 0.65 ^b	33.46 ± 0.52 ^b	0.09 ± 0.76 ^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 ± S.D., $n = 5$).

Result for *P. nigrum* relative leaf chlorophyll content (SPAD) is shown in Table 2. Chlorophyll (SPAD) content was increased by 23% in the Fermented Plant Juice (FPJ) treatment when compared to the control. The result is similar to a study done by Cabrera (2004) and can be attributed to higher soil N concentration combined with the presence of soil moisture (Table 1) for cytoplasmic fluid development at the leaf cellular level. Sulok et al. (2012) observed that plants with higher nitrogen content and presence of sufficient fluid tend to have darker green leaves.

Table 2: Effect of different natural farming (NF) liquid fertilizers on *Piper nigrum*'s NDVI and chlorophyll (SPAD).

Treatment	NDVI	SPAD
F0	0.79 ± 0.75 ^a	67.10 ± 0.68 ^a
F1	0.90 ± 0.39 ^b	76.66 ± 0.71 ^b
F2	0.91 ± 0.42 ^b	82.78 ± 0.95 ^b
F3	0.94 ± 0.88 ^b	81.96 ± 0.93 ^b
F4	0.89 ± 0.72 ^b	80.14 ± 0.79 ^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 ± S.D., $n = 5$).

Figure 1 shows the relationship between *P. nigrum* NDVI and soil total N. The close relationship between NDVI and soil total N regardless of treatments shows a polynomial cubic regression line of zero intercept with $r^2 = 0.82$ indicating that higher N content in the soil increased NDVI in black pepper. A similar study done by Cai et al. (2002) concluded that higher NDVI values were associated with higher soil nitrogen rates. Carter (1994) added that available nitrogen from the soil may be utilized by the plant in the production of nucleic acids and proteins which is essential for the plant's growth.

Chlorophyll production is also dependent on nitrogen availability. Values of black pepper foliar relative chlorophyll concentration increased as soil total N increased while the relationship was best described by a polynomial cubic regression line of zero intercept (Figure 2). Correlation coefficient value of $r^2 = 0.64$ indicating a correlation between leaves chlorophyll concentration and soil total N (Figure 2). Previous report by Tucker (2004) and Daughtry et al. (2000) revealed that because N is a structural element of chlorophyll, thereby it affects formation of chloroplasts and accumulation of chlorophyll in them.

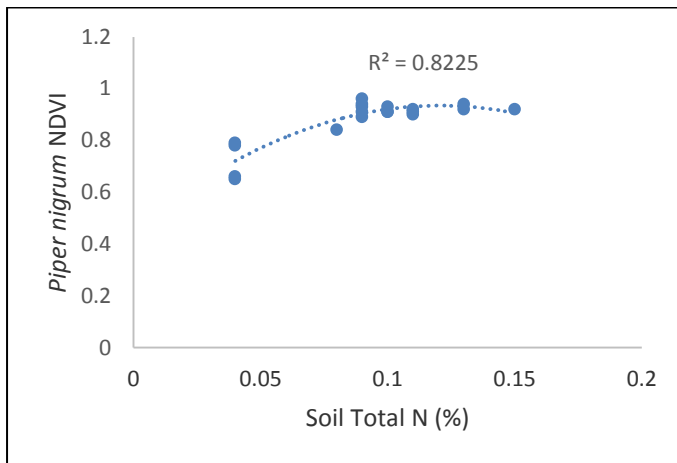


Figure 1: Relationship between *Piper nigrum* NDVI and soil total N subjected to different NF treatments. Values are means \pm s.e. of five leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determination coefficient are included.

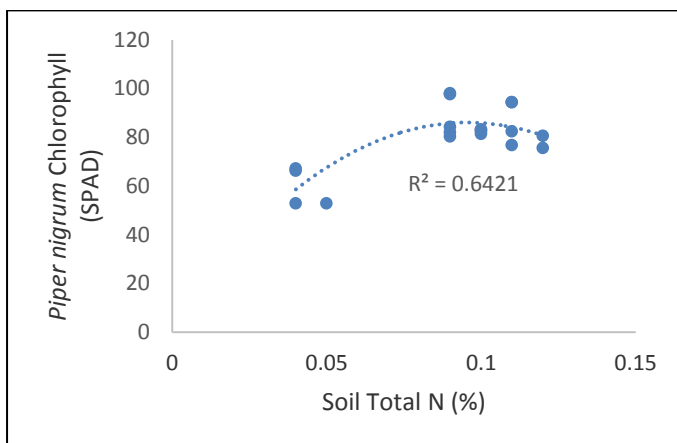


Figure 2: Relationship between *Piper nigrum* chlorophyll (SPAD) and soil total N subjected to different NF treatments. Values are means \pm s.e. of five leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determination coefficient are included.

Conclusion

The establishment of F1 through F4 plot using different NF practices responded better in terms of its selected soil properties as well as NDVI and relative chlorophyll content (SPAD) of black pepper. Except for soil bulk density, application of IMO, FPJ, FFJ and LABS improved the selected soil properties considerably by showing comparatively higher SOM, soil VWC, and soil total N. The NDVI and relative chlorophyll content of *P. nigrum* grown under the NF approach were significantly higher than the control. Furthermore, it was found that NDVI and chlorophyll content of black pepper were significantly correlated to higher nitrogen availability in the soil. Through the action of effective microorganism (EM), applying various NF liquid fertilizers in black pepper vineyards can greatly improve soil properties which in turn lead to positive pepper physiological growth.

Acknowledgements

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Effect of Cytokinins on Shoot Induction from Nodal Explant of Pecah Beling (*Strobilanthes crispus*)

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Introduction

Strobilanthes crispus or also known as Pecah Beling is a medicinal plant of Acanthaceae family widely used for treatment of various ailments and boosting immune systems (Sunarto, 1997). This plant composed of various essential bioactives such as β -sitosterol and taraxerol that has anti-diabetic (Sangeetha et al., 2013), anti-inflammatory (Yoon et al., 2015) and anticancer properties (Nik Suriani et al., 2014). This plant is highly demanded for raw material used in developing various natural products such as supplements and functional foods. Conventional propagation through cutting is not convenient due to slow growth rate, limited plantlet production and inconsistent production of plant raw material due to external environmental factors such as weather and soil conditions. To ensure continuous supply of this plant, tissue culture technique offers rapid, high, consistent and controlled production of plantlets (Murthy et al., 2014). So far, there are no literatures reporting on tissue culture technique for *S. crispus*. Micropropagation of other *Strobilanthes* species such as *S. flaccidifolius* (Deb and Arenmongla, 2012) and *S. cusia* (Kabita et al., 2015) is, however, frequently discussed.

Successful micropropagation is controlled by several factors such as growth regulators, media and physical factors. Plant growth regulator, especially cytokinin, plays a role in plant development such as shoot formation and multiplication as well as promotion of cell division and expansion (Mok and Mok, 2001). However, the requirement of growth regulators is reported to be species dependant due to the presence of endogenous hormone in Acanthaceae species, as reported by Thomas and Yochiro (2010) in *Justicia gendarussa* and Cheruvatur and Thomas (2014) in *Rhinacanthus nasutus*. Kabita et al. (2015) reported shoots multiplication of *S. cusia* (Nees) using benzylaminopurine (BAP) while Deb and Arenmongla (2012) used combinations of α -naphthalene acetic acid (NAA) and BAP for shoot multiplication of *S. flaccidifolius*. Therefore, the objective of this research is to study the effect of single or combination of cytokinins in culture media for shoot induction from nodal explant of *S. crispus*.

Materials and Methods

Establishment of aseptic plant material

The plant material was bought from local vendor in Gaya Street, Kota Kinabalu, Sabah. Shoot tips (0.5 – 1 cm) that emerged from the nodes were used as explants. Explants were initially washed with running tap waters and surface sterilised with Chlorox[®] (20% v/v) for 7 minutes followed by five rinses with sterile distilled waters. Finally, the shoot tips were cultured in Murashige and Skoog (MS) medium containing 3% (w/v) sucrose and 0.8% (w/v) agar (Sigma). The pH of the °C media was adjusted to 5.7 using 0.1M HCl and/or 0.1M NaOH prior to autoclaving at 121 °C for 20 min. The cultures were incubated at 25 \pm 2 °C under 16 h photoperiod for 30 days, before used for further treatments.

Effect of cytokinins on shoot induction from nodal explant

Nodal parts from 30 days old aseptic plantlets were used as explants. The nodes were cut into 1 cm in length and cultured on MS basal medium supplemented with single BAP, Kn (Kinetin) and TDZ

(Thidiazuron) at concentrations of 0.5, 1, 2 and 4 mg/L respectively. Selected treatments (0.5 mg/L BAP and 0.5 mg/L Kn) were separately combined with 0.05, 0.1, 0.2 and 0.4 mg/L TDZ for further optimisation of shoot induction and multiplication. All cultures were incubated in growth chamber (Convion) at 25 ± 2 °C under 16 h photoperiod provided by cool white fluorescent lamps ($36 \mu\text{mol m}^{-2} \text{s}^{-1}$). Data such as percentage of shoot induction, number of shoots per explant, shoot length, number of days for shoot induction and other morphological changes were recorded every 4 days for up to 30 days of culture.

Statistical analysis

All experiments were arranged in a Completely Randomised Design (CRD). Data were subjected to one way Analysis of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software version 23. The mean values were statistically compared using Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Results and Discussion

Shoot induction started with breaking of axillary shoot buds from nodal explant, followed by shoot initiation within 3 to 5 days after culture. Treatments with single BAP, Kn and control medium were able to induce 100% shoot regeneration at all concentrations (Table 1). In addition, treatment with BAP and Kn at 0.5 mg/L accelerated axillary shoot budbreak to 3 days. Among all single cytokinin treatments, TDZ was able to induce multiple shoots, whereby 2.9-3.1 shoots per explant was successfully induced on medium supplemented with 0.5-1 mg/L TDZ. Although TDZ allowed highest number of shoots, BAP, Kn and control treatment had consistent shoot regeneration frequency and produced longer shoots. TDZ, unlike BAP and Kn, stimulated biosynthesis of endogenous cytokinins, thus promoting cell division and subsequently shoot multiplication. This made the nodal explants continue to differentiate into more shoots, but they failed to elongate (Guo et al., 2011). However, increasing cytokinin concentrations from 2 to 4 mg/L reduced number of shoots and shoot length, and delayed shoot regeneration to 5 days (Figure 1f). Introducing cytokinins in high concentration promoted programmed cell death (Kunikowska et al., 2013), which explained decreased number of shoots and shoot length with increased BAP and Kn concentrations. However, elevated cytokinin concentration also promoted callus formation (Figures 2a and 2b). It has been reported that cytokinin signalling in plant cells was increased in wounded plant tissues to promote callusing (Iwase et al., 2011; Ikeuchi et al., 2013). TDZ in high concentrations also promoted ethylene production, a harmful plant by product that is toxic to the plant itself (Figure 1g) (Hutchinson and Saxena, 1996; Guo et al., 2011). Therefore, cytokinin-cytokinin combination has been employed for further optimisation of shoot multiplication.

BAP or Kn at 0.5 mg/L in combination with TDZ ranging from 0.05 mg/L to 0.4 mg/L were employed for cytokinin-cytokinin combination. For such treatment, Kn 0.5 mg/L + TDZ 0.1 mg/L showed highest number of shoots induced (9.4 ± 10.3 shoots per explant) with shoots 4.3 ± 5.1 mm in length (Table 2) (Figures 1a-1e). However, both BAP and Kn in combination with increasing TDZ concentration reduced shoot induction percentage to as low as 59.6% number of shoots and shoot length. Callus formation was observed in BAP and Kn in combination with 0.05 and 0.1 mg/L TDZ, where globular callus was formed in Kn 0.5 mg/L + TDZ 0.05 mg/L (Figure 2c). Saha et al. (2007) reported stimulatory effect of Kn through its action in inhibiting ethylene production in plants. Therefore, higher rate of shoot multiplication in Kn + TDZ combination could likely be due to its inhibitory effect on ethylene released by TDZ in the medium. In short, cytokinin-cytokinin combination was effective in promoting shoot multiplication compared to single cytokinin treatment but with slightly decreased shoot regeneration frequency. Similar period (3 to 5 days) was required for shoot induction in single cytokinin and cytokinin combination treatment.

Combination of cytokinin-cytokinin treatment has been shown to induce more shoots apart from auxin-cytokinin combination as demonstrated by Ashraf et al. (2014) on micropropagation of *Chlorophytum* sp., i.e. 8.88 μ M BAP and 26.6 μ M Kn combination induced more shoots than treatment with single cytokinin. This may be attributed to synergistic actions from both cytokinins on shoot induction of the nodal explant. Formation of globular callus was also observed in Kn 0.5 mg/L + TDZ 0.05 mg/L treatment. The use of TDZ in combination with other cytokinin generated stress effect and later promoted cell differentiation (Fehe'r et al., 2003).

Table 1: The effect of single cytokinin on multiple shoot induction from nodal explant of *S. crispus* after 30 days of culture. [Culture medium: MS medium with pH 5.7, supplemented with 3% sucrose, 0.8% agar (Sigma) and various cytokinins. Photoperiod: 16 h].

Cytokinin	Concentration (mg/L)	Days required for shoot induction	Shoot induction (%)	No. of shoots	Length of shoots (mm)	Other morphological changes
Control (MSO)	-	5	100 ^a	2.0 \pm 0 ^b	4.9 \pm 0.9 ^a	None observed
BAP	0.5	3	100 ^a	2.0 \pm 0 ^b	4.9 \pm 0.9 ^a	None observed
	1	4	100 ^a	2.0 \pm 0 ^b	5.2 \pm 1.3 ^a	None observed
	2	5	100 ^a	1.9 \pm 0.3 ^b	3.2 \pm 0.6 ^b	Callus formation observed
	4	5	100 ^a	1.7 \pm 1.1 ^b	2.9 \pm 0.5 ^b	Callus formation observed
KN	0.5	3	100 ^a	2.0 \pm 0.0 ^b	5.1 \pm 1.2 ^a	None observed
	1	4	100 ^a	1.8 \pm 0.4 ^b	4.8 \pm 0.8 ^a	None observed
	2	5	100 ^a	1.6 \pm 0.5 ^{bc}	3.1 \pm 1.2 ^b	Callus formation observed
	4	5	100 ^a	1.3 \pm 0.5 ^{cd}	2.8 \pm 0.6 ^b	Profused callus formation
TDZ	0.5	4	100 ^a	3.1 \pm 0.8 ^a	1.9 \pm 0.7 ^c	None observed
	1	4	100 ^a	2.9 \pm 0.8 ^a	2.1 \pm 0.8 ^c	None observed
	2	5	73.3 ^b	1.2 \pm 0.4 ^d	1.5 \pm 0.5 ^{cd}	None observed
	4	5	59.8 ^c	1.0 \pm 0.0 ^d	1.1 \pm 0.6 ^d	None observed

Value represents mean \pm standard deviation.

Table 2: The effect of cytokinin combination on multiple shoot induction from nodal explant of *S. crispus* after 30 days of culture. [Culture medium: MS medium with pH 5.7, supplemented with 3% sucrose, 0.8% agar (Sigma) and various cytokinins. Photoperiod: 16 h].

PGR (concentration in mg/L)			Days required for shoot induction	Shoot induction (%)	No. of shoots	Length of shoots (mm)	Other morphological changes
BAP	KN	TDZ					
0.5	-	0.05	4	93.2 ^a	2.1 \pm 0.7 ^d	3.4 \pm 2.0 ^b	Callus formation
0.5	-	0.1	4	86.4 ^a	5.3 \pm 1.8 ^c	5.1 \pm 1.5 ^a	None observed
0.5	-	0.2	5	79.8 ^{ab}	6.6 \pm 1.4 ^b	4.3 \pm 1.8 ^{ab}	None observed
0.5	-	0.4	5	66.2 ^{bc}	2.0 \pm 0.0 ^d	2.0 \pm 1.0 ^c	None observed
-	0.5	0.05	4	93.2 ^a	5.6 \pm 1.9 ^{bc}	3.5 \pm 1.7 ^b	Globular callus formation observed
-	0.5	0.1	4	86.4 ^a	9.4 \pm 2.8 ^a	5.1 \pm 0.6 ^a	None observed
-	0.5	0.2	5	79.8 ^{ab}	10.3 \pm 2.6 ^a	4.3 \pm 1.6 ^{ab}	None observed
-	0.5	0.4	5	59.6 ^c	2.1 \pm 0.3 ^d	2.2 \pm 1.1 ^c	None observed

Value represents mean \pm standard deviation.



Figure 1: The effects of cytokinins on shoot induction from nodal explant of *S. crispus* cultured in MS medium supplemented with various cytokinins after 30 days of culture. (a-e) Multiple shoot induction from nodal explant after 30 days of culture in MS + KN 0.5 mg/L + TDZ 0.2 mg/L. (f) Adverse effects of BAP and Kn in higher concentrations (2-4 mg/L). Arrow shows formation of callus. (g) explants cultured in TDZ (2-4 mg/L) showed toxicity effect of TDZ in higher concentration. (Bar = 3 mm).



Figure 2: The effects of cytokinins on shoot induction from nodal explant of *S. crispus* cultured in MS medium supplemented with various cytokinins after 30 days of culture. (a) Callus formation observed in 2-4 mg/L BAP and 2 mg/L Kn as indicated by arrow, (b) Profused callus formation in 4 mg/L KN, as indicated by arrow, (c) Formation of globular callus in Kn 0.5 mg/L + TDZ 0.05 mg/L. (Bar = 1 mm).

Conclusion

Through this study, shoot induction from nodal explant of *S. crispus* was best achieved in MS medium supplemented with a cytokinin combination, i.e. 0.5 mg/L Kn + 0.1 mg/L TDZ treatment. Single cytokinin treatment was also able to induce shoot but produced fewer shoots (1.3 to 3.1) per explant. The result may be important as a pioneer study on tissue culture of this medicinal plant.

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Effect of Rootstock Age on Success and Growth of Softwood Grafting for Harumanis Mango

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Introduction

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae, is an important tropical fruit and has grown in more than 100 countries of the world (Alam et al., 2006; Ram et al., 2012). Harumanis mango is one of the famous fruits that has high economic demand and potential for Malaysia export business especially in the Perlis State in Malaysia (Farook et al., 2013). It is pleasant in smell and sweet in taste with orange pulp (Farook et al., 2013). Harumanis mango cultivar is included in the national agenda as a specialty fruit from Perlis, Malaysia, for the world (Farook et al., 2013). Usually, mango can be propagated by seeds or by grafting (Ram et al., 2012). 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 current methods used are time consuming and low in success. One of the major requirements for achieving the increased production of Harumanis planting materials would be the rapid multiplication and distribution of superior clones. Softwood grafting is one of the successfully economic efficient and rapid methods for the propagation of Harumanis.

However, the success of grafting depends on different factors such as influence of environmental parameters, source of rootstock, rootstock age, grafting time and method of grafting (Shantagouda et al., 2008). The selection of suitable rootstock is as important as the selection of the scion cultivar. It has a strong influence on the growth, yield, fruit maturity and soil adaptability, among other things (Mandal et al., 2011). Hence, it is highly essential to evaluate the effect of rootstocks age on success and growth of softwood grafting for Harumanis planting materials.

Materials and Methods

Mangifera indica cultivar Telur was used as source of rootstocks. *Mangifera indica* cultivar Telur was collected from commercial mango plot at the Malaysian Agriculture Research and Development Institute (MARDI), Sintok, Kedah. Before sowing, the skin and the flesh of 80 mature fruit of Telur cultivar were removed and the seeds were washed with clean water and air-dried for one day. The seeds were later sown in seedbed containing sand. The seedbed was shaded with black netting and watered daily. The seedlings of Telur cultivar were selected and transplanted into polibags (8" x 12"), containing a mixture of soil, sand and cow manure in 3:2:1 (v/v). The scion shoots of Harumanis (10-15 cm lengths) at the same size with the rootstock were collected on the same day for grafting purposes. Different age (2, 4, 6, 8 and 10 months) of Telur rootstocks were grafted with Harumanis scion using wedge grafting method under netted structure. Forty rootstocks for each rootstocks age were used for grafted with Harumanis scion. Grafted plants were watered regularly and general plant protection measures were taken by applying fungicides and pesticides to control the pest and diseases. The observations were recorded three months after grafting (MAG) for graft success and six months MAG for graft survival percentage and monthly for graft growth parameters such as shoot height, graft diameter and number of leaves.

Survival rate of the grafts was calculated after six MAG by the following formula:

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

Results and Discussion

Among the different of age tested, Harumanis scion grafted on four months old rootstocks exhibited a maximum graft success (80.23%) and two months old were observed a minimum success (30.61%) (Table 1). Harumanis scion grafted on four months old of rootstocks showed a maximum graft survival (75.02%) while a minimum graft success was observed on two months old rootstocks graft survival (41.52%) after three and six months of grafting. Reddy and Melanta (2001) reported that three months old rootstocks recorded a maximum graft union success followed by four month old rootstocks in mango. Phadnis (1991) in cashew recorded a maximum success in softwood grafting with rootstocks less than five months age. Similar results were recorded in cashew by Nagabhushanam and Rao (2005). The reason might be due to the fact that, the optimum thickness of the rootstocks influenced the graft union formation satisfactorily. Similar results were obtained by Mandal et al. (2011), when grafting was done in four to six months old rootstock in mango. The age of rootstocks has a positive relationship with regenerating ability of a plant part, which is found in younger rootstocks and this is due to a higher activity of meristematic cells resulting in faster formation of callus and quick healing of grafting union. In general, 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 rootstocks age on graft success and graft survival of *M. indica* var. Harumanis.

Treatments (Age of rootstock)	Graft success (%)	Graft survival (%)
	3 MAG	6 MAG
T1	30.61	41.52
T2	80.23	75.02
T3	77.50	65.65
T4	44.80	53.79
T5	40.55	49.99

T1=2 Months; T2=4 Months; T3=6 Months; T4=8 Months; T5=10 Months; MAG=Months after grafting

Table 2: Effect of rootstocks age on shoot height (cm), graft diameter (mm) and number of leaves of *M. indica* var. Harumanis.

Treatments (Age of rootstock)	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	2.53	3.53	4.40	5.58	6.49	7.40	4.42	6.88	7.70
T2	3.58	5.29	6.03	7.21	7.69	7.79	7.15	8.65	10.13
T3	3.00	3.98	5.89	5.38	7.38	7.79	6.85	8.40	9.70
T4	3.38	5.02	5.76	6.60	6.66	8.01	7.30	8.70	9.65
T5	3.99	5.00	5.66	6.89	6.60	8.23	6.15	8.20	9.16

T1=2 Months; T2=4 Months; T3=6 Months; T4=8 Months; T5=10 Months; MAG=Months after grafting

Harumanis scion grafted on four months old of rootstocks showed a maximum mean shoot height (6.03 cm) while a minimum mean shoot height was observed on two months old rootstocks (4.40 cm) after 90 days of grafting (Table 2). For graft diameter, Harumanis scion grafted on 10 months old rootstocks showed a maximum mean graft diameter (8.23 mm) while a minimum mean graft diameter was observed on Harumanis scion grafted on two months old rootstocks (7.40 mm) after 90 days of grafting. A maximum mean number of leaves was observed on Harumanis scion grafted with four

months old rootstocks (10.13) and a minimum mean number of leaves was observed on Harumanis scion grafted with two months old rootstocks (7.70) after 90 days of grafting. According to Reddy (1998) and Reddy and Melanta (2001), a maximum number of leaves was recorded in four to six months old rootstocks and it was due to younger stocks could store more carbohydrates and other food substances and this leads to more vegetative growth in terms of number of leaves. These results are in confirmatory with Reddy (1998), who reported that maximum number of leaves on graft was prepared on three months old rootstock in mango. Maheswari and Nivetha (2015) reported maximum leaves were produced on four months old rootstock used for softwood grafting in Jack. Therefore, using four months old rootstocks of Telur cultivar as a source of rootstocks for grafted with Harumanis scion can increase production of planting materials.

Conclusion

Based on the results, four months old rootstocks of Telur cultivar as a source of rootstocks for grafted with Harumanis scion can increase success of grafting and growth for Harumanis mango.

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Effect of Ethephon and Phosphorus Levels on Plant Growth Parameters, Total Phenolic Content and Plant Dry Matter of Sweet Corn (*Zea mays* L. *saccharata*) Grown on Rasau Soil Series

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Introduction

Ethephon (2-chloroethyl-phosphonic acid) is a systemic plant growth regulator that can be used directly either by soil drenching or foliar application to the plant. It will penetrate into plant tissue and decompose to ethylene, chloride ion and phosphonic acid (Bhat et al., 2010). At present, it is widely used for specific function such as to hasten fruit ripening, stimulate flowering emergence and improved plant resistant to lodging. Ethephon treatment resulted in a significant reduction of corn plant height which was attributed to decrease in internode length (Mischeck and Fanuel, 2014). Moreover, ethephon will breakdown to release ethylene that will be involved to enhance root growth at low soil nutrient concentration such as nitrogen, phosphorus and potassium (Postma and Lynch, 2011). In Malaysia, most of mineral soil are grouped in Oxisols which are acidic in nature and developed from a range of parent materials which are dominated by kaolinite and oxides of iron (Fe) and aluminium (Al) (sesquioxides) (Shamsuddin and Markus, 2014). The high content of Al and Fe will lead to fixation of soluble inorganic phosphorus (P) and would affect availability of P in the soil that is required for better plant growth (Adnan et al., 2003). In order to achieve higher crop productivity, soil fertility and management play an important role. Thus, proper crop and nutrient management should be adjusted according to the soil condition so that plant requirement will be sufficient to complete their cycle. Consequently, application of ethephon to marginal soil would be a promising approach to improve crop growth performance. Therefore, the effect of ethephon application at several P levels on performances of sweet corn (*Zea mays* L. *saccharata*) grown on Rasau soil series was investigated.

Materials and Methods

The trial was conducted at Malaysian Agricultural Research and Development Institute (MARDI), Sintok located in Bukit Kayu Hitam, Kedah. About 15 kg of Rasau soil series was filled into polybag and arranged in Randomized Complete Block Design (RCBD) with six replicates. Vigorous and healthy sweet corn seedlings were selected for transplanting. Four phosphorus (P) levels were 0, 15, 45 and 60 kg P₂O₅ ha⁻¹ and standard recommendation of ethephon at 270 ppm was applied once equally to all treatments (200 mL per polybag) by soil drench technique at 20 days after transplanting. The nitrogen (N) and potassium (K) were fixed at 120 kg N ha⁻¹ and 90 kg K₂O ha⁻¹ for all treatments. Plant maintenance such as pest and diseases control followed standard procedure for sweet corn cultivation. Weed control was done manually when necessary. The seedlings were irrigated manually twice daily until field capacity level. The data collection was carried out during planting until tasseling (45 days after transplanting). Plant height was recorded at each vegetative stage while plant dry matter (leaf, stem and root) was measured once at harvest. The SPAD value data of 8th leaf (V8) was recorded at harvest by using Minolta SPAD 502 chlorophyll meter. Soil physico-chemical properties such as soil pH, bulk density, particle density, porosity, soil moisture content, carbon, nitrogen, phosphorus and potassium were measured before conducting the experiment. Specific method of physico-chemical properties is given in Table 1. Dry samples of leaf were prepared for

total phenolic and chlorophyll content. The total phenolic content of the dry sample was determined by the Folin–Ciocalteu reagent while chlorophyll concentration was determined by using Harbone's method (1998). The absorbance of both total phenolic content and chlorophyll concentration was measured using UV-spectrophotometer. Statistical analysis was performed using the Statistical Analysis System (SAS 9.3.1) and tested for mean comparison using Least Significant Different (LSD) at $P \leq 0.05$.

Results and Discussions

Soil physical and chemical properties

The physical and chemical properties of the Rasau soil are given in Table 1. The pH of the soil before experiment was slightly acidic at 5.07. The organic carbon was < 1% which indicated the soil is low in organic matter (1.15 % OM). According to Baldock and Skjemsta (1999), different soil types have different ranges of organic matter ranging from 0.9%-1.7% for sandy soil, 1.2%->2.4% for sandy loam soil, 1.6%->3.1% for loamy soil and 2.1%->3.4% for clay and clay loam soil types. Moreover, Rasau soil development is derived from sub recent riverine alluvium that contained high percentage of sand compared with silt and clay particle. Soil moisture data for Rasau soil depicted that it is able to retain moisture up to 13.60 % with 23.74% porosity.

Table 1: Soil physico-chemical properties of Rasau soil series at 0-20 cm depth.

Soil properties	Value	Method/Extractant
pH	5.07	Water 1:2.5
Carbon (C), %	0.67	Combustion
Nitrogen (N), %	0.08	Combustion
Phosphorus (P), mg L ⁻¹	45.22	Bray and Kurtz II
Potassium (K), cmol (+) kg ⁻¹	3.77	Ammonium acetate
Bulk density, g cm ³	1.82	Core
Particle density, g cm ³	2.38	Pycnometer
Porosity, %	23.74	Volumetric
Moisture, %	13.60	Gravimetric

Plant height

The effect of application ethephon and P on plant height of sweet corn is presented in Figure 1. Overall, application of ethephon at different rates of P fertilizer had significant effect on plant height at all growth stages. However, at V6 until V8 stages, there was significant plant height increment at 45 kg P₂O₅ ha⁻¹ compared with other treatments. The highest plant height recorded was 125.1 cm while the lowest plant height was 90.6 cm obtained from control treatment. The results indicated ethephon application might have decomposed to phosphoric acid and oxidized within the soil to orthophosphate and thus provided sufficient P for plant growth. Furthermore, at standard rate of P fertilizer application with addition of ethephon tends to decrease the plant height, probably due to excessive concentration of P in the soil that would interact with trace element such as iron (Fe) and consequently ethylene would inhibit stem elongation. Chandiposha and Chivende (2014) reported that ethephon interaction with planting density affect plant height of corn plant which resulted in decrease in internode length. Meanwhile, Turuko and Mohammed (2014) reported that, when available P is above the optimum level, it might interrupt other nutrients which in turn would cause decrease in growth of common bean (*Phaseolus vulgaris* L.).

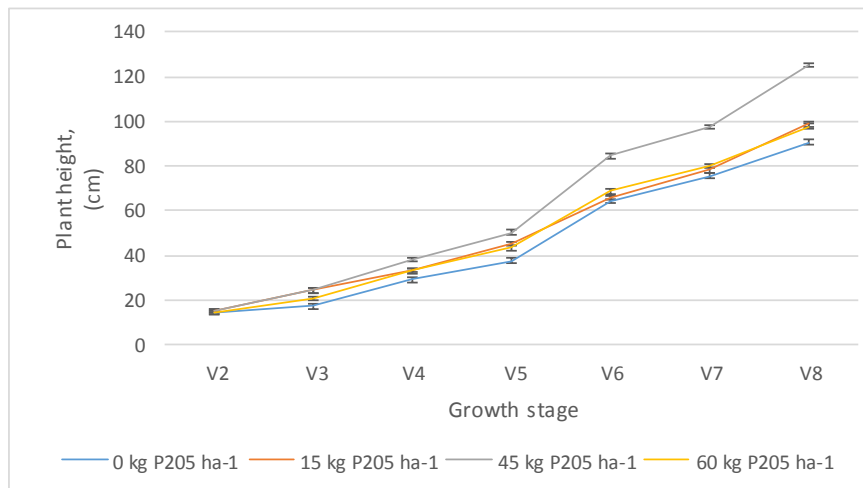


Figure 1: Plant height of sweet corn at vegetative stage 2 (V2) until tasselling stage (V8) treated with 270 ppm ethephon and four levels of P fertilizer.

Relative growth rate, chlorophyll concentration and total phenolic content

The relative growth rate of height (RGR^h), SPAD reading, chlorophyll concentration and total phenolic content (TPC) of sweet corn treated with ethephon at different levels of P are presented in Table 2. There were significant differences of RGR^h among treatments at $P \leq 0.05$ tested with LSD for mean comparison. The highest RGR^h was from sweet corn treated with ethephon at 45 kg P₂O₅ ha⁻¹ with 2.93 cm day⁻¹ growth rate. However, there were no significant differences at $P \geq 0.05$ among treatments for SPAD value, chlorophyll concentration and TPC in leaves samples recorded during the experiment. The results showed that application of P fertilizer with ethephon at the early growth stage might improve plant growth without occurrence of stress symptom to the plant. However, Iqbal et al. (2011) reported that ethephon application at 200 $\mu\text{l l}^{-1}$ with optimal nitrogen of 80 mg N kg⁻¹ soil showed greater growth including increase in photosynthesis of mustard (*Brassica juncea* L.). Their results that contradicted with that of P application might be due to nitrogen involvement directly in the ethylene-mediated changes in ribulose 1,5-bisphosphate carboxylase (Rubisco), carboxylation efficiency and an indirect effect of ethylene on stomatal aperture interaction.

Table 2: Height relative growth rate, SPAD reading, chlorophyll concentration and total phenolic content of sweet corn at four levels of phosphorus fertilizer rate treated with 270 ppm ethephon.

Phosphorus	RGR ^h	SPAD reading	Chlorophyll _{a+b}	Total phenolic content
— kg ha ⁻¹ —	— cm day ⁻¹ —	— value —	— mg g ⁻¹ —	mg GAE/g leaf dry weight
0	2.27 b	47.06 a	94.17 a	1.84 a
15	2.53 b	50.40 a	94.88 a	1.83 a
45	2.93 a	47.27 a	93.82 a	1.71 a
60	2.48 b	52.54 a	91.02 a	1.98 a

* Means with similar alphabet in a column were significantly different at $P \leq 0.05$ of Least Significant Different (LSD)

Plant dry matter

The dry matter weight of leaf, stem and root are presented in Table 3. There were significant differences at $P \leq 0.05$ among treatments for leaf, stem, root and total dry matter weight. The

application of ethephon (270 ppm) at 45 kg P₂O₅ ha⁻¹ showed highest dry matter weight of leaf, stem and total dry weight while at 15 kg P₂O₅ ha⁻¹ showed highest root dry matter weight. Yagoob (2015) reported chickpea plant (*Cicer arietinum* L.) root dry weight was highest at 15 mg P kg⁻¹ soil compared to 5 mg P kg⁻¹ soil and 10 mg P kg⁻¹ soil and concluded that the root traits were increased with increasing P rates in the presence of Arbuscular mycorrhizal fungi. Total dry matter weight of sweet corn in this study seem to decreased at 100% standard fertilizer rates while at 75% was observed to show highest value that suggested that ethephon application would reduce 15% of sweet corn P fertilizer requirement. The results were probably caused by phosphate ion being produced during ethephon breakdown which subsequently enhanced phosphorus concentration available in soil for plant growth. Root to shoot dry weight ratio showed greater value of 0.82 at 15 kg P₂O₅ ha⁻¹ as compared with other levels of P fertilizer. Formation of root greater than shoot in this study might indicate that under limited soil P condition, plants developed more roots to absorb nutrients.

Table 3: Dry matter weight of leaf, stem and root of sweet corn at different levels of phosphorus treated with 270 ppm ethephon.

Phosphorus — kg ha ⁻¹ —	Dry matter weight				Root : Shoot ratio
	Leaf	Stem	Root	Total	
	g				
0	29.31 c	58.67 b	17.77 b	105.74 c	0.61 b
15	37.65 b	85.58 a	30.70 a	153.92 b	0.82 a
45	52.35 a	96.56 a	29.28 a	178.19 a	0.56 c
60	48.18 a	94.70 a	24.01 ab	166.88 a	0.50 c

* Means with similar alphabet in a column were significantly different at $P \leq 0.05$ of Least Significant Different (LSD)

Conclusion

Plant height and dry matter content of sweet corn from vegetative stages until tasseling stage showed significant differences among treatments except for SPAD reading, chlorophyll concentration and total phenolic content. It depicted that at 45 kg P₂O₅ ha⁻¹ with ethephon application consequently affect growth performance as compared with recommended fertilizer rate of 60 kg P₂O₅ ha⁻¹. Thus, application of ethephon would be useful in managing marginal soils that are deficient in soil P with extra benefit of reduction of fertilizer requirement and fertilizer cost of sweet corn cultivation.

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Effects of Seed Priming Durations and Potassium Nitrate Concentrations on Germination of Eksotika Papaya

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Introduction

Germination of papaya seed is usually slow and erratic due to inhibition effects of phenols in the sarcotesta layer of its seed. These phenolic compounds accumulated in esclerotesta and sarcotesta layer but negligible amount presence in the embryo and endosperm. Nonetheless, seed dormancy was observed even with removal of sarcotesta layer (Tokuhisa, 2007). Poor seed storage and handling are identified to influence seed emergence and vigour, causing total failure of seed to germinate (Ibrahim et al., 2011). Attempts have been made to increase seed germination by pre-treatment at low temperature (Yahiro, 1979), gibberellic acid (Anburani and Shakila, 2010), drying (Chan and Tan, 1990) and potassium priming (Owino and Ouma, 2011).

Seed priming technique comprises of hydro-priming, osmopriming, halo priming and hormonal priming (Nawaz et al., 2013). Seed priming was able to reduce seed emergence time and increase emergence synchronicity (Parera and Cantliffe, 1994). Use of inorganic salts such as sodium chloride (NaCl), calcium chloride (CaCl₂) and calcium sulphate (CaSO₄) as soaking solutions is considered as halo priming (Parera and Cantliffe, 1994). Halo priming of papaya seed var. Kenya using potassium chloride (KCl), potassium hydroxide (KOH), potassium nitrate (KNO₃) and potassium sulphate (K₂SO₄) increased seed germination significantly (Owino and Ouma, 2011). This practice effectively improved seed germination of *Capsicum annuum*, *Oryza sativa*, *Triticum aestivum*, *Sorghum bicolor* and *Cajanus cajan* (Nawaz et al., 2013).

Many studies on halo priming are using specific concentration of KNO₃ with specific priming duration. Owino and Ouma (2011) conducted the experiment with 0.04 mol of potassium ion with 30 min of priming duration, while Sehrawat et al. (2010) performed priming treatment on papaya seed with 0.5 M and 1.0 M of KNO₃ in 24 hours of priming duration. Zanotti et al. (2014) experimented “Solo” papaya seeds with 1.0 M of KNO₃ in one hour priming duration, giving high percentage of abnormal seedlings probably due to high concentration of KNO₃. However, the treatment increased emergence speed index in “Solo” papaya seeds. Thus, the objective of this study was to evaluate the effects of various concentrations of KNO₃ and priming durations on papaya seed germination and vigour.

Materials and Methods

Seed source

The seeds used in the study were obtained from hermaphrodite papaya fruit cv. eksotika obtained from the Genebank and Seed Centre, MARDI, Pontian, Johor, Malaysia. The fruit was harvested at maturity index 2 and kept at room temperature until the fruit reached maturity index of 5. At this stage, the fruit produces the best quality seeds (Yogeesha et al., 2013). The seeds were extracted from halve fruit by scooping out the seeds by using a spoon. In order to remove the gelatinous sarcotesta layer, seeds were washed under running tap water and rubbed gently on fine metal mesh. Floatation test was carried out to separate floated seeds where it had low viability (Hartmann et al., 2001). The

seeds were pat dried to remove excess water by using paper towel. PRADA sieve with 3.15 mm aperture was used to separate and remove the smaller seeds.

Priming treatment

The washed seeds were divided equally into 17 lots and each lot was treated differently. One seed lot was used as control treated with fungicide for 5 minutes. The seeds were treated with 0.0125 M, 0.0250 M, 0.050 M or 0.10 M of KNO₃ solution and primed at 30 min, 1 hr, 2 hr or 4 hr of priming duration. All treatments were added with fungicide (a.i. benomyl) at 2 g per liter solution. The seeds were pat dried again using paper towel to remove excess solution and air-dried at room temperature (75±10% R.H.) until it reached moisture content of 8-12% (Yogeesha et al., 2013). Moisture content of the seed was determined with a Moisture Analyzer MX-50 (A&D Company Limited, Japan).

Germination tests

Germination tests were conducted immediately after the seed reached suitable moisture content of 8-12%. The test was conducted with four replicates of 25 seeds each for a total of 100 seeds per treatments. Seeds were placed on paper towel saturated with distilled water and germinated at 28±2 °C under a 12-hour photoperiod (Salomao and Mundim, 2000). Seed germination was recorded daily for 14 days where only radicle protrusion of length more than 1.0 cm will be the criterion of successful germination. Germination percentage (GP) was calculated based on germination percentage on Day 14. The experiment was carried out in Completely Randomized Design (CRD) with four replicates. The results were expressed as germination percentage (GP), mean germination time (MGT), time to 50% germination (T₅₀) and germination index (GI).

The mean germination time (MGT) was calculated based on the equation of Ellis and Roberts (1981) modified by Moradi et al. (2008).

$$MGT = \frac{\sum Dn}{\sum n}$$

Where n is the number of seed which germinated on day D, while D is the number of days counted from the beginning of germination (Moradi et al., 2008). The time to 50% germination (T₅₀) was calculated according to the formula of Coolbear et al. (1984) as modified by Farooq et al. (2005).

$$T_{50} = t_i + \frac{[(N/2) - n_i][t_i - t_j]}{n_i - n_j}$$

Where N is the final number of germination and n_i, n_j are cumulative number of seeds germinated by adjacent counts at times t_i and t_j when n_i < N/2 < n_j (Moradi et al., 2008). The germination index (GI) was calculated as described by the Association of Official Seed Analysis (AOSA, 1983).

$$GI = \frac{\text{No of germinated seed}}{\text{Days of first count}} + \dots + \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

Statistical analysis

Data collected was analysed with the statistical package SAS (Statistical Analysis System) through the analysis of variance (ANOVA) and the treatment means were compared using Least Significant Difference (LSD) at 0.05 probabilities.

Results and Discussions

The effects of KNO₃ concentration and priming duration treatment were evaluated by analysing the germination data collected over 14 days of experiment (Table 1). The T₅₀, MGT and GI are highly significant ($P < 0.001$) for KNO₃ concentration but not significant for priming duration.

Table 1: Analysis of variance for papaya seed germination as influenced by KNO₃ concentration and priming duration.

Sources of variation	df	GP (%)	T ₅₀ (d)	MGT (d)	GI
KNO ₃ concentration	3	1391.30***	6.85***	5.94***	120.0***
Priming Duration	3	83.67 ^{ns}	0.37 ^{ns}	0.098 ^{ns}	5.50 ^{ns}
Error		41.0	0.33	0.25	3.62

^{ns} Not significant; *** Significantly different at $p < 0.001$; GP = germination percentage; T₅₀ = time to 50% germination; MGT = mean germination time; GI = germination index

Seed germination

Different priming concentration and priming duration of KNO₃ did affects the germination percentage compared to untreated seed. The highest germination percentage, 85% achieved from combination treatment of 0.0250 M of KNO₃ with 1 hr of priming (Table 2). Means comparison of different KNO₃ concentration and priming duration revealed 0.0250 M of KNO₃ and 2 hr of priming duration achieved the highest germination percentage of 80% and 78.5%, respectively (Figure 1). Many reported findings supported the improvement of seed germination percentage by priming treatment. Papaya seed cv. Kapoho Solo had more than 50% seedling emergence when the seed was soaked with 1.0 M of KNO₃ for 15 minutes (Furutani et al., 1993). Potassium priming treatments especially KNO₃ and K₂SO₄ increased the germination percentage to 86% compared to control 66.7% when the seed was treated with 0.04 mol of potassium ion for 30 minutes (Owino and Ouma, 2011). Papaya seed primed with 0.5 M and 1.0 M of KNO₃ had higher germination percentage even the seed undergone accelerated aging conditions (Sehrawat et al., 2010). According to Nawaz et al., (2013), priming with inorganic salts increased activity of most enzymes involved in seed germination in mobilizing organics substances to different parts of the embryo. The presence of KNO₃ helps the germination process therefore increase the germination percentage.

Seed germination rate

Priming treatment significantly affects the germination rate of papaya seed. The highest germination rate indicated by lowest value of mean germination time, MGT and time for 50% germination, T₅₀ were accomplished with combination of 0.10 M KNO₃ and 1 hr of priming with value of 6.55 D and 7.28 D respectively (Table 2). Means comparison of different KNO₃ concentrations and priming durations for T₅₀ revealed 0.0250 M of KNO₃ and 1 hr of priming duration achieved the lowest values of 6.97 D and 6.85 D, respectively, while for MGT revealed 0.10 M of KNO₃ and 1 hr of priming duration achieved the lowest MGT values of 7.63 D and 7.59 D, respectively (Figure 2). Zanotti et al. (2014) observed the same trend for KNO₃ treated “Solo” papaya seed where the radicle protrusion speed rate was higher compared to control even the seed was harvested at different maturity stage.

Seed vigour

Seed vigour can be described in term of GI which is an adaptation of the daily counting method to evaluate the seedling vigour which represented seed vigour more precisely. The GI predicted the relative vigour of samples with the same amount of seeds. High value of GI signified higher seedling vigour of one sample in relation to another (Ranal and Santana, 2006). Priming treatment influenced the GI value significantly where the highest value of GI, 15.78 attained from the combination of 0.0250 M and 1 hr priming of KNO₃ (Table 2). Means comparison of different KNO₃ concentration

and priming duration for GI revealed 0.0250 M of KNO_3 and 2 hr of priming duration achieved the highest GI value of 14.59 and 14.36, respectively (Figure 3). Increase in seed vigour usually related to the activation of enzymes crucial for germination process. Halo priming with KNO_3 solution for *S. bicolour*, *P. americanum* and *O. sativa* seeds activated α -amylase, p-amylase and proteases activity significantly (Kadiri and Hussaini, 1999; Chang-Zheng et al., 2002). In seed germination especially cereal type, α -amylase is crucial to hydrolyze the starch contained in endosperm into metabolize sugars as energy for roots and shoots growth (Kaneko et al., 2002).

Table 2: Effects of seed priming duration and KNO_3 concentration on germination of eksotika papaya seed.

Treatment	GP (%)	T_{50} (d)	MGT (d)	GI
Control	56 ^d	8.50 ^a	9.08 ^a	7.80 ^c
0.0125 M 0.5 hr	70 ^{bc}	7.10 ^{bc}	7.80 ^b	12.50 ^{ab}
0.0125 M 1 hr	83 ^{ab}	7.20 ^{bc}	7.93 ^b	14.40 ^{ab}
0.0125 M 2 hr	80 ^{abc}	6.83 ^{bc}	7.43 ^b	15.28 ^{ab}
0.0125 M 4 hr	74 ^{abc}	7.80 ^{ab}	8.08 ^b	12.60 ^{ab}
0.0250 M 0.5 hr	74 ^{abc}	7.33 ^{bc}	7.95 ^b	12.88 ^{ab}
0.0250 M 1 hr	85 ^a	6.80 ^{bc}	7.60 ^b	15.78 ^a
0.0250 M 2 hr	84 ^{ab}	6.93 ^{bc}	7.60 ^b	15.55 ^{ab}
0.0250 M 4 hr	77 ^{abc}	6.88 ^{bc}	7.78 ^b	14.10 ^{ab}
0.050 M 0.5 hr	71 ^{abc}	7.18 ^{bc}	7.80 ^b	12.63 ^{ab}
0.050 M 1 hr	68 ^c	6.88 ^{bc}	7.58 ^b	12.70 ^{ab}
0.050 M 2 hr	80 ^{abc}	6.90 ^{bc}	7.68 ^b	14.63 ^{ab}
0.050 M 4 hr	71 ^{abc}	7.05 ^{bc}	7.60 ^b	13.08 ^{ab}
0.100 M 0.5 hr	79 ^{abc}	6.95 ^{bc}	7.63 ^b	14.48 ^{ab}
0.100 M 1 hr	72 ^{abc}	6.55 ^c	7.28 ^b	14.10 ^{ab}
0.100 M 2 hr	70 ^{bc}	7.55 ^{abc}	8.13 ^b	11.95 ^b
0.100 M 4 hr	76 ^{abc}	7.08 ^{bc}	7.53 ^b	14.40 ^{ab}
LSD at 0.05	11.8	0.93	0.79	3.12

Means with the same letter(s) in the column do not differ at $p < 0.05$; GP = germination percentage; T_{50} = time to 50% germination; MGT = mean germination time; GI = germination index

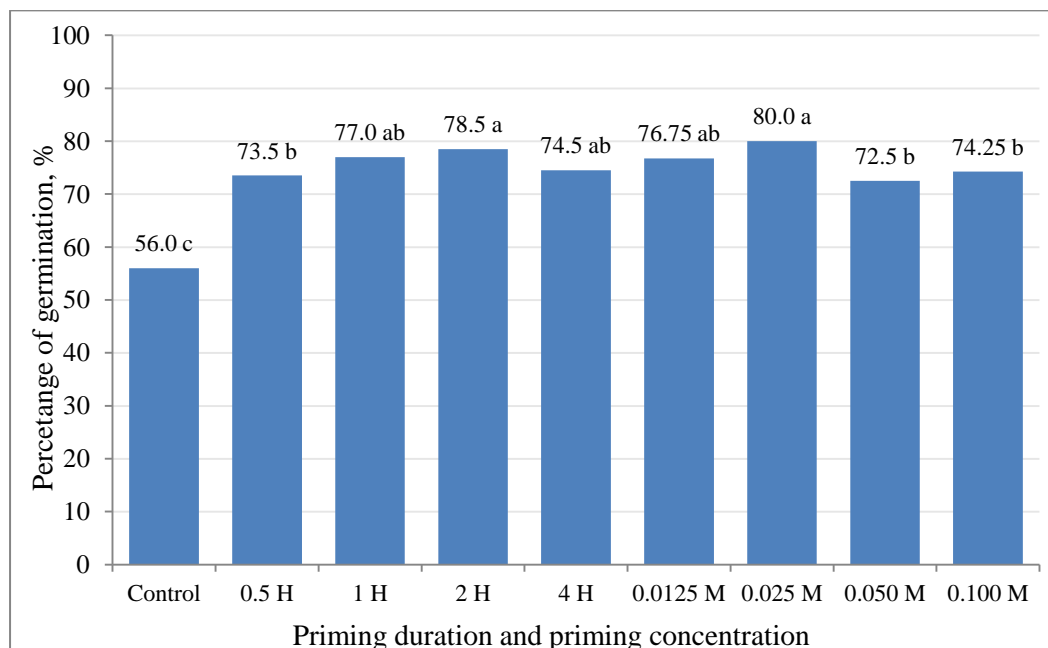


Figure 1: Germination percentage of papaya seed as influenced by KNO_3 concentration and priming duration.

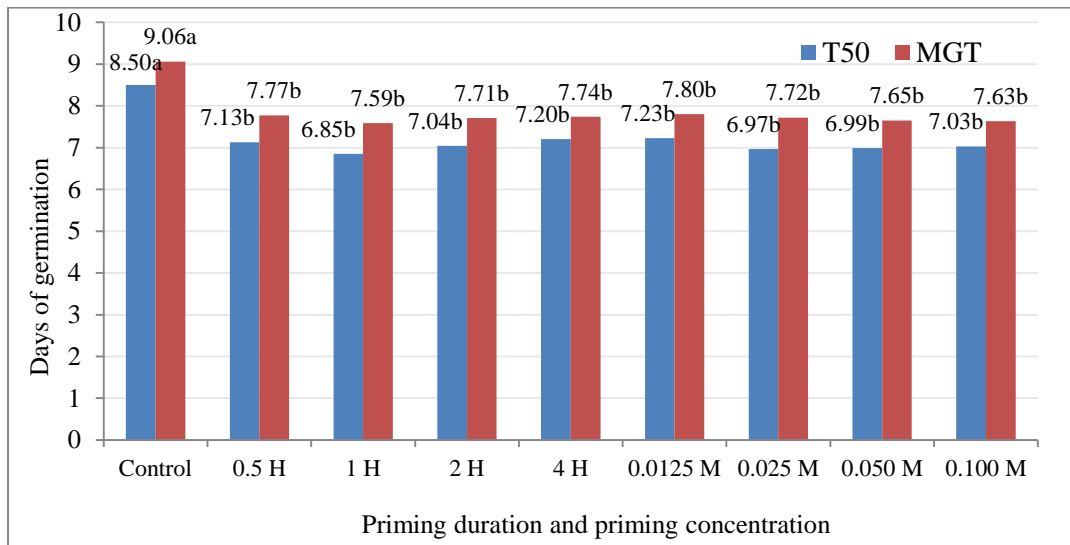


Figure 2: Germination rate of papaya seed as influenced by KNO_3 concentration and priming duration. T_{50} = time for 50% germination; MGT = mean germination time.

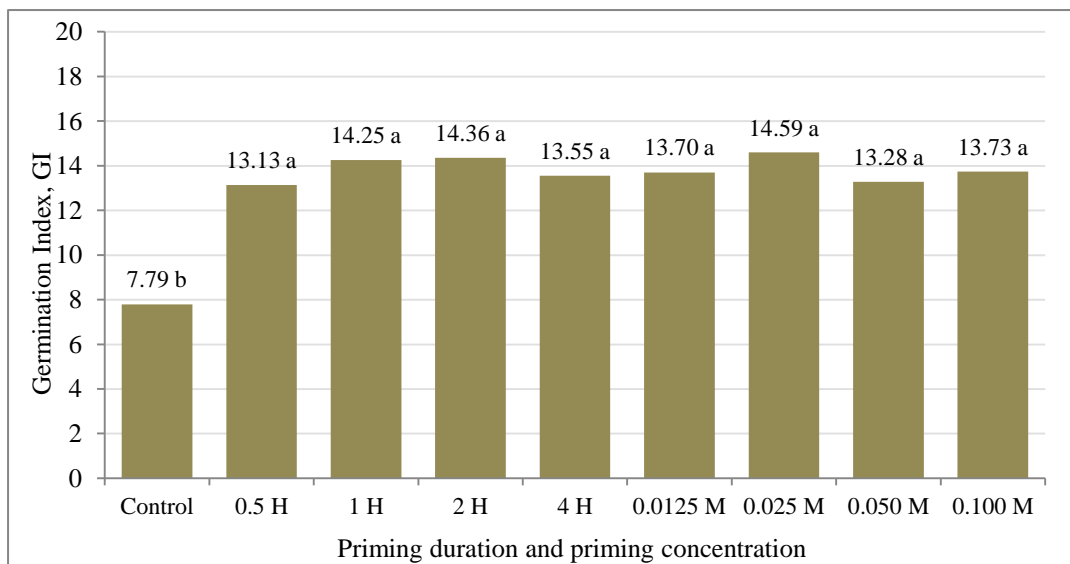


Figure 3: Germination index of papaya seed as influenced by KNO_3 concentration and priming duration.

Conclusion

Priming treatment affected seed germination percentage, germination rate and seed vigour positively. Treatment combination of 0.025 M KNO_3 with one hour of priming duration is sufficient to enhance papaya seed germination and vigour.

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A Note on the Survival of *Aquilaria malaccensis* and Other Tree Species in an Enrichment Planting in Greened Slime Tailings in the Peninsular Malaysia

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Introduction

Forest Research Institute Malaysia has developed rehabilitation technologies for the greening ex-tin mine (Ang et al., 2006). The greened site has been a model for rehabilitation of harsh sites in Malaysia (Ang, 2012). ASEAN Korea Environmental Cooperation Project (AKECOP) has been playing necessary support to the planting and tending of the trees grown in FRIM's research station. In Phase II of AKECOP and also with the financial support from ASEAN-Korean Forest Cooperation (AFoCo), we observed poor regeneration on greened slime tailings site comprising mixed planting of *Acacia mangium*, *Acacia auriculiformis*, *Hopea odorata* and *Khaya ivorensis*. Woody tree and shrub species that are dispersed by avian dispersal agents were found under the mixed stands but the isolated greened ex-tin mine remained lack of primary forest species that produce fruits, which are not edible by birds or bats. An enrichment planting using indigenous rainforest tree species that produce seeds that are not dispersed by avian group was carried out in Tin Tailings Afforestation Centre in Bidor. This is part of the activities to fulfill the blue print of the TTAC (Zol et al., 2012). The objective of this study is to determine the survival and growth performance of selected indigenous rainforest species

Materials and Methods

A 1.5 ha of slime tailings located in Tin Tailings Afforestation Centre (TTAC) had been enriched with 20 indigenous tree species. The site was loosened to 1 m depth and applied with ashes of 1 kg per planting point.

The seedlings of the 20 selected indigenous rainforest species were acclimatized in the TTAC for a period of three months. They have an average height of 45-50 cm and collar diameter of < 1 cm. They were planted during the wet season in December 2011. The species planted in the enrichment program are listed in Table 1. Tending practices including line weeding followed by circle weeding of 50 cm radius were carried out for each planting point bi-monthly, and then with application of mixture of organic fertilizer of 80% chicken manure with 10 g NPK (15:15:15) at three month intervals.

Table 1: Species planted in the greened slime tailings.

Non-dipterocarps	Dipterocarps
<i>Garcinia homobronia</i>	<i>Shorea parvifolia</i>
<i>Melaleuca cajuputi</i>	<i>Shorea leprosula</i>
<i>Sindora coriacea</i>	<i>Shorea curtisii</i>
<i>Careya arborea</i>	<i>Dryobalanops aromatica</i>
<i>Cananga odorata</i>	<i>Hopea pubescens</i>
<i>Agathis borneensis</i>	<i>Neobalanocarpus heimii</i>
<i>Streblus elongatus</i>	<i>Shorea roxburghii</i>
<i>Palaquium gutta</i>	<i>Shorea platyclados</i>
<i>Aquilaria malaccensis</i>	<i>Shorea ovalis</i>
<i>Pentaspadon motleyi</i>	<i>Shorea macroptera</i>

Results and Discussion

Effects of ash treatment

Survival count, total length and collar diameter were carried out in June 2012, and May 2013. Only survival is presented. The survival of the enrichment species is reduced from an average of 91.8% to 59.3% from 2012 to 2015 (Table 2). The effects of ash treatment did not significantly affect the survival of the rainforest species. Mortality of the planting was due to destruction of seedlings by wild boars.

Table 2: Survival of tropical rainforest species seedlings grown on greened slime tailings.

Treatment	Slime Site	Planted seedlings	Survival (%) (Jun 2012)	Survival (%) (May 2013)	Survival (%) (Jul 2014)	Survival (%) (Jul 2015)
Control	Jln Biodiversiti	360	97.78	73.33	62.78	50.00
	Jln Pasir	360	98.89	76.94	61.67	57.78
	Total	720	98.33	75.14	62.22	53.89
Ash	Jln Biodiversiti	360	91.11	74.44	70.00	58.61
	Jln Pasir	360	92.50	75.28	67.75	60.00
	Total	720	91.81	74.86	68.89	59.31

Seedlings of ash treatment were planted in Nov 2011 and replanted in May 2012. Seedlings of control treatment were planted in May 2012.

Species performance

Generally, dipterocarp species had higher survival count than non-dipterocarps grown on the greened slime tailings (Table 3). *Shorea roxburghii* had survival more than 80%. This species is known to adapt well in open conditions especially in the enrichment planting program and also park planting. *Shorea curtisii*, *S. platyclados* and *S. parvifolia* had the lowest survival range as they require more shaded environment.

Table 3: Effects of ash treatments on survival (%) of rainforest species grown on the greened slime tailings (2015).

Species	Control			Ash		
	Jln Biod (%)	Jln Pasir (%)	Mean (%)	Jln Biod (%)	Jln Pasir (%)	Mean (%)
Dipterocarps						
<i>Dryobalanops beccarii</i>	61.11	77.78	69.44	77.78	72.22	75.00
<i>Hopea pubescens</i>	50.00	72.22	61.11	50.00	55.56	52.78
<i>Neobalanocarpus heimii</i>	72.22	72.22	72.22	66.67	83.33	75.00
<i>Shorea curtisii</i>	33.33	38.89	36.11	77.78	61.11	69.44
<i>Shorea leprosula</i>	55.56	77.78	66.67	61.11	66.67	63.89
<i>Shorea macroptera</i>	55.56	61.11	58.33	55.56	50.00	52.78
<i>Shorea ovalis</i>	55.56	50.00	52.78	77.78	72.22	75.00
<i>Shorea parvifolia</i>	66.67	77.78	72.22	55.56	72.22	63.89
<i>Shorea platyclados</i>	38.89	55.56	47.22	77.78	66.67	72.22
<i>Shorea roxburghii</i>	72.22	88.89	80.56	94.44	100	97.22
Non-dipterocarps						
<i>Agathis borneensis</i>	55.56	44.44	50.00	44.44	55.56	50.00
<i>Aquilaria malaccensis</i>	83.33	61.11	72.22	33.33	50.00	41.67
<i>Cananga odorata</i>	5.56	38.89	22.22	27.78	44.44	36.11
<i>Careya arborea</i>	55.56	33.33	44.44	44.44	44.44	44.44
<i>Garcinia homobroniana</i>	44.44	61.11	52.78	100	50.00	75.00
<i>Melaleuca cajuputi</i>	5.56	0.00	2.78	0.00	16.67	8.33
<i>Palaquium gutta</i>	50.00	44.44	47.22	22.22	61.11	41.67
<i>Pentaspadon motleyi</i>	83.33	83.33	83.33	100.00	88.89	94.44
<i>Sindora coriacea</i>	44.44	77.78	61.11	66.67	55.56	61.11
<i>Streblus elongatus</i>	11.11	38.89	25.00	38.89	33.33	36.11

For the non-dipterocarps planted under the *Hopea odorata* stand, *Pentaspadon motleyi* had the highest survival, and the lower survival group of species comprising *Cananga odorata*, *Melaleuca cajuputi* and *Streblus elongatus*.

Conclusion

The early survival of the planting shows promising results for some climax rainforest tree species but tending program must be continued. Method to prevent further damage of wild boars or feral pigs to the seedlings is being developed.

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Effects of Arbuscular Mycorrhiza on Growth and Postharvest Performances of Hempedu Bumi (*Andrographis paniculata* Nees) Grown on BRIS Soil

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Introduction

In Malaysia, the herbal industry has become a new source of wealth due to the increased demand in herbal supplements, health functional food, herb-based energy drinks and skin cares. Currently, there are 11 types of herbs identified as highly potential to be commercialized which include tongkat ali, kacip fatimah, misai kucing, dukung anak, hempedu bumi, rosel, pegaga, mengkudu, ginger (MOA, 2015) and belalai gajah (Ramlan et al., 2015). Among these top 11 herbs, hempedu bumi (*Andrographis paniculata* Nees) is on the rise for consumers' demand for health care product, botanical drug, herbal remedies, pharmaceuticals, nutraceuticals, food and beverages.

Andrographis paniculata, also known as king of bitter, sambiloto, taikila or kalmegh, and is classified under family Acanthaceae, is widely cultivated in southern Asian including Malaysia, Indonesia, Thailand and India. In order to maintain a constant supply of *A. paniculata* to various industries as mentioned above, the growth and postharvest performances of this herb were investigated. In Terengganu, the cultivation of *A. paniculata* as a commercial crop is not yet developed, which might be due to the occurrence of infertile Beach Ridges Interspersed with Swales (BRIS) soil. This soil has more than 98% sand, excessive drainage, high surface soil temperature, low moisture and nutrient content (Wan Rasidah et al., 2010). It covers 67,582.61 ha in Terengganu (Mohd Ekhwan and Mazlin, 2009). In addition, Affendy (2010) claimed that this sandy area is not suitable for cultivation due to afore mentioned problems. Therefore, this study was aimed at evaluating the effectiveness of different species and rates of arbuscular mycorrhizal (AM) in improving the growth and quality of *A. paniculata* planted on BRIS soil. The suitable species specifically for *A. paniculata* was also investigated. To the best of the current knowledge, the information on pre- and postharvest performance of this local herb planted on BRIS soil is scarce. The application of AM is believed to increase the growth performance of the plant due to its symbiosis characteristics with the plant roots. It is well documented that AM plays a key role in enhancing growth performance of various crops. Besides, the application of AM could also contribute to the short planting period, higher yield and quality of this local herb.

Materials and Methods

A total of 36 *A. paniculata* plants were grown in this experiment. The experiment was conducted in a greenhouse at the Universiti Malaysia Terengganu with average temperature of 35 – 45 °C. Five-node stem cuttings were transferred into polybag containing sterilized (90 - 110 °C for 8 hours) BRIS soil collected from Stesyen Pembangunan Komoditi Rhu Tapai, Terengganu. The experiment was arranged in a randomized complete block design (RCBD) with three replicates. Single plant represented one experimental unit. The treatments included Control (0 g of *Glomus* sp.), 10 g *Glomus* sp., 50 g *Glomus* sp., 0 g *Scutellospora* sp., 50 g *Scutellospora* sp., 0 g *Gigaspora* sp., 10 g *Gigaspora* sp., 50 g *Gigaspora* sp., 0 g mixed sp., 10 g mixed sp., and 50 g mixed sp. All plants experienced similar cultural practices such as irrigation, fertilization, weed, pest and disease control.

The experimental period was 77 days (September 29, 2015 – November 26, 2015). The preharvest parameter such as stem diameter was recorded weekly at 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and 77 days after transplanting (DAT) while spore count and root infection were measured at day 14, 42 and 70 DAT. Meanwhile, postharvest parameters assessed at harvest were fresh and dry weight of leaves and stems, leaf area, chlorophyll content and total phenolic content of *A. paniculata* leaves. The data were subjected to two-way analysis of variance (ANOVA) using General Linear Models (GLM) procedures and further separated by Tukey for minimum significant difference at $P \leq 0.05$ (SAS Institute Inc., 1991).

Results and Discussion

In the present study, different species and rates of AM had a significant interaction in increasing the number of branches, spores and percentage of root infection of *A. paniculata* (Figures 1, 2 and 3). Inoculation of 50 g *Gigaspora* sp. on *A. paniculata* showed the highest number of branches on day 28 and 70 (19 and 132, respectively) as compared to non-inoculated *A. paniculata* (16 and 66, respectively) (Figure 1). This finding was in agreement with Dhritiman et al. (2014) who claimed that AM-inoculated plants showed a significant increase in the growth and production of sickle pod, peacock flower, and Egyptian mimosa over non AM-inoculated plants. Meanwhile, Shinde and Vaidya (2014) claimed that higher number of branches led to higher number of leaves produced. This is mainly caused by enhanced cytokinin formation of the plant which leads to more cell division (Ramkrishana and Selvakumar, 2012; Shinde and Vaidya, 2014). On 42 DAT, the highest spore number was identified in soil with 50 g mixed species followed by 10 g mixed species and 10 g *Gigaspora* sp. (160, 86 and 82 spores, respectively) (Figure 2). Later on 70 DAT, the highest spore number was recorded in soil treated with 10 g mixed species followed by 50 g mixed species and 50 g *Scutellospora* sp. (281, 264 and 263 spores, respectively).

For root infection, 91.6% *A. paniculata* roots were infected with 10 g mixed species followed by 50 g mixed species, *Scutellospora* sp. and *Glomus* sp. (85%, 83% and 83%, respectively) on 70 DAT (Figure 3). Similarly, Nur Amirah et al. (2013) reported that high percentage of root infection was recorded in misai kucing (*O. stamineus*) inoculated with mixed species of AM fungi than non AM plants. Meanwhile, Mohammad Saharkhiz et al. (2011) claimed that *O. sanctum* inoculated with combination of *G. mossae* and *G. versiforme* had higher root colonization, chlorophyll content and flower stem length than non-inoculated. It was presumed that AM fungi could have allowed more active translocation of minerals along the extrametrical hyphae which resulted in more effective fungal exploitation on volume of soil (Rhodes and Gerdemann, 1975).

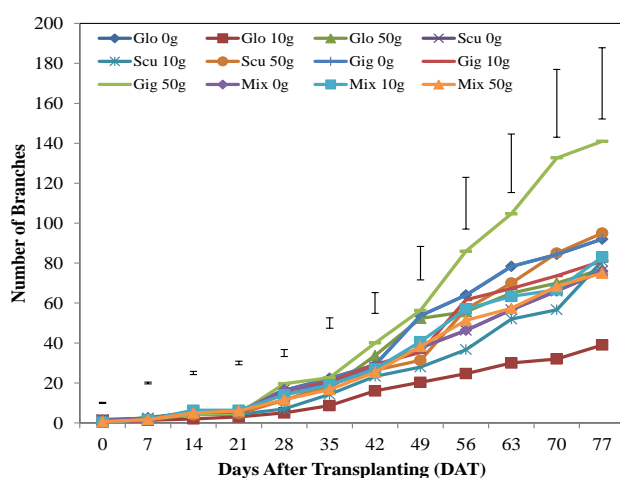


Figure 1: Effects of different species and rates of AM on number of *A. paniculata* branches. Vertical bars represent HSD values at 5% significance level.

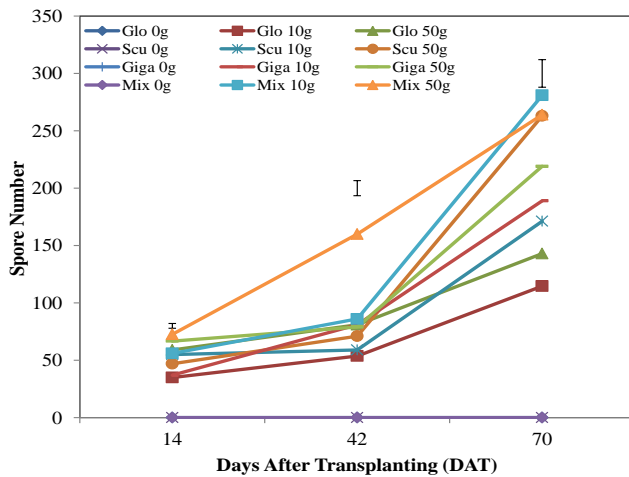


Figure 2: Effects of different species and rates of AM on spore number of *A. paniculata*. Vertical bars represent HSD values at 5% significance level.

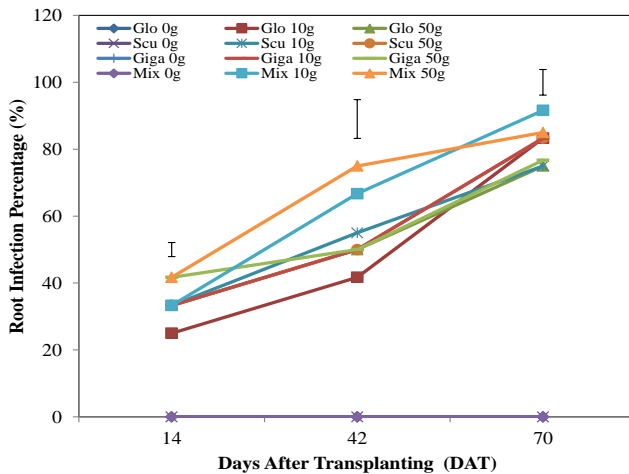


Figure 3: Effects of different species and rates of AM on percentage of root infection on *A. paniculata*. Vertical bars represent HSD values at 5% significance level.

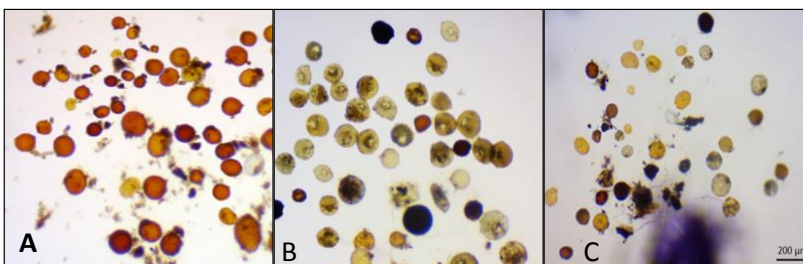


Figure 4: Arbuscular mycorrhiza spore: A) *Glomus* sp., B) *Scutellospora* sp. and C) mixed species.

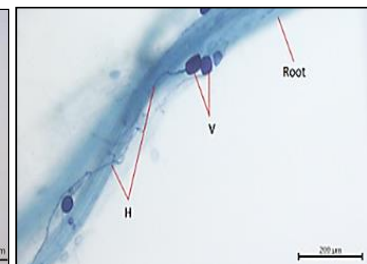


Figure 5: Arbuscular mycorrhiza fungi infection on root of *A. paniculata* showing (H) hyphae and (V) vesicle.

No significant interaction was observed between two factors for stem diameter, leaf fresh and dry weight, cumulative fresh weight, leaf area, total chlorophylls and total phenolic content (Figures 6, 7 and Table 1). Leaf fresh and dry weight, leaf area and total chlorophyll content were, respectively, statistically similar with the application of different AM species and rates (Table 1). For stem diameter, irrespective of AM species, diameter size of *A. paniculata* was only significant on day 77 by

the inoculation of different rates of AM. Plants inoculated with 50 g AM showed the biggest stem diameter (7.55 mm) as compared to 10 g AM inoculated plants (7.00 mm). The different AM species resulted in insignificant stem diameter growth of *A. paniculata*, i.e. 7.42 mm with mixed sp., 7.14 mm with *Gigaspora* sp., 7.12 mm with *Glomus* sp. and 6.78 mm with *Scutellospora* sp. Similarly, different AM species did not enhance the stem diameter of ironwood (*Libidibia ferrea*) as reported by Silva et al. (2014). In contrast, Pagano et al. (2010) found that AM inoculation stimulated diameter growth of candeia (*Eremanthus incanus*) as compared to non-innoculated plants. Similarly, Wu and Xia (2004) claimed that inoculation of *Glomus mossae* on trifoliate orange increased the plant height and stem diameter. The sporadic outcomes might be associated to the availability of soil phosphorus (P) that limits AM colonisation (Ryan and Graham, 2002), thereby affects the plant growth.

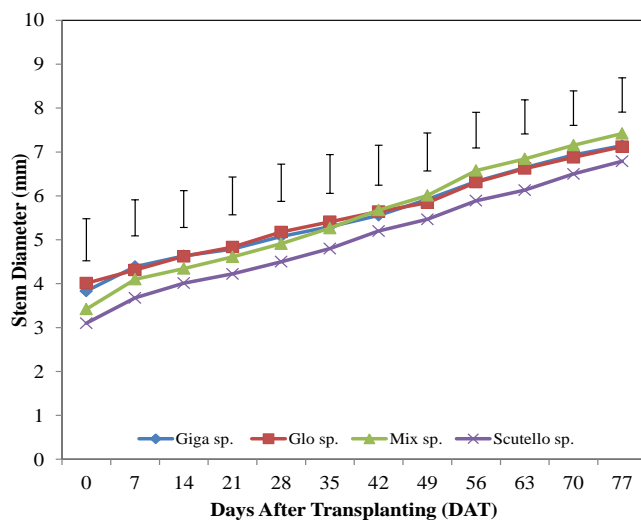


Figure 6: Effects of different rates of AM on stem diameter of *A. paniculata*. Vertical bars represent HSD values at 5% significance level.

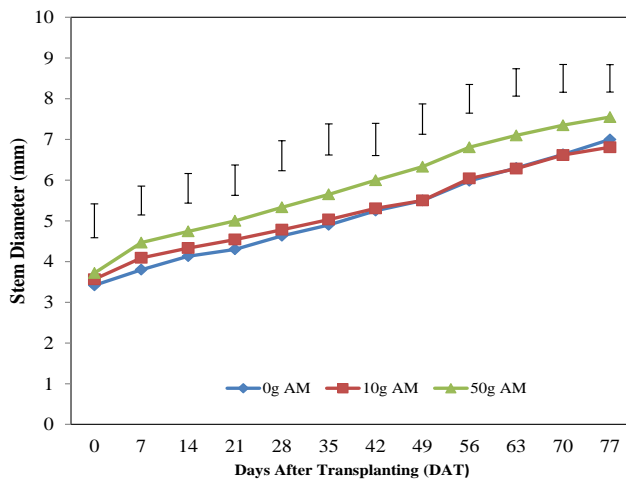


Figure 7: Effects of different species of AM on stem diameter of *A. paniculata*. Vertical bars represent HSD values at 5% significance level.

Meanwhile, cumulative fresh weight of *A. paniculata* increased with the increased of AM level (Table 1). AM at 50 g had the highest cumulative fresh weight (25.8 g) as compared to control plants (5.8 g). However, different species of AM were not noticeable in increasing cumulative fresh weight of *A. paniculata*. Irrespective of AM species, *A. paniculata* inoculated with 10 g AM resulted in higher amount of total phenolic content than control plants (Table 1). Similarly, Engel et al. (2016) found mycorrhizal influence on the secondary metabolites of three medicinal plants. The possible reasons of

changes in phenolic content induced by mycorrhization might be associated to mineral nutrient uptake (Toussaint et al., 2007). Besides, AM fungi-induced changes in phytohormone level in host plants could also be the background of this phenomenon (Toussaint et al., 2007). In addition, the sporadic outcomes of phenolic acid content in AM plants might also be due to the polyphenols that act as signaling and/or regulatory compounds in plant-microbe symbioses (Larose et al., 2002; Mandal et al., 2010).

Table 1: Effects of three rates and four species of arbuscular mycorrhiza on fresh and dry weight, cumulative fresh weight, leaf area, total chlorophyll and total phenolic content of *A. paniculata* grown on BRIS soil.

Factor	Leaf fresh weight (g)	Leaf dry weight (g)	Cumulative leaves fresh weight (g)	Leaf area (cm ²)	Total chlorophylls	Total phenolic
AM rates (R)						
0	4.77 a	1.56 a	14.85 b	30.32 a	0.95 a	0.05 b
10	6.28 a	2.22 a	22.70 a	46.74 a	0.88 a	0.16 a
50	6.37 a	2.05 a	22.79 a	47.08 a	1.02 a	0.06ab
F-test significance	NS	NS	*	NS	NS	*
AM species (S)						
<i>Gigaspora</i> sp.	6.12 a	1.96 a	20.12 a	46.47 a	0.87 a	0.03 a
<i>Scutellopora</i> sp.	6.02 a	2.08 a	20.41 a	45.38 a	0.81 a	0.10 a
<i>Glomus</i> sp.	6.81 a	2.22 a	23.38 a	47.18 a	1.14 a	0.07 a
Mixed sp.	4.28 a	1.52 a	16.54 a	26.51 a	0.97 a	0.02 a
F-test significance	NS	NS	NS	NS	NS	NS
Interaction, RxS	NS	NS	NS	NS	NS	NS

NS: not significant; *: significant at $P < 0.05$. Mean separation within columns and factors followed by the same letter are not significantly different by HSD at $P \leq 0.05$.

Conclusion

Irrespective of AM species, 50 g AM was pronounced in enhancing the growth of *A. paniculata* planted on BRIS soil. However, no specific species can be deduced in improving the development of *A. paniculata* as all species had similar effect.

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Growth and Quality of Lowland Cherry Tomato (*Solanum lycopersicum* var. *cerasiforme*) as Influenced by Different Substrates of Biochar in Soilless Culture Media

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Introduction

Over the centuries, cherry tomato (*Solanum lycopersicon* var. *cerasiforme*) had become popular and important fruit vegetable among the world's communities not only because of large consumption but also high in nutritional value for health. It is well documented that chery tomato contain high concentrations of sugars, acids, vitamins, minerals, lycopene and other carotenoids (Simonne et al., 2006). Cherry tomatoes often have higher vitamin C (George et al., 2004) and lycopene (Kuti and Konuru, 2005) concentration when comparing to other varieties. Vitamin C is needed for healing wounds, and for repairing and maintaining bones and teeth. Meanwhile, lycopene is a pigment that gives the red colour of the tomato. It is also an antioxidant that can eliminate free radical cell which will reduce the risk of getting cancer. Due to its market demand and nutritional value, tomatoes are always insufficient in supply. In order to fulfil the market demand, many researches have been conducted to improve the growth, yield and quality of cherry tomato. According to Umesha et al. (2013) the controlled cultivation of tomato in soilless media has more benefits, in terms of yield, water and fertilizer use efficiency.

Cherry tomatoes are highly recommended to be planted using this method as it is easier and more likely to avoid soil-borne disease and pest. Most widely used growing medium is coco peat (CP). It is an agricultural by-product obtained after the extraction of fibre from the coconut husk. It has high ability to absorb water but lack of nutrient content (Arachchi and Somasiri, 1997; Awang et al., 2009; Ain Najwa et al., 2014). Thus, CP requires addition of mineral nutrients from fertilizer, manure, biochar. Biochar is a charcoal-like which applied to the soil, enhances soil properties and increases soil fertility (Lehmann et al., 2006). Application of biochar optimizes crop growth by improving soil properties and fertilizer-use efficiency, especially in marginal and problematic soils (Rosenani et al., 2012). Biochar is the carbon-rich product obtained when biomass such as wood, manure, or leaves is heated in a closed container with little or no available air (Nartey and Zhao, 2014). Many reports focus on the effects of biochar on crop production on soils with low nutrients (Crane-Droesch et al., 2013) and acid soils (Liu et al., 2013) and alkaline soils (Borchard et al., 2014). However, the benefits of biochar on crop productions in soilless cultivation system is scarce. Hence, this research was conducted to investigate the effect of different substrates of biochar on the growth and quality of lowland cherry tomato under soilless media system.

Materials and Methods

The experiment was conducted in a greenhouse at the School of Food Science and Technology Universiti Malaysia Terengganu. The tomato seeds (F1-Hybrid) were purchased from Leckat Corporation Sdn. Bhd. Coco peat (CP) was purchased from Bumi Agro Enterprise. Rice husks (RH) was obtained from Kilang Beras Bernas, Tumpat, Kelantan. Sugarcane bagasse (SB) and coconut shells (CS) were obtained from local markets around Kuala Terengganu. All of the agriculture wastes as mentioned above were converted into biochar by conventional pyrolysis process.

Forty two cherry tomato seeds were grown in seedling tray before being transferred into polybags filled with different treatments of soilless growing media after 3 weeks. Each polybags contain 3 kg of

growing media either alone or combinations. Fertilizers used in this study were type A and B. Irrigation has being equipped with 8 L/hr dripper and scheduled for 6-7 min/day. The experiment was laid out in a randomized complete block design (RCBD), with seven different treatments i.e. i) 2.85 kg coco peat (CP) + 150g biochar (rice husk, RH), ii) 2.85 kg CP + 150 g biochar (sugarcane bagasse, SB), iii) 2.85 kg coco peat + 150 g biochar (coconut shells, CS), iv) 2.85 kg coco peat + 75 g biochar RH + 75 g biochar SB, v) 2.85 kg coco peat + 75 g biochar RH + 75 g biochar CS, vi) 2.85 kg coco peat + 75 g biochar SB + 75 g biochar CS, vii) 50 g biochar RH + 50 g SB + 50g CS, with three replications. Each replicate was represented by two cherry tomato plants. The amount of biochar incorporation (5% from total weight of growing medium) and the control treatment (CP + RH) were based on Ain Najwa et al. (2014). All postharvest parameter assessments were assessed at the Postharvest Technology Laboratory, Universiti Malaysia Terengganu (UMT). The data collected were subjected to one-way ANOVA using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

The current price of CP is between RM 500 and RM 1000 per tonne (Sabran, 2013) and widely used as growing media in soilless cultivation system. Meanwhile, rice husk (RH), sugarcane bagasse (SB) and coconut shell (CS) is agriculture wastes that become a problem in agriculture sector. Thus, conversion of these agricultural wastes into a useful product (biochar) may overcome the current environmental pollution issue. In this study, different combinations of biochar substrates such as RH, SB and CS were used together with CP to improve quality of the growing media as well as the cherry tomato plant performances. All biochar substrates and its combinations with CP resulted in a comparable stem diameter, fresh weight and skin colour development of cherry tomato (Figure 1; Table 1). A comparable value of above attributes could be the possible reason to replace the use of CP only. Previously, Ain Najwa et al. (2014) reported that the use of CP and oil palm fruit bunch (OPFB) alone resulted in small stem diameter, low yield and poor colour development. However, the combinations of CP with biochar substrates increased the stem diameter, fruit number and fresh weight of cherry tomato (Ain Najwa et al., 2014) which might be ascribed to the changes in media structure, organic content and aeration capacity (Wira et al., 2011). In addition, Hochmuth et al. (1998) claimed that perlite and CP had a positive correlation in increasing the yield components of strawberry. The sporadic outcomes might be associated to different physical characteristics of biochar; texture, particle size, surface area and water holding capacity which can vary with different substrates and pyrolysis temperature (Zhang et al., 2013). Thus, chemical properties and nutrient compositions of the biochar can vary greatly with different substrates. However, all the above biochar substrates are suitable to be used and commercialised as an excellent growing media amendment. In addition, among these three biochar substrates, RH is the most widely studied and used as soil improvement.

Fruit colour parameters such as lightness (L^*), chromaticity value a^* and b^* , hue angle (h°) and chroma (C^*) exhibited no significant influence from different biochar substrates applied. Each colour attribute showed a comparable value except for h° . CS treated plants had the highest value of h° ($37.07 h^\circ$) as compared to other treatments. As reported by Ain Najwa et al. (2014), a reduction in h° , L^* , chromaticity value b^* and higher value a^* indicate more saturated red on fruit skin colour. Cherry tomato grown on CS showed significantly high h° which leads to a less saturated red colour of the skin. In contrast, fruit colour development in cherry tomato was enhanced with the application CP and biochar as reported by Ain Najwa et al. (2014). Similarly, Luoto (1984) also claimed that tomatoes grown in peat based had redder skin colour, softer and tastier. Possibly, the intense red skin colour on tomato may be attributed to the increased of lycopene concentrations. In addition, the degradation of chlorophyll will induce chloroplasts change to chromoplasts which indicates red skin colour of tomato (Frazer et al., 1994). In the present study, combination of CP and different substrates of biochar resulted in similar red skin colour development.

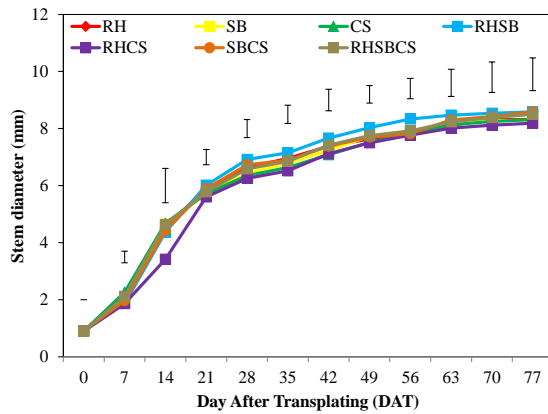


Figure 1: Effect of different biochars on stem diameter of cherry tomato plant. Vertical bars represent LSD at $P \geq 0.05$. (RH: Rice husk biochar, SB: Sugarcane bagasse biochar, CS: Coconut shell biochar, RHSB = RH + SB, RHCS: RH + CS, SBCS: SB + CS, RHSBCS: RH + SB + CS).

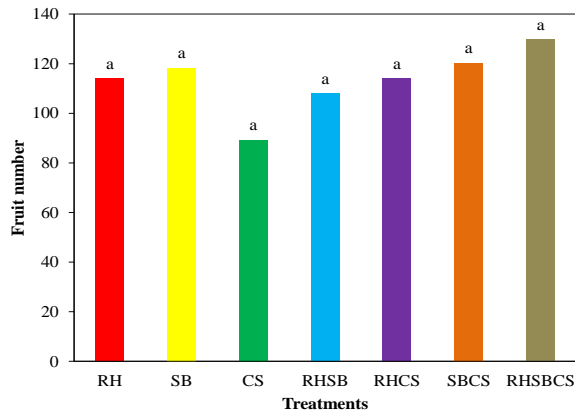


Figure 2: Effect of different biochars on fruit number of cherry tomato plant. Means with the same letter are not significantly different at $P > 0.05$. (RH: Rice husk biochar, SB: Sugarcane bagasse biochar, CS: Coconut shell biochar, RHSB = RH + SB, RHCS: RH + CS, SBCS: SB + CS, RHSBCS: RH + SB + CS).

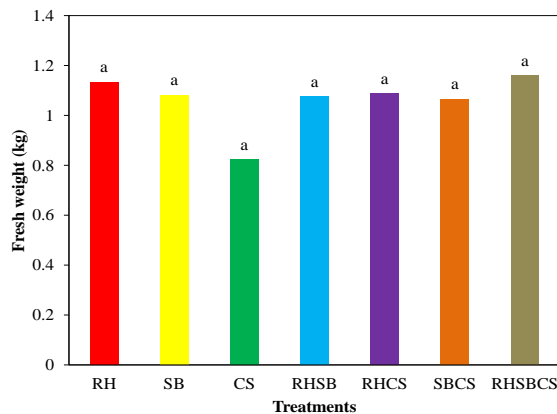


Figure 3: Effect of different biochars on fresh weight of cherry tomato plant. Means with the same letter are not significantly different at $P > 0.05$. (RH: Rice husk biochar, SB: Sugarcane bagasse biochar, CS: Coconut shell biochar, RHSB = RH + SB, RHCS: RH + CS, SBCS: SB + CS, RHSBCS: RH + SB + CS).

Table 1: Effects of different biochar substrates on cherry tomato fruit colour.

Treatment	Lightness (L*)	Chromaticity a*	Chromaticity b*	Hue angle (h°)	Chroma (C*)
RH (Control)	38.52 ^a	29.49 ^a	20.88 ^a	35.30 ^b	36.13 ^a
SB	38.76 ^a	29.05 ^a	21.17 ^a	36.08 ^b	35.94 ^a
CS	38.61 ^a	28.15 ^a	21.29 ^a	37.07 ^a	35.30 ^a
RHSB	38.60 ^a	28.37 ^a	20.57 ^a	35.94 ^b	35.05 ^a
RHCS	38.22 ^a	27.81 ^a	20.56 ^a	36.44 ^b	34.59 ^a
SBCS	38.67 ^a	29.05 ^a	21.02 ^a	35.88 ^b	35.86 ^a
RHSBCS	38.77 ^a	28.91 ^a	20.98 ^a	35.96 ^b	35.72 ^a

Means with different letters are significantly different at the 5% level according to LSD test. (RH: Rice husk biochar, SB: Sugarcane bagasse biochar, CS: Coconut shell biochar, RHSB = RH + SB, RHCS: RH + CS, SBCS: SB + CS, RHSBCS: RH + SB + CS).

Other fruit quality attributes such as fruit firmness, titratable acidity (TA) and soluble solids concentration (SSC) were significantly affected by different biochar substrates (Table 2). In processing tomato, one of the important qualities is TA (Anthon et al., 2011) which has been reported to play a significant role in preventing calcium oxalate kidney stones (Rodgers et al., 2014). Rice husk biochar treated-plant resulted in the highest SSC and TA among all of the treatments. Meanwhile, fruit firmness of CS treated-plant was the highest among all biochar treated-plants. In general, regardless of biochar substrates, the values of SSC, TA and fruit firmness of cherry tomato were in the normal range. In addition, no specific biochar substrate can be deduced in improving cherry tomato fruit quality as different substrates increase different fruit quality parameters. This suggest that the effect of biochar application is more dependent on soil fertility status and climate than on biochar types as recently reported by Jay et al. (2015). However, in the present study, all biochar types has the potential benefit as an excellent growth media in soilless culture system specifically for cherry tomato.

Table 2: Effects of different biochar substrates on soluble solids concentration (SSC), titratable acidity (TA) and fruit firmness of cherry tomato.

Treatment	SSC (%)	TA (% malic acid)	Fruit firmness (N)
RH (Control)	6.53 ^a	1.20 ^a	4.65 ^{ab}
SB	5.67 ^{ab}	1.06 ^{ab}	4.54 ^{ab}
CS	5.13 ^b	1.09 ^{ab}	4.85 ^a
RHSB	6.17 ^{ab}	1.00 ^b	4.73 ^{ab}
RHCS	5.90 ^{ab}	1.12 ^{ab}	4.40 ^{ab}
SBCS	5.65 ^{ab}	1.00 ^b	4.21 ^b
RHSBCS	6.02 ^{ab}	1.06 ^{ab}	4.32 ^{ab}

Means with different letters are significantly different at the 5% level according to LSD test. (RH: Rice husk biochar, SB: Sugarcane bagasse biochar, CS: Coconut shell biochar, RHSB = RH + SB, RHCS: RH + CS, SBCS: SB + CS, RHSBCS: RH + SB + CS).

Conclusion

Various substrates of biochar such as sugarcane bagasse and coconut shell and its combinations are able to replace rice husk biochar that widely used recently as they had a comparable growth performance and postharvest quality without causing adverse effect. All of the substrates can be developed into commercial growth media specifically for cherry tomato. The best biochar substrate could not be revealed as all the substrates had promising effect as explained above.

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Early Detection of Visual Deficiency Symptoms on *Hibiscus* Plants in Perdana Botanical Garden, Kuala Lumpur

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Introduction

The Perdana Botanical Garden which was formally known as the Lake Gardens, is strategically located in the city of Kuala Lumpur. It was built in 1888 by A.R. Venning, the state treasurer of Selangor during that time. In 2011, it was re-launched as the Perdana Botanical Garden by the Malaysian Prime Minister to revamp the park into a Botanical Garden. In early May 2016, FRIM scientists were invited to assist in detecting visual deficiency symptoms on hibiscus ornamental plants planted in this garden. The park management requested for recommendations on fertilization procedures in order to correct the early nutrient stress manifested by the ornamental plants. The objective of the visit was to detect the cause of deficiency symptoms, to assess the overall health of the affected hibiscus plants and to recommend mitigation steps in rehabilitating the ornamental plants.

Materials and Methods

The site selected was in the Hibiscus Garden area within the Perdana Botanical Garden, Kuala Lumpur. The hibiscus plants are known to be 17 months old and were treated with the NPK Humus 8:8:8 fertilizer at the rate of 10 g/ plant. Soil amendments using organic sheep dung fertilizer was also applied at the rate of 50 g/ plant in late April 2016. Two areas with persistent visual symptoms were selected, which were the Fountain (T2) and the Pergola (T3) area. Another area which had healthy hibiscus plants in the Walkway was selected as a control (T1). Firstly, the visual deficiency symptoms of the plants were recorded. The *in situ* soil pH and soil salinity (Electrical conductivity) measurements were recorded at all three sites using a Walklab pH meter T1900 and Field Scout Direct EC probe at 5 cm from soil surface. Soil samples according to profile depths up to 90 cm were retrieved at all sites and sent to the Soil Chemistry Laboratory in FRIM for analysis of P, K, Mg, CEC, Fe, Al for soil. Two replicates of foliar tissue samples were taken from the shrubs for all sites for the analysis of N, P, K, Mg and Fe.

Results and Discussion

In the area selected as control, the selected hibiscus shrubs were healthy with well developed leaves at the height of 2 meters (Plate 1). Top soil was loose and black in colour (30 cm), but the subsoil layer was moist, mixed with sand and clayey texture. No visual deficiency symptoms on plants were detected. The fountain area had severe stunting (Plate 2), experienced leaf chlorosis and green veins symptoms on young leaves (Plate 3). The top soil was friable and black in color but the subsoil was very firm, clayey and sticky. There were external attacks of mealybugs on the stem and branches. Shrubs of hibiscus in the Pergola area had beaked leaves symptom and the matured leaves were purplish black (Plate 4). Some of the leaves experienced chlorosis at the edges (Plate 5).

The top soil at the Pergola (50 cm) was similar to the fountain area and the subsoil was reddish, very clayey and sticky (Plate 6). Based on our observations, the soil pH was within suitable levels for all 3 sites for hibiscus plants (Table 1). The EC measurements were also within permissible levels of 1.5-2.5 mS cm⁻¹ (Spectrum Technologies, 2009) for flowering plants. The top soil (\pm 50 cm depth) was friable, well drained with high organic matter content due to the sheep dung and the presence of sand. However, the subsoil ($>$ 50 cm depth) needs to be incorporated with sand for better aeration and moisture retention for rooting since the clayey texture impeded suitable growth. Based on our early

visual observations, we believe that the hibiscus shrubs in the fountain area showed green veins and stunting due to iron deficiency and probably other micronutrients (Marschner, 1995). The purplish black leaves with beaked leaves in the Pergola area is due to magnesium deficiency symptoms.



Plate 1: Healthy hibiscus plant



Plate 2: Plants are severely stunted



Plate 3: Leaf symptoms of green veins



Plate 4: Beaked leaves and purplish black symptoms



Plate 5: Chlorosis at leaf edges



Plate 6: Clayey type of subsoil

Table 1: Results of *in situ* soil pH and EC measurements in the Hibiscus Garden

Site/ Field	Soil temperature (°C)	Soil pH	Soil EC (mS cm ⁻¹)
Walkway (T1,Control)	29.6	6.7	1.5
Fountain (T2)	29.7	6.6 – 6.7	1.5-1.6
Pergola (T3)	30.3	6.6-6.8	1.4-1.6

Conclusion

A micronutrient fertilizer which contains ferrous sulphate can be used to correct the iron deficiency symptoms in the fountain area. The visual deficiency symptoms in the Pergola area can be corrected with magnesium fertilizer sprayed as a foliar for rapid correction. Further recommendations will be done upon results on soil and plant analysis.

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Identification of Vegetative and Reproductive Growth Stages in *Xanthostemon chrysanthus* (F. Muell.) Benth. using BBCH Scale

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Introduction

Phenology is a study of periodic biological events, such as bud emergence, flushing, flowering and fruiting, closely regulated by environmental changes (Beuker et al., 2010). It contributes to management of various disciplines from biodiversity conservation, agriculture, forestry, zoology to climate change impact on flora and fauna. The information of phenological events offers invaluable evidence towards appropriate planning and management of certain practices associated to plant development. Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scales are widely used in observing the growth and development of plants. BBCH scales have been used to define various crops, for instance, *Elaeis guineensis* (Forero et al., 2011), *Camelina sativa* (Martinelli and Galasso, 2011), *Xanthostemon chrysanthus* (Ahmad Nazarudin et al., 2012), *Annona squamosa* (Liu et al., 2015) and *Ziziphus jujube* (Hernández et al., 2015). The scale is divided into 10 clearly identifiable developmental phases which demonstrate the entire growth of plants. These phases are considered as 10 principal growth stages numbered from 0 to 9 and each principal growth stage considers 10 secondary growth stages numbered from 0 to 9 (Hack et al., 1992).

The objective of this study was to determine the duration of vegetative and reproductive growth stages of *X. chrysanthus* (Myrtaceae) using BBCH scale developed for the species by Ahmad Nazarudin et al. (2012). This species is natives to tropical northern Australia, New Guinea, Indonesia and the Philippines and planted for beautification purposes due to its distinctive flowers.

Materials and Methods

A research plot was established at Metropolitan Batu Recreational Park, MBP (3° 12' 49" N/101° 40' 43" E), Kuala Lumpur, Malaysia. Six years old trees after planting were sampled and taken at a distance of 1-1.5 m from the road shoulder of the park. The planting distance was 10 m with an average tree height and diameter at breast height of 6 m and 11 cm, respectively. A total of ten *X. chrysanthus* were randomly selected and monitored from the population of 21 trees available. All trees were free from pest and disease symptoms.

Vegetative and reproductive organs were randomly selected and tagged. Observations made depend on the frequency of formation and changes for the tagged organs. In the case of inflorescence bud swelling and flowering, daily observation was carried out due to the rapid development. The total annual rainfall received was 3,181.7 mm. The temperature ranged between 22.9-33.3 °C and a relative humidity of 76.4%. The soil texture was loamy sand, comprises of 67% sand, 15% clay and 18% silt, and with soil pH 6.1.

Results and Discussions

There are nine principal growth stages described in *X. chrysanthus* (Ahmad Nazarudin et al., 2012). Four of these principal growth stages were recorded in the vegetative stage. These include bud development (stage 0), leaf development on tree branches (stage 1), formation of side shoots (stage 2), and shoot elongation (stage 3). However, in stage 2, no data was recorded due to its restriction in describing side shoot emergence at the main stem. As this woody tree branching is very extensive and it goes beyond the scope of BBCH scale, it is better to describe using tree architectural models (Finn et al., 2007). Development of harvestable vegetative plant parts (stage 4) was not relevant for this ornamental species, hence, this stage was also not accounted. Meanwhile, five principal growth stages were recognised in reproductive stage, i.e. inflorescence emergence (stage 5), flowering (stage 6), fruit development (stage 7), fruit and seed maturity or ripening (stage 8) and senescence/beginning of dormancy (stage 9). Figure 1 shows the macrophotographs of the development stages of *X. chrysanthus*.

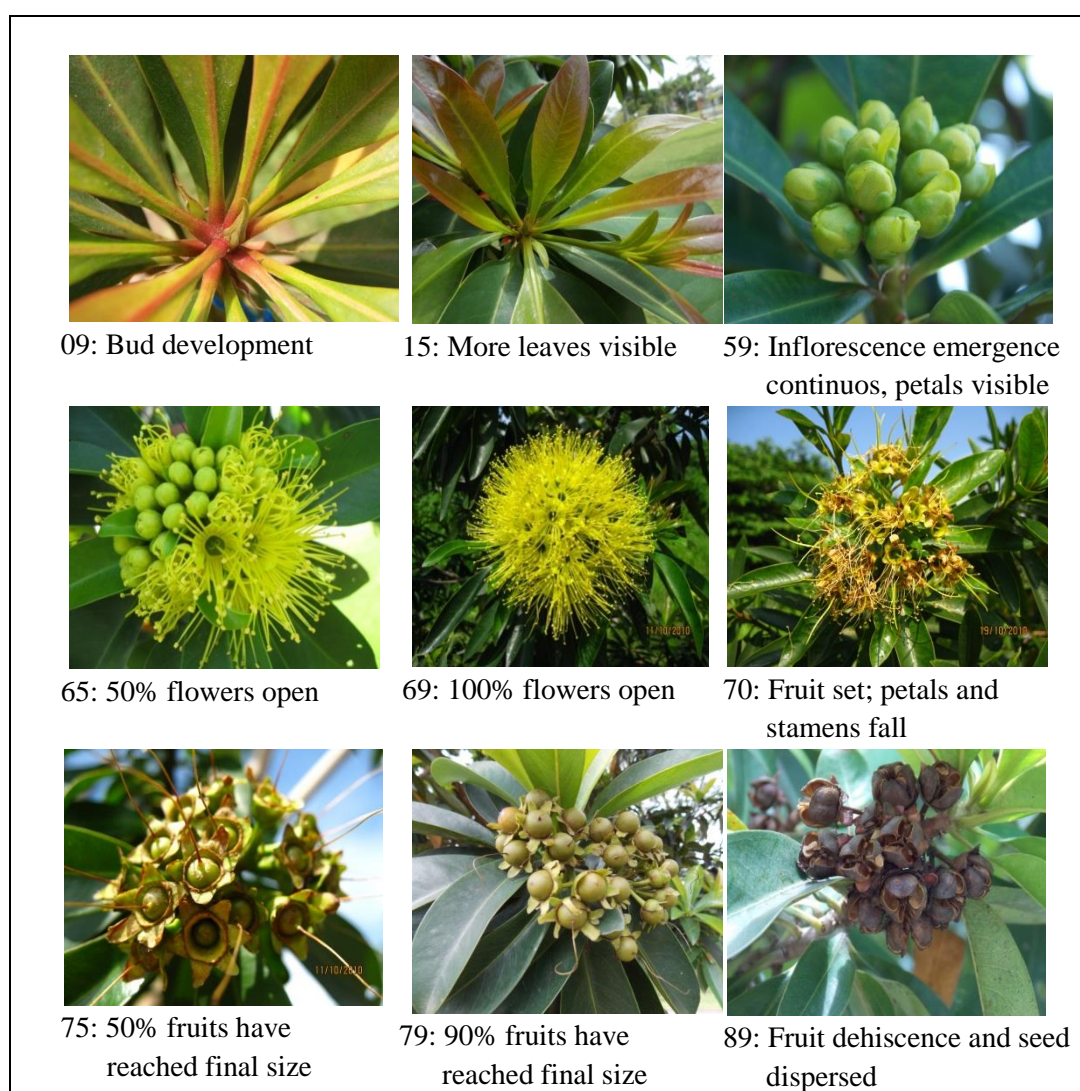


Figure 1: Vegetative and reproductive stages of *X. chrysanthus* according to BBCH-scale.

Vegetative and reproductive development stages of this species took 198 and 176 days, respectively (Table 1). At reproductive stage, the flowering period required 40 days, beginning from inflorescence bud swelling (stage 51) and was completed at the end of flowering or beginning of ovary growth (stage 69). The flowers conspicuously last for 29 days (stage 51-69), thus representing 16.5% of the

whole reproductive stage. The aesthetic value of the flowers declined after the stamens dried out and dropped, indicating the initial stage of fruiting. Fruit development and maturity took 136 days, representing 77.3% of the whole reproductive stage. Upon completion of fruit ripening, the tiny seeds were explosively dispersed.

Table 1: Vegetative and reproductive periods of *X. chrysanthus* according to BBCH-scale.

Growth stage	BBCH scale	*Number of days	Duration (Accumulated days)
Vegetative			
Bud development	01-10	14 ± 0.5	14
Leaf development on tree branches	10-11	18 ± 1.7	32
Shoot elongation	31-39	166 ± 12	198
Timeline (days)			198
Reproductive			
Inflorescence emergence	51-54	14 ± 1.3	14
	54-61	4 ± 0.7	18
Flowering	61-65	6 ± 0.7	24
	65-69	5 ± 0.5	29
	69-70	11 ± 0.7	40
Fruit development	70-75	11 ± 0.8	51
	75-79	22 ± 1.4	73
	79-81	32 ± 1.9	105
Fruit/seed maturity or ripening	81-89	71 ± 1.3	176
Timeline (days)			176

*Mean value ± standard deviation

Conclusion

Vegetative and reproductive development stages of *X. chrysanthus* were found to have completed within 198 and 176 days, respectively. The flowering period of the species was 40 days. Identification of vegetative and reproductive growth stages of landscape trees can be useful for defining better management practices such as the correct timing for fertilising and controlling pests and diseases. It may benefit future research on urban horticulture and arboriculture.

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Effects of Different Growing Media on Germination, Growth Performance and Biomass of Papaya Seedlings at Nursery Stage

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Introduction

Papaya (*Carica papaya* L.) belongs to the Caricaceae family and is originated from tropical America (Kumawat et al., 2014). It is widely cultivated in Malaysia for many purposes such as for food production, downstream or processing products and lately has been discovered to have contributed as health values. Eksotika papaya is a well-known hybrid product of MARDI that has been a preference for the export market. It is commercially propagated by seeds before field transplanting. Raising seedlings in nursery assures that orchards are established with superior quality planting materials which have distinct advantages of better survival and growth. Recently, production of high quality Eksotikas at nursery stage was found to have a problem which assumed to have started from germination and growth performance phase. It is due to the unfavorable selection of substrate makes the seeds or seedling growth became slow, erratic and incomplete (Nwofia et al., 2015). Similar cases with Red Lady papaya also requires appropriate growing media to expand the growth performance in the nursery (Bhardwal, 2014). Selection the accurate growing media such as in single or mixture form will provide sufficient support, serves as a reservoir for nutrients and water, allow oxygen diffusion to the roots and permit gaseous exchange between roots and atmosphere outside the substrate (Bhardwal et al., 2014). Currently, the use of peat moss as a rooting and growing media for papaya is well established. Peat moss has been reported to improved aeration condition with forming greater root system, promoted shoot nutrition uptake and increased yield (Rabeea et al., 2013). Furthermore, Hafiz (2012) stated that peat moss has an availability to promote rooting of papaya cuttings due to better physical properties such as appropriate porosity and moisture content. An application of peat moss to the Strawberry which has been done in Pakistan has proven can promote growth at vegetative and reproductive stage (Rabeea et al., 2013). Using of rice husk as one of the substrate in this study is due to the precursor materials which granular structure, insolubility in water, better mechanical strength and local availability at almost no cost (Haytham et al., 2014). Haytham (2014) reported that the mixture of rice husk with another substrate may produce a promising growth and yield component. Generally, growing media is categorized as a critical factor determining the seedling quality of papaya in the nursery stage. To achieve such improvement, proper agronomic procedures and proper selection for planting materials needs to be developed.

Therefore, the objective of this study was to determine the effect of different growing media on germination, growth performance and biomass of papaya seedling.

Materials and Methods

Research location

An experiment was conducted in a nursery located in MARDI Sintok, Kedah. The rain shelter structure consist of 50% shading was used as an experimental site.

Preparation of papaya seedlings

Seeds of papaya cv. Eksotika was used in this study and it was soaked overnight in water. After about 24 hours, the floated seeds were taken for germination process. Seeds were germinated in a different

growing media according to the treatments as specified below by using germination trays and placed on a bench.

Transplanting and maintenance of papaya seedlings

All the germinated seeds moisture was consistently maintained daily using controlled mist sprayers. Seedlings which have 6 leaves were transplanted into polybags containing different growing media. There were 15 selected samples comprises of uniform sizes and shapes before transplanting process. The entire transplanted seedling was labelled as in the following treatments and replications below. Each seedling was also treated by same amount of fertilizer comprises of 30g per seedling for every two weeks and daily watering by drenching using watering can.

Treatments application and experimental design

There are five treatments, comprising five replications and each consists of three samples for each replication. All papaya seedlings were arranged in the rain shelter structure using Complete Randomized Design (CRD). The treatments used in this study were:

Control – Soil and Sand (1:1, v:v)

T1 – Peat moss (100%)

T2 – Treated Rice Husk (100%)

T3 – Peat moss, Soil and Sand (1:1:1, v:v:v)

T4 – Treated Rice Husk, Soil and Sand (1:1:1, v:v:v)

The data was analyzed using ANOVA SAS 9.3 TS Level 1M1. Differences within the means were compared by using Duncan's test at 5% probability level.

Parameters taken

Several of parameters were taken which classified as different parameters phase as germination, growth and biomass parameters. Germination percentage (%) was measured in 14 days after sowing. An equation to calculate the germination percentage as using this formula:

$$\frac{\text{Number of seeds germinated}}{\text{Number of seeds sowing}} \times 100 = \text{Germination Percentage (\%)}$$

The growth parameters taken were seedling height (cm), leaves canopy (cm) and stem diameter (mm) at 3 cm from basal. Number of leaves and node are also taken. Growth data was measured weekly and first measurement started seven days after transplant. The growth data was taken for the period of 35 days after transplanted. However, biomass parameters taken were leaves fresh weight, stem and root (g) which was measured at 45 days after transplanted.

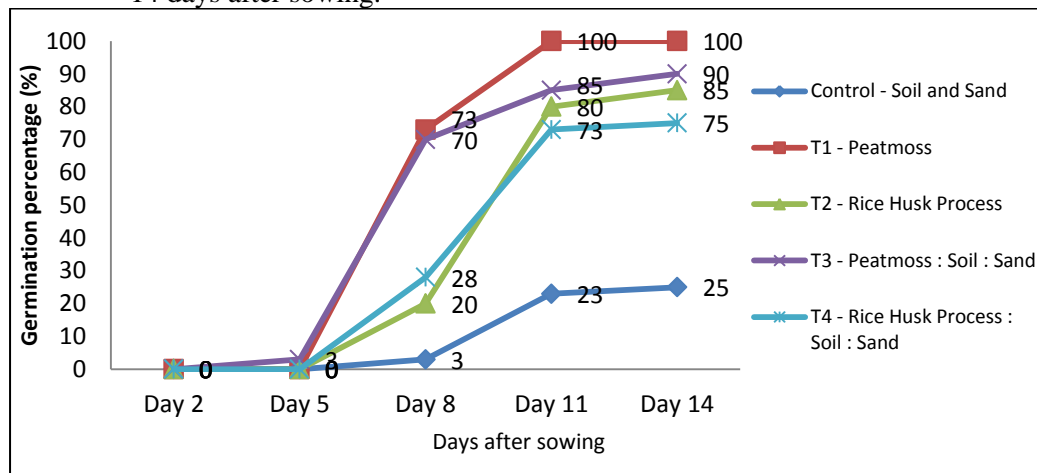
Results and Discussion

Germination percentage

It is evident from the data in Figure 1, that there is a trend of germination percentage (%) at day 8 after sowing. Maximum value was achieved by peat moss (T1) as 73%, followed by mixture of peat moss, soil and sand (T3) as 70%, mixture of treated rice husk, soil and sand (T4) as 28%, 100% treated rice husk (T2) as 20% and as common growing media as mixture of soil and sand (Control) at 3%. Maximum data recorded at day 11, as peat moss (T1) has achieved complete germination of 100%, while control as use mixture of soil and sand is 3%. At day 14 after sowing, mixture of peat moss, soil and sand has reached to 90%, followed by treated rice husk process (T2) as 85%, mixture of treated rice husk, soil and sand (T4) as 75% and mixture of soil and sand (Control) as 25%. Thus,

the proper physical properties of growing media are important. Moisture content is the main factor that may influence the completion of papaya germination. In this case, peat moss shows the highest value due to the appropriate physical properties which is more porous, proper moist condition, good infiltration and aeration (Rabeea, 2013). Mixture of peat moss, soil and sand reach 90% of total germination percentage due to the media combination that not suitable to provide the moisture content required for the papaya seeds. Mixture of sand and soil is considered as bulky and not provide better aeration for rooting system of papaya. Thus, create an uneven moisture level to the substrate. Similar results obtain in application of soil and sand to papaya seedling which provides the least result in percentage of germination from the sowing time (Bhardwaj, 2014).

Figure 1: Effects of different growing media on germination percentage of papaya cv. Eksotika II in 14 days after sowing.



Growth parameters

Plate 1 shows the growth performance of papaya seedling between treatments: T1 (1a), T2 (1b), T3 (1c), and T4 (1d) at 7 days after transplanted into polybags. At day 15 after sowing, the entire seedling from mixture soil and sand that transplanted into polybags did not survive. Thus, none of growth and biomass data was collected for all control samples. It was due to slow growth performance of seedling and low germination rate of the seeds. Unfavorable physical properties of soil and sand mixture has disturbed the root performance, thus has affected growth of seedling (Hafiz et al., 2012). Parameters recorded in Table 1 as seedling height, leaves canopy, stem girth, and number node shows the evidence that the usage of peat moss (T1) shows the maximum value and significantly difference at $p < 0.05$ amongst the treatments. There were 29.4 cm for seedling height, 20.39 cm for leaves canopy, 8.32 mm for stem girth and 16.79 for number of node. However, the combination of peat moss, soil and sand (T3) was significantly different compared to treated rice husk (T2) and mixture of treated rice husk, soil and sand (T4) for three parameters as seedling height (cm) as 15.27 cm, leaves canopy (cm) as 5.53 cm and number of node as 14.36. Parameter for leaves number shows that there is no significant difference between peat moss (T1) with 10.14 and mixture of peat moss, soil and sand (T3) with 11.43. However, those treatments are significantly different compare to treated rice husk (T2) as 5.1 and mixture of treated rice husk, soil and sand (T4) as 6.4. Survival rate of the seedling from soil and sand mixture are decreased after transplanting was done due to the poor physical properties which have disturbed the expansion of rooting system. Water holding capacity in the media combination of soil and sand are low where increase the bulk density and the substrate did not facilitate holding of water to supply moisture for seedling (Kumawat et al., 2014). Peat moss indicated that, it is most suitable growing media to enhance growth at vegetative stage as seedling height, stem diameter and leaves canopy. It is fine textured compare with other treatments with better moisture content and aeration flow between the particles (Hafiz et al., 2013). Nevertheless, the mixture of peat moss, soil and sand is found not significantly difference compare with peat moss. It is due to the characteristics

of papaya leaves which will abort every weeks and this was happened followed by the leaves maturity. Thus, the data comparison was not significant but if we compared the number of node, resulting the significant different between T1 and T3. That was indicated, the seedling from peat moss treatment is more vigorous and matured. Rice husk is not suitable to enhance vegetative growth of papaya in nursery either in a single form (T2) or by mixture component (T4). Research done in cucumber by using the single growing media of rice husk and mixture of rice husk with soil and sand indicated, the lowest result for all vegetative growth and yield (Haytham et al., 2014). Treated rice husk should have to be incorporated with others growing media to improve the porosity and bulk density of the substrate for making more suitable for nutrient supply (Wang et al., 2004).

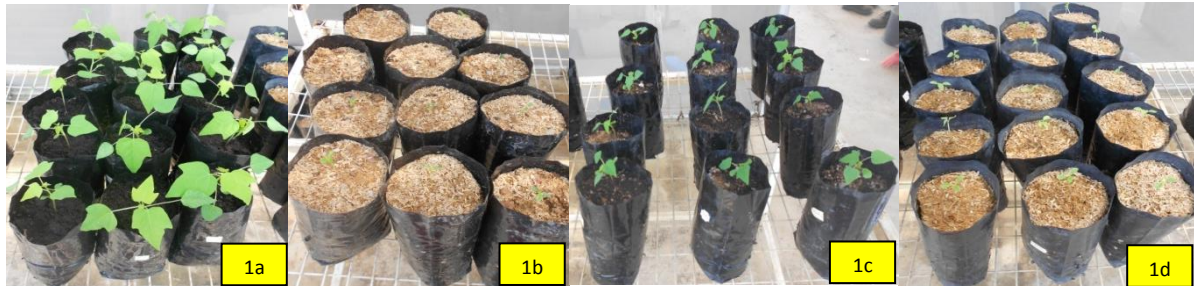


Plate 1: Growth performance of papaya seedling between treatments as T1 (1a), T2 (1b), T3 (1c) and T4 (1d) at 7 days after transplanted into polybags

Table 1: Effects of different growing media on vegetative growth of papaya cv. Eksotika II at 35 days after transplanted.

Treatment	Seedling height (cm)	Leaves canopy (cm)	Stem girth (mm)	Leaves number	Node number
T1	29.4a	20.39a	8.32a	10.14a	16.79a
T2	5.08c	3.64c	1.59c	5.1b	7.1d
T3	15.27b	17.54b	4.79b	11.43a	14.36b
T4	4.74c	3.94c	1.26c	6.4b	8.4c

Treatments with the same letters do not differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test.

Biomass parameters

Table 2 has found that, for fresh weight of leaves, stem and root, there is significantly difference amongst the treatment as peat moss (T1) is 11.67 g. However, the mixture of peat moss, soil and sand (T3) is significantly different compared with treated rice husk (T2) and mixture of treated rice husk, soil and sand (T4) for all fresh weight recorded from leaves, stem and root. However, mixture of peat moss, soil and sand (T3) has shown significant difference compare with treated rice husk (T2) and mixture of treated rice husk, soil and sand (T4) for parameters of leaves, stem and root fresh weight. By measurement of root length (cm), peat moss (T1) as 24.43 cm and mixture of peat moss, soil and sand (T3) as 27.67 cm are not significantly difference. However, those two treatments are significantly difference compare with treated rice husk (T2) as 9.87 cm and mixture of treated rice husk, soil and sand (T4) as 5.87 cm. Peat moss showed favorable results for all fresh weight component as leaves, stem and root. In research done for strawberry has stated that, the usage of peat moss might provide good physical, chemical and biological characteristics that improve aeration with forming greater root system, promoted shoot nutrient uptake and increased yield (Rabeea, 2013). Rabeea (2013) also stated the use of peat moss may promote better fresh weight of plant. This indicates that peat moss is more suitable and high quality growing media to boost growth performance. The high fresh weight of the plant is an indicator of the qualities of substrates (Hafiz et al., 2013).

Table 2: Effect of different growing media on biomass of papaya cv. Eksotika II at 35 days after transplanted.

Treatment	Fresh weight of leaves (g)	Fresh weight of stem (g)	Fresh weight of root (g)	Root length (cm)
T1	11.67a	21.59a	21.428a	24.43a
T2	0.408c	0.23c	0.598c	9.33b
T3	4.825b	5.53b	11.173b	27.67a
T4	0.12c	0.12c	0.127c	5.87b

Treatments with the same letters do not differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test.

Conclusion

The use of single growing media as peat moss with 100% portion in polybags for papaya before field transplanting process (45-50 days after transplanted into polybags) is most promising to be applied. It is due to the enhancement of better germination, growth and biomass parameters measurements. It is also convenient for growers where there is no need to mix peat moss media with another portion of another media resulting in management and cost saving. Common use of growing media for papaya as mixture of soil and sand gives low germination percentage thus is not recommended. Treated rice husk process is not recommended to be use as single growing media or mixtures for papaya due to the slow growth and low biomass measurement.

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Growth Performance and Production of *Neptunia oleracea* Lourerio using Different Plant Materials

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Introduction

Herbaceous perennial plants reproduce by seeds and asexually by means of vegetative organs. Plants from habitat that are unfavorable to seedling establishment tend to rely largely on vegetative reproduction (Sculthorpe, 1967; Fenner and Thompson, 2005). Asexual reproduction is the dominant form of reproduction for wetland plants, i.e. by shoot fragments (*Ceratophyllum*), turions (*Utricularia*), inflorescence plantlets (*Echinodorus*), runners or stolons (*Cryptocoryne*), rhizomes (*Typha*), stem tubers (*Sagittaria*), root tubers (*Nymphoides*) and corm (*Aponogeton*) (Sculthorpe, 1967; Cronk and Fennessy, 2001).

Neptunia oleracea Lourerio (daun tangki), although less common as a vegetable, is cultivated in inundated fields or canals (Edwards, 1980; Paisooksantivatana, 1994). It is harvested at three to four weeks after planting and 250 shoots are gathered into a bunch for trading in local market. About 30,000 – 50,000 shoots can be harvested in each harvest in one ha area (Paisooksantivatana, 1994).

The sexual reproduction of *N. oleracea* is initiated when the seeds are released by opening of two sutures from the dry pod and dropped off to the wet ground followed by germination (Windler, 1966; Cook, 1996; Cronk and Fennessy, 2001). This species also generates new plants through auxiliary buds and lenticels at stem (Paisooksantivatana, 1994; Holtum and Ivan, 2002; DPI, 2009).

However, in Sarawak, Malaysia, *N. oleracea* is only gathered from wild and offered for sale in local markets as a leafy vegetable (Voon et al., 1990; Suzalina Akma, 2008; Dayangku Alifah, 2009; Mohd Syahrul, 2009; Muhd Arif, 2009). This study was conducted to evaluate the propagation methods for culturing this potential vegetable using the different forms of plant materials, by seedling and stem cutting. The study was also conducted to evaluate the yield of *N. oleracea* as well as harvesting period per cycle propagation.

Materials and Methods

Plant materials

The study was conducted for a period of 17 weeks at Net House Complex, Universiti Putra Malaysia Bintulu Sarawak Campus where seed as reproductive plant form and stem cutting as vegetative plant material were tested. Seeds and matured stems were collected from Kg Penipah, Mukah water channel and allowed to germinate and grown for two weeks. Thirty seedlings and 30 cuttings were transplanted into 3 top soil: 2 sand: 1 compost by weight media in 240 cm x 120 cm x 50 cm fiber glass tank. The planting distance used in this study was 30 cm x 30 cm following the method of *N. oleracea* culturing in inundated rice field in Thailand as described by Paisooksantivatana (1994).

Observation on the growth performance

The plant length and the number of shoots formed were recorded weekly for four weeks after transplanting to determine the growth performance between different plant materials, i.e. seed and stem cutting. Water from culture tanks was collected at weekly intervals for determination of the concentration of NO_3^- , NO_2^- , NH_3 , and PO_4^{3-} content by using a portable spectrophotometer- Hach DR/2400 with a 2.5 cm cell length.

Yield

The tender young shoots of *N. oleracea* were harvested at five weeks after transplanting. The fresh weight, length and diameter of harvested *N. oleracea* were recorded. Thirty shoots were bundled with rubber band. Harvesting of the plant was performed until the production declined. In this study, eight harvestings were performed at two-week intervals. The fertilizer of 15% N: 15% P: 15%K (200 g/tank) was applied after the 4th harvesting to maintain the growth performance.

Statistical analysis

Independent T-test ($p \leq 0.05$) was used to compare growth performances, i.e. increased in plant length and number of shoots of *N. oleracea*. The parameters and nutrient contents of water used during the cultivation were also compared by using Plymouth Routines in the Multivariate Ecological Research (PRIMER) version 5 (Clark and Warwick, 2001) to verify the nutrient or nutrients in the water sources that explained the correlation between the increment in growth performance. The yields and the dimensions of harvested shoots of *N. oleracea* from seeds and stem cuttings were tested by using independent T-test ($p \leq 0.05$) using Statistical Analysis System (SAS) version 9.2 for Windows 2008.

Results and Discussion

Growth performance

The results on growth performance of *N. oleracea* from two forms of plant materials, i.e. seed and stem cutting were recorded in Figure 1. The plant gradually increased in the growth with no significantly difference between the two types of plant materials. At the end of seed and stem cutting propagations, the plants differed significantly as they elongated to length of 95.40 cm and 97.80 cm, respectively. In contrast to the increment in plant length, there was a significant difference in formation of shoots in weekly observations. In the first week, more shoots were produced from stem cutting as compared to seed. After three weeks, the stem cutting recorded similar number of shoots as the seed. At the end of the study, the average number of shoots produced was 23 and 25 for the seed and stem cutting, respectively. Stem cutting produced higher number of shoots and had a tendency to gain more yield in production.

N. oleracea may not selectively absorb one nutrient at a time, but the uptake may involve a combination of absorbable nutrients in the water sources (Table 1). The results showed a combination of more than one nutrient in water sources that explained the observed increment in plant length but there was no indication that nutrients had an effect on the number of shoots produced (Table 2). Only NO_3^- was responsible for the increment in plant length. The rate of NO_3^- uptake increases during vegetative growth in legume crops (Imsande and Touraine, 1994). The development of plant towards availability status of water nutrients is demonstrated in Figure 2 that shows the progress of the plants cultured in tank.

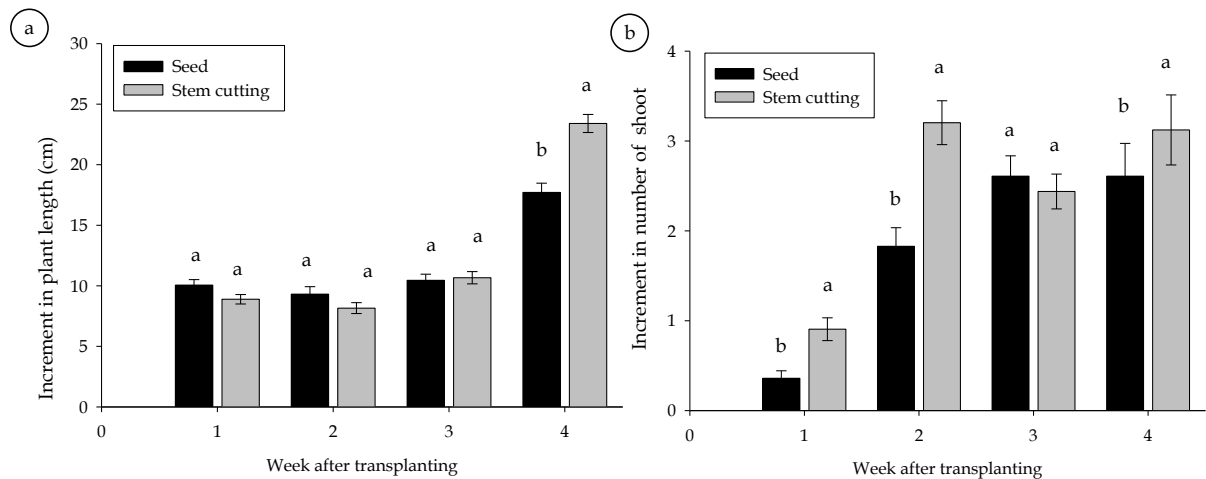


Figure 1: The growth performance of *N. oleracea* from different plant materials. (a) Increment of plant length and (b) increment of number of shoots (given as mean \pm s.e, n=30). The bars sharing a common letter in same week are not statistically significant between planting materials according to T-test ($p \leq 0.05$).

Table 1: Nutrient content in water for *N. oleracea* seeds and stem cuttings. Different superscript alphabets in the same column within the same week indicate significant difference at $p \leq 0.05$ (T-test). All values are given as mean \pm s.e, n=6.

Week	NH ₃ (ppm)		NO ₂ ⁻ (ppm)		NO ₃ ⁻ (ppm)		PO ₄ ³⁻ (ppm)	
	Plant material		Plant material		Plant material		Plant material	
	Seed	Stem cutting	Seed	Stem cutting	Seed	Stem cutting	Seed	Stem cutting
1	0.13 \pm 0.01 ^b	0.19 \pm 0.01 ^a	0.07 \pm 0.01 ^b	0.09 \pm 0.01 ^a	27.37 \pm 2.61 ^b	25.87 \pm 2.27 ^a	0.88 \pm 0.04 ^b	2.61 \pm 0.10 ^a
2	0.13 \pm 0.01 ^a	0.15 \pm 0.01 ^a	0.07 \pm 0.01 ^a	0.06 \pm 0.02 ^a	21.37 \pm 1.78 ^a	22.02 \pm 1.81 ^a	0.85 \pm 0.04 ^b	1.99 \pm 0.06 ^a
3	0.11 \pm 0.01 ^a	0.14 \pm 0.01 ^a	0.06 \pm 0.01 ^a	0.06 \pm 0.02 ^a	19.42 \pm 0.41 ^b	21.05 \pm 1.76 ^a	0.83 ^b \pm 0.01	1.83 ^a \pm 0.03
4	0.15 \pm 0.01 ^b	0.19 \pm 0.03 ^a	0.05 \pm 0.01 ^a	0.06 \pm 0.01 ^a	18.28 \pm 0.34 ^a	19.73 \pm 1.73 ^a	0.77 ^b \pm 0.01	1.74 ^a \pm 0.04

Table 2: The drivers, nutrient combination related to the observed growth performance in *N. oleracea* throughout the experiment as produced by BV-STEP analysis.

Parameter	Plant material	Number of nutrients	Spearman rank correlation (<i>p</i>)	Best nutrient combination
Increment in plant length	Seed	1	0.657	NO ₃ ⁻
	Stem cutting	1	0.878	
Increment in number of shoots	Seed	0	0.000	No trend was obtained
	Stem cutting	0	0.000	No trend was obtained

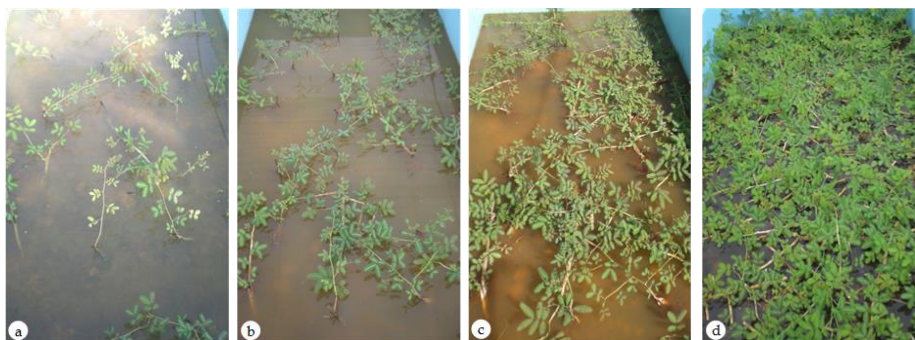


Figure 2: The development of *N. oleracea* in tank. (a) Initial planting with 2 – 3 nodes, (b) week 2, elongation of stems covering 50% of the tank culture, (c) week 3, coverage of approximately 75% of the culture area and (d) week 4, coverage was almost 100% and harvesting was conducted at week 5.

Yield

Eight harvestings were done after five weeks from transplanting to investigate the production of *N. oleracea* at weekly intervals in the under study 5.76 m² area (Figure 2). The plant formed from stem cutting showed higher yield productions when compared to the plant propagated from seed. Most of the portions of plants harvested were intact with white spongy structure similar to the traded shoots. The tender shoots produced from the culture were shorter than those in native market, probably attributed to the limited space for growth in the tank compared to open area (Table 3).

In this study *N. oleracea* were grown in tank system for five months. Table 4 shows the comparison of culturing and production of this plant with other production study (Paisooksantivatana, 1994). However, there are no reports on yield of *N. oleracea* grown in tank so far. The high growth performance of vegetative part was also demonstrated in *N. oleracea* by stem cutting materials. Besides, stem cuttings produce more shoots leading to a tendency to gain more yields. The plant that is frequently cut is also stimulated to develop more side shoots (Palada and Crossman, 1999). In Thailand, both stem cutting and seed are cultivated (Paisooksantivatana, 1994). This present study showed that the yield was almost ten times higher than in field culture as reported by Paisooksantivatana (1994).

Table 3: Shoot measurement of *N. oleracea* grown from different plant materials. Different superscript alphabets with shoot parameter within the same harvesting week indicate significant different at $p \leq 0.05$ (T-test), $n=60$. All values are given as mean \pm s.e.

Harvesting	Shoot weight (g)		Shoot length (cm)		Shoot diameter (mm)	
	Plant materials		Plant materials		Plant materials	
	Seed	Stem cutting	Seed	Stem cutting	Seed	Stem cutting
1	3.60 \pm 0.141 ^a	3.31 \pm 0.138 ^a	20.49 \pm 0.43 ^a	20.05 \pm 0.46 ^a	3.95 \pm 0.10 ^a	3.80 \pm 0.08 ^a
2	2.93 \pm 0.11 ^b	3.23 \pm 0.13 ^a	15.67 \pm 0.27 ^b	18.75 \pm 0.35 ^a	3.60 \pm 0.08 ^a	3.72 \pm 0.07 ^a
3	4.28 \pm 0.16 ^a	4.46 \pm 0.16 ^a	18.30 \pm 0.32 ^b	20.21 \pm 0.41 ^a	4.36 \pm 0.09 ^a	4.33 \pm 0.08 ^a
4	4.17 \pm 0.13 ^b	4.51 \pm 0.14 ^a	19.00 \pm 0.29 ^a	19.40 \pm 0.30 ^a	4.40 \pm 0.08 ^a	4.21 \pm 0.07 ^b
5	3.11 \pm 0.12 ^b	3.86 \pm 0.12 ^a	16.13 \pm 2.26 ^b	17.58 \pm 0.32 ^a	3.89 \pm 0.07 ^b	4.08 \pm 0.06 ^a
6	2.37 \pm 0.06 ^a	2.21 \pm 0.05 ^a	13.77 \pm 0.16 ^a	13.85 \pm 0.18 ^a	3.48 \pm 0.04 ^a	3.14 \pm 0.05 ^b
7	2.24 \pm 0.04 ^a	2.10 \pm 0.05 ^a	13.37 \pm 0.19 ^a	13.68 \pm 0.16 ^a	3.42 \pm 0.03 ^a	3.35 \pm 0.03 ^a
8	1.45 \pm 0.06 ^a	1.28 \pm 0.05 ^b	10.76 \pm 0.20 ^a	10.33 \pm 0.21 ^b	3.01 \pm 0.05 ^a	2.98 \pm 0.04 ^a

Table 4: The agronomic comparison of yield production for *N. oleracea*.

Culture method	Plant material	Fresh weight (g/m ²)	Yield (shoots/m ²)	Number of bunches of harvested shoot produced (bunch/m ²)	Cropping season (months)	References
Inundated rice field	Stem cutting	-	3 - 5	-	4 - 6	Paisooksantivatana (1994)
Tank	Seed	44.97 - 371.01	27 - 148	1 - 4	5	This present study
Tank	Stem cutting	26.39 - 416.84	18 - 144	1 - 4	5	This present study

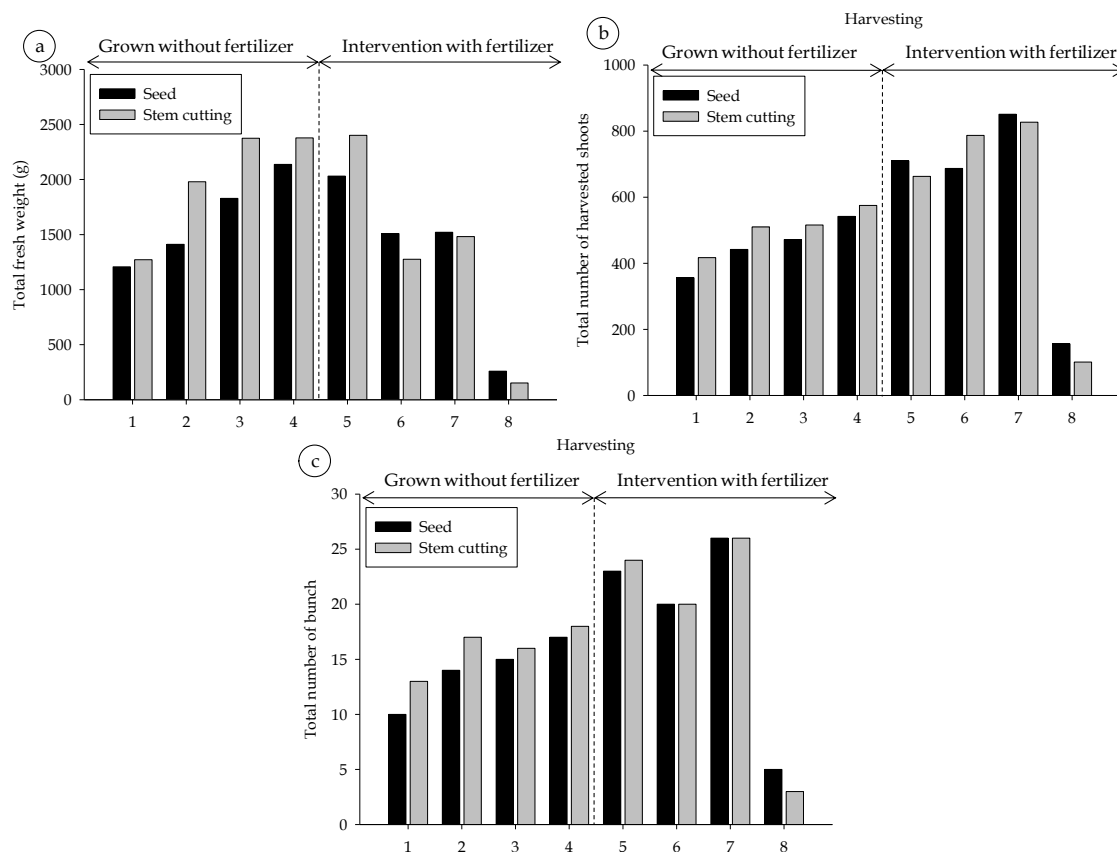


Figure 3: Yield and production of *N. oleracea* propagated from different plant materials in 5.76 m². (a) Total of harvested fresh weight, (b) total number of harvested shoots and (c) total number of bunches.

Conclusion

The plant gradually increased in growth with no significant difference between plant materials of seeds or stem cuttings but then the plant grown from stem cutting was relatively longer and produced more shoots than seed propagation. Therefore, stem cutting propagation has great potential to gain more yield in production. Only NO₃⁻ was responsible in increasing the plant length. Eight harvestings were done starting from five weeks after transplanting to investigate the production of *N. oleracea* at weekly intervals and stem cutting propagation produced relatively higher yield compared to seed propagation.

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Preliminary Study on Leaf Growth of *Ficus carica* var. Brunswick Planted at Open Field System

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Introduction

Ficus carica is a gynodioecious plant (Lisci and Pacini, 1994) and commonly known as fig. According to Stover et al. (2007), fig trees are thought to be originated from Western Asia. The fig fruit characteristic is a syconium, which is a complex inflorescence consisting of many fruit drupelets (Lisci and Pacini, 1994). Nowadays, there are increases of fig cultivation in Malaysia either it is grown as a hobby for urban community or as a commercial production for fruit and planting material. There are several fig varieties which suitable to be planted in Malaysia namely Brunswick, Black Jack and Improved Brown Turkey.

Brunswick is a variety that is found to have large pear-shaped fruit with an outer yellow and greenish skin made brown under the hot sun. The fruit flesh is brown to reddish in colour. It is a robust plant that can tolerate the heat and humidity in Malaysia (Fig Direct Resources, 2015). Brunswick is among one of the popular fig varieties that are planted and sold here in Malaysia.

Fig plants are commonly grown under rain shelter and open field system. Production under rain shelter is a promising system due to uncertain climate condition and pest and disease control, but at the same time, this system requires a high cost for the structure construction. Meanwhile, open field system is more economical and practical for up-scaling production. Furthermore, differences of cultivation system will affect the plant growth development including leaf growth. Leaf growth development of fig plant is important due to fig fruits developed in the axils of their leaves (Crane 1986), suggested that information on leaf growth performance of fig grown at open field system is essential to improve their yield and fruit quality. Therefore, this study was conducted to investigate leaf development of Brunswick variety under open field system.

Materials and Methods

Location and planting materials

The study was carried out at MARDI Sintok located in Bukit Kayu Hitam, Kedah. The research plot has been established since October 2015 and planted with 327 fig plants from Brunswick variety. The advance planting materials were supplied by FigDirect Resources (AS0367807-A).

Crop maintenance

The treatment plants were applied with 30g granular fertiliser, NPK (15:15:15) weekly. Water was applied once a day using water drip irrigation system until field capacity level. Pest and disease control were applied when necessary followed standard practices.

Sampling and study procedure

A total of 10 newly emerged leaf buds were selected randomly and labelled. The observation of leaf growth was conducted every 1-3 days by photograph and measurement of the leaf length and width were collected weekly until the leaves sample was aborted.

Data analysis

The relative growth rates were calculated using modified Hoffmann's formula (2002):

$$\text{Relative growth rate for length} = (L_2 - L_1) / (t_2 - t_1)$$

Where; t_1 = time one (in days); t_2 = time two (in days); L_1 : Length of leaf at time one (in cm); L_2 : Length of leaf at time two (in cm)

$$\text{Relative growth rate for width} = (Wd_2 - Wd_1) / (t_2 - t_1)$$

Where; t_1 = time one (in days); t_2 = time two (in days); Wd_1 : Width of leaf at time one (in cm); Wd_2 : Width of leaf at time two (in cm)

The analysis of variance (ANOVA) for mean comparison was done by using Duncan Multiple Range's Test (DMRT).

Results and Discussion

Leaf development stage from week 0 to week 7 is presented in Table 1. The maximum leaf length ranges from 5.5 to 7.6 cm and 5.7 to 8.1 cm for leaf width. The results showed that under open system cultivation, Brunswick had smaller leaf length and width as compared to several fig varieties namely Bidaneh, Paizeh, Zard, Siah bolol riz, Siah zoodras, Siah diras, Morabaii, Hallavi riz and Hallavi dourousht that are planted in Iran ranging from 12.0 cm to 26.5 cm for leaf length measurement and 8.25 cm to 18.60 cm of leaf width measurement (Darjazi, 2011).

The leaf length and width growth pattern of Brunswick variety can be explained by equation $y = 0.043x^3 - 0.6555x^2 + 3.0373x + 1.0653$ ($R^2 = 0.9908$) for leaf length (Figure 1), and $y = 0.0482x^3 - 0.7326x^2 + 3.4696x + 0.4317$ ($R^2 = 0.9854$) for leaf width (Figure 2). The growth pattern for both parameters showed gradual increase in size until 5 weeks after emergence, and their growth were constant until 10 weeks, before leaf abortion. It shows that vigorous leaf performance was until 5 weeks after leaf bud emergence suggested that to improve plant growth in term of leaf growth performance adequate water and nutrient should be sufficient during early shoot emergence until 5 weeks. Moreover, during constant leaf growth period probably the fruit bud formation will be developed.

There was significant difference ($P < 0.05$) on relative growth rate (RGR) of leaf in the early four weeks period (Table 2). The RGR for leaf length was highest during the first week with a mean of $0.25 \text{ cm week}^{-1}$. Meanwhile, the growth slightly decreased at the fourth week which is $0.14 \text{ cm week}^{-1}$. A similar trend was observed on RGR of leaf width with $0.27 \text{ cm week}^{-1}$ growth development at the first week of measurement and $0.15 \text{ cm week}^{-1}$ at week four.

The high RGR of leaf growth during early weeks, followed by low RGR during the middle weeks commonly occurs for monocotyledons and dicotyledons plants (Munns et al., 2010). Similar growth pattern was shown on *Arabidopsis thaliana* (Vanhaere et. al., 2015), *Acacia longifolia*, *Cytisus grandifloras* and *Ulex europaeus* (Crisóstomo et al., 2007).

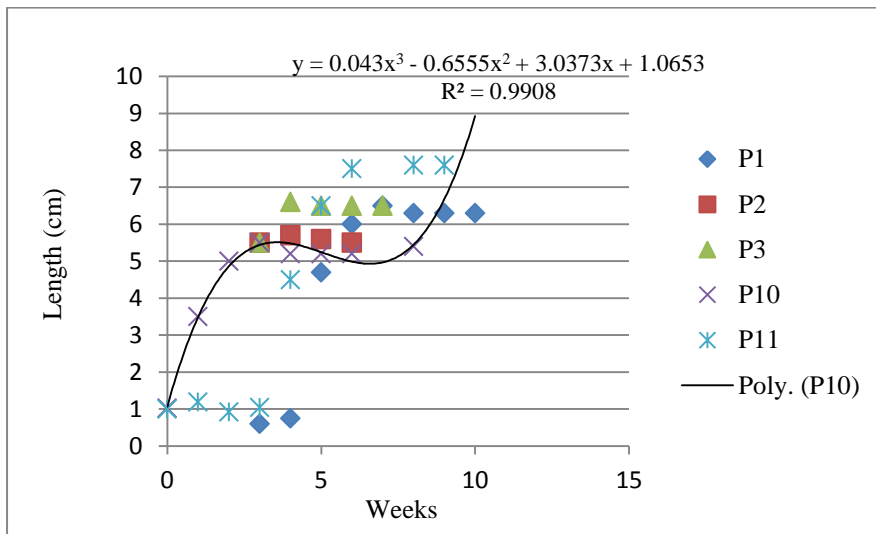


Figure 1: Leaf length growth performance of Brunswick variety grown under open system condition.

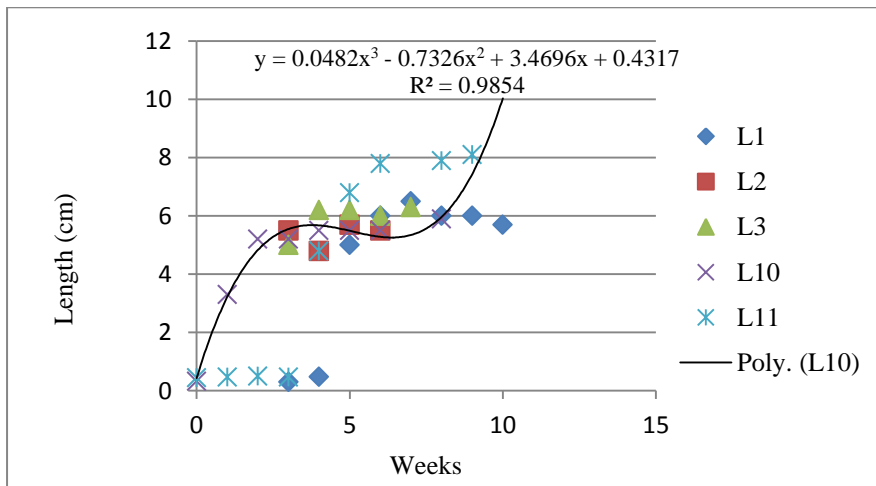
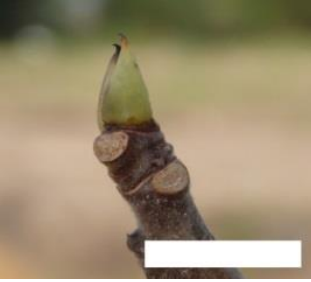




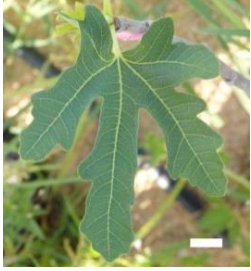




Figure 2: Leaf width growth performance of Brunswick variety grown under open system condition.

Table 1: Leaf growth stage of Brunswick fig variety from week 0 to week 7 under open field system.

Week	Picture	Week	Picture	Week	Picture
0		1		2	
3		4		5	
6		7			

*line bar in each picture represent 10 mm

Table 2: Summary of the variance analysis on the relative growth rate of leaf length and width of Brunswick fig variety under open field system.

Week	Relative growth rate (cm week ⁻¹)	
	Leaf length	Leaf width
1	0.250a	0.272a
2	0.220ab	0.230ab
3	0.150bc	0.162b
4	0.138c	0.146b

Means followed by the same letter in the same column are not significantly different by DMRT at $P \leq 0.05$

Conclusion

The full cycle of leaf growth development of Brunswick variety under open field condition was 10 weeks before abortion. Vigorous leaf growth performance was observed after 5 weeks of leaf bud emergence and the information on fig leaf growth can be used to improve yield of production including crop management in term of fertilization and water application.

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Growth Performance of Selected Pineapple Hybrids on Peat Soil

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Introduction

Pineapple is the third most important tropical fruit in the world production after banana and citrus. Pineapple is a popular non-seasonal fruit and widely cultivated on peat soil in Johor, Peninsular Malaysia. It is one of the major horticultural crops in Malaysia. About 90% of the planting area is on peat soil and the remainder is planted on mineral soil (Raziah and Alam, 2010). The world pineapple production was increased by 16 million metric tonne to 25 million metric tonne since 10 years ago (MPIB, 2015). The acreage of pineapple cultivation also was increased from 841,381 ha in 2006 to 1,024,508 ha in 2013 (MIPB, 2015). Malaysia was ranked at 19th with a share of 1.18% of the total world pineapple production. The total pineapple cultivation area in Malaysia is 9,827 ha (MPIB, 2013). Local pineapple varieties were planted throughout the year with 334,400 metric tonne of pineapple production. 84% of the local varieties are fresh consumption for domestic market while 10% is for processing industry and the other 6% is fresh consumption for export.

Pineapple is used either for fresh consumption or processing purposes into canned pineapple, jam, juices, pickles and candy. Pineapple requires 12-18 months from planting to harvesting but different variety had different planting cycle. The popular pineapple cultivar for fresh fruit was developed by MARDI is known as Josapine. It was developed from pineapple hybrid of 'Johor' (Spanish) and 'Sarawak' (Cayenne). This variety is released by MARDI in 1996 and it had been commercialized successfully. In this study, evaluation of the growth performance was carried out for twelve different hybrids developed from Josapine and Australian 'piping-leaf' genotypes 53-116 and 59-656.

Materials and Methods

Plant materials and field experiment

The study was carried out in pineapple experimental field at MARDI Pontian, Johor. Twelve pineapple hybrids were selected from the F₂ hybrid population consisting of 10,000 seedlings derived from crosses between local pineapple variety and piping-leaf genotypes. All planting materials in this experiment were propagated by quartering techniques to reduce error due to propagule age and size. The plants were raised to a height of 45 cm before they were planted in the experimental field. The plants were planted in a randomized complete block design with 3 replicates. In each plot, there were 40 plants grown in one double-row bed. Flower induction was carried out using 50 mL Ethrel solution and 360 g urea in 18 L water.

Morphological measurement

Data on the growth and yield performance i.e. plant height, D-leaf length, panicle length, crown length, fruit length, fruit diameter, crown weight, fruit weight, core size, the number of suckers or slips and percentage of total soluble solid content (% TSS) were recorded at near maturity. TSS was recorded using a hand refractometer (0-25% Brix). The data were recorded from 10 sample plants taken at random of each plot.

Parameters observed in this study were as follows:

- a. Plant height (cm), measured from the base of the plant to top of the longest leaf.
- b. D-leaf length, measured from the base of the longest leaf to tip of the leaf.
- c. Peduncle length.
- d. Crown length, measured the length of the crown from the base to the tip of the crown.
- e. Fruit length (cm), measured from fruit base to top of fruit, excluding crown.
- f. Fruit diameter (cm), measured at maximum breadth of fruit.
- g. Crown weight (kg), weigh crown without fruit and peduncle.
- h. Fruit weight (kg), weigh without crown and peduncle.
- i. Core size (cm), the fruit was horizontal sliced and then measured at maximum breadth of heart.
- j. Percentage of TSS ($^{\circ}$ Brix), some juice from the base, middle and top part of fruit with yellow skin were assessed its TSS using Hand Refractometer.
- k. Number of suckers/slips.

Statistical analysis

Values tabulated on the tables and figures are the means of three replicates. Differences between means were evaluated by Duncan as indicated at $p < 0.01$ and 0.05 levels of error using SAS Statistical package.

Results and Discussion

The results of the study are tabulated in Table 1 and 2. The analyses of variance in Table 1 showed that the mean squares for all characters were significantly different between hybrids with the exception of fruit length, fruit diameter, crown weight and slip number. The mean values of the twelve hybrids for the eleven characters are shown in Table 2.

Plant height and D-leaf length

Results obtained showed that the plant height and D-leaf length at maturity were significantly different for all hybrids. The vigourousity of the plants was affected by the plant height. The higher plant height showed that the plant is more vigorous compared to other plant.

Peduncle length

The length was ranging from 21.75 cm to 43.67 cm. The drawback of the hybrids that have long peduncle (>35 cm) and it will affect the stature and fruit dislodging would occur due to the heavy top containing fruit.

Crown length and weight

Crown size is measured in weight unit and it will affect the package and transportation in which the lighter the crown weight, the easier the fruits are packaged and transported (Hadiati et al., 2011). The ranges of crown length are from 12.99 cm to 26.32 cm. Hybrid J53-126 had the longest crown with 25.61cm and 0.24 kg of crown weight.

Fruit length and diameter

Fruit length and diameter were not significantly different between hybrids. With regards to fruit length and diameter, J53-65 was the most superior. It had the highest mean with 18.50 cm in length and 19.68 cm in diameter. However, this hybrid was not found as the heaviest fruit weight among the hybrids. This showed that the fruit weight was not significantly affected by the fruit weight and its diameter.

Table 1. ANOVA. Mean square values of eleven characters in twelve pineapple hybrids.

	df	Plant height	D-leaf length	Panicle length	Crown length	Fruit length	Fruit diameter	Crown weight	Fruit weight	Core size	TSS	Slip number
Hybrids	11	187.25**	249.56**	137.78*	46.08*	13.21ns	19.39ns	0.019ns	0.27**	1.13**	26.91**	0.20ns
Rep	2	54.62ns	64.96ns	84.22ns	3.73ns	39.11*	22.23ns	0.005ns	0.13*	0.02ns	0.98ns	0.69ns
Error	24	20.92	24.55	56.57	12.77	10.15	17.31	0.011	0.04	0.08	2.25	0.21
Total	37											

** significant at $p = 0.01$; ns = not significant

Table 2. Mean values of eleven characters in twelve pineapple hybrids.

Hybrids	Plant height (cm)	D-leaf length (cm)	Peduncle length (cm)	Crown length (cm)	Fruit length (cm)	Fruit diameter (cm)	Crown weight (kg)	Fruit weight (kg)	Core size (cm)	TSS (%)
J53-126 (1)	85.63e	76.31cde	31.00abc	25.61a	11.05b	10.87b	0.24abc	0.75e	2.05ef	15.23ab
J59-48 (2)	102.89a	96.53a	27.12bcd	26.32a	13.56ab	10.68b	0.16abc	0.75	1.91efg	10.77cd
J53-67 (3)	73.87f	63.88f	26.73bcd	24.63ab	14.27ab	11.86b	0.37a	1.05cde	2.25def	14.74ab
J53-31 (4)	100.40ab	90.46ab	30.34abcd	22.283ab	16.56ab	12.71ab	0.16abc	1.63a	2.43cde	15.85ab
J59-74 (5)	98.13abc	89.79ab	26.37bcd	12.99c	13.47ab	13.47ab	0.20abc	1.35abc	3.12ab	15.63ab
J59-103 (6)	90.16cde	83.56bcd	23.40cd	22.31ab	12.07b	9.97b	0.28abc	0.70e	1.72fg	14.38b
J59-142 (7)	98.07abc	91.09ab	39.52ab	20.85ab	14.45ab	10.87b	0.14bc	0.98de	1.45g	11.60c
J59-66 (8)	87.09de	74.45e	25.62bcd	19.89ab	11.84b	11.38b	0.183abc	0.93de	2.37cde	15.73ab
J53-94 (9)	95.33abcd	86.33b	32.83abcd	21.43ab	15.10ab	12.06ab	0.35ab	1.47ab	3.56a	17.26a
J53-65 (10)	92.22bcde	86.07b	26.35bcd	25.72a	18.50a	19.68a	0.21abc	0.84de	2.84bc	10.09cd
J59-149 (11)	87.46de	75.43de	21.75d	25.49a	12.87ab	11.71b	0.14bc	1.15bcd	2.62bcd	9.67cd
J29-24 (12)	92.81bcde	84.96bc	43.67a	17.86bc	12.77b	11.04b	0.13c	0.93de	1.82fg	8.2d

*values within a column with the same letter are not significantly different at $p < 0.01$ according to DMRT

Fruit weight

Weight of fruits produced by all hybrid plants in this study varied from 0.33 kg to 2.71 kg. The hybrid with the heaviest fruit was J53-31 (1.63 kg) while J59-103 had the smallest fruit (0.70 kg). The fruit weights of the other hybrids were ranged from 0.75 kg to 1.35 kg. The fruit weights of all hybrids exception of three hybrids viz. J53-126, J59-48, J59-103 appeared to be acceptable either for canning or fresh market. The small fruits may only be suitable for fresh consumption.

Core size

The core size of all the hybrids was significantly varied from each other. The ranges of the core size are from 1.45 cm to 3.12 cm. Two of the hybrids viz. J59-74 and J53-94 have large core of the fruit (3.12 cm and 3.56 cm) and unacceptable core size for processing industry. However, these can be accepted for fresh consumption if the cores in the slices are not fibrous and unsightly. Acceptable core size for cannery processing is should be less than 3 cm because of the coring knife would not be able to remove the core completely.

Percent total soluble solid (TSS)

According to Hadiati et al., (2011), TSS is one of the parameters used to predict eating quality and Chan and Lee (1999) stated that high TSS would impart the good taste of pineapple. The most prominent feature of the five hybrids viz. J53-126, J53-31, J59-74, J59-66 and J53-94 is the very high TSS in the range of 15-17% which was significantly higher than other hybrids. The other hybrids have acceptable TSS with the exception of J59-24 (9.67%) and J59-149 (8.26%) which is a canning variety whose low TSS can be resolved by addition of sugar during processing.

Conclusion

The result showed that there were three hybrids viz. J59-66, J53-94 and J59-74 were performed better than the other hybrids in this evaluation cycle. These hybrids would be recommended for the genotypex environment evaluation (GXE trial) at multi-location both in peat and mineral soil to determine their stability across different environment.

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Paddy Cultivation Physical Analysis Studies for the Determination of Nanofertilizer Efficiency Test

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Introduction

Fertilizer is a substance added to soil to improve plants growth and yield. Fertilizer technology has been developed tremendously based on farmers' demand for their crops. Modern synthetic fertilizer is composed mainly of nitrogen, phosphorus and potassium with secondary nutrient added according to plants requirement which has proven to improve the quality and quantity of food nowadays. But a long term excessive use of synthetic fertilizer may cause environmental issues such as Eutrophication which resulted from oversupply of nutrients, which induces explosive growth of plants and algae. When such organisms die, bacteria begin to decompose the remains, using up oxygen for respiration which consumes the oxygen in the body of water, thereby creating the state of hypoxia. Larger life forms, such as fish will suffocate to death.

Therefore, nanofertilizer is an alternative technology which would reduce the possibility of excessive use of commercial fertilizer. Nanofertilizers are design to improve adsorption ability because of its small size (10^9) and high surface area. A smart-carrier fertilizer is most technically advanced way of supplying nutrients to crops and can improves fertilizer use efficiency. Compared to conventional fertilizers, their gradual pattern of nutrient supply meets plant needs and minimizes leaching. A nano strategy involving a smart carrier fertilizer based on modification of fertilizer using hydroxyapatite (HA) nanoparticles was studied. HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) nanoparticles are rated as one of the prominent materials in agricultural applications, which can provide phosphorus nutrient.

The proposed fertilizer composition may maximize the fertilizer use efficiency while minimizing the adverse effects to the environment due to use of large quantities of fertilizer in agriculture (Trenkel et al., 1997; Sopyan et al., 2008)

Materials and Methods

A pot study of rice cultivation was carried out at Horticulture Research Centre green house for fertilizer efficiency test. Two type of nitrogen fertilizer was used which is urea and urea-modified hydroxyapatite nano-particles with three replications for both treatments. A few parameters such as plant height, total of leaves, total of tiller, panicle length, fresh weight, dry weight and chlorophyll content for dry and old leaves had been set.

Data for all parameters were taken once in every 15 days from the first treatment of fertilization process for the total of 105 days of treatment. These parameters were fresh and dry weight, plant height, chlorophyll content, panicle length, tiller number, filled grain, empty-grain weights and nutrient analysis. Total fresh weight (g): Leaves and stems will be separated and then weight by using electronic balance. Total dry weight (g): Total dry weight of leaves and stems will be weighed by using electronic balance after the samples dry for three days in the oven at 60 °C. Plant height (cm): Plant height will be measured from soil surface until the highest shoot. Chlorophyll content: Chlorophyll content in the leaves will be measured by using Minolta Chlorophyll Meter. Three readings will take at young and older leaves for every treatment and an average will be recorded. Leaves number: Every leaves on whole plant was calculated including young leaves. Tiller number:

Every tiller on every plant was recorded. Panicle length;_Panicle length for every plant recorded. Nutrient analysis: Collect all part of plant (roots, stems and leaves). Use clean container (paper bag). Never use a metal container because it will contaminate the samples. Clean the sample with dry brush. Wash them with distilled water or deionized water. Do not prolong when wash it because can leach the nutrient. Air dried the sample under the shade not under the sun. Use clean paper bags for mailing the sample to the lab. Do not use plastic bags because it does not allow samples to dry so sample may be decomposed.

The experimental design was a completely randomized design (CBD) with three replications and three treatments including control (no fertilizer) (Table 1). The data collected was statistically analysed using Analysis of Variance (ANOVA) procedures and calculated by using SAS program. The treatment means were compared by Tukey test. There were nine experiment pots for this study. The pots were arranged with distance about 30 cm between plant and row for easily growth and filled up to 15 cm height with medium prepared. Each pot was sown with three seedlings. Weeds were controlled manually. Watering was done manually twice a day. These cultivation activities were done for 105 days using all the parameters as mentioned above and harvested for further physical and chemical analysis.

Table 1: Experimental treatment labelling.

Treatment	Replication 1 (R1)	Replication 2 (R2)	Replication 3 (R3)
Control (No Urea) T1	T1R1	T1R2	T1R3
Urea T2	T2R1	T2R2	T2R3
Urea-Modified HA T3	T3R1	T3R2	T3R3

Results and Discussions

All parameters were monitored according to interval of 15 days of treatment which is 15, 30, 45, 60, 75, 90 and 105 days of treatment. After 105 days, samples were harvested and physical analysis was done as shows below.

Each one of the parameters portrays in Figure 1 shows same trends that urea itself is higher compared to control and HA-urea. Similar result for urea and HA-urea in chlorophyll content (young leaves) were obtained. Further investigation on bonding percentage between urea and HA nano particles has to be done. Nutrient content in HA-urea possibly less than urea itself due to poor bonding percentage of urea and HA. Based on these preliminary results, nutrient uptake studies has to be done with other micro nutrient to enhance it performance.

The data collected has been statistically analysed using ANOVA procedures and calculated by using SAS program. The treatment means has been compared by Tukey test. Data obtained were shown in Table 2 as below. From data shown in Table 2, we can conclude that through physical analysis; there is no significant result of HA-urea compared to control and urea itself. This may cause by low percentage of bonding between HA and urea. More studies has to be done regarding this matter.

Table 2: Statistical analisis using ANOVA.

Treatment	Chlorophyll content	Plant weight	Plant leaves	Plant weight	Nitrogen content
Urea	34.478A	14.469A	6.8700A	0.7850A	2.8989A
Urea-HA	32.522A	12.741A	6.2667A	0.4992B	2.7522A

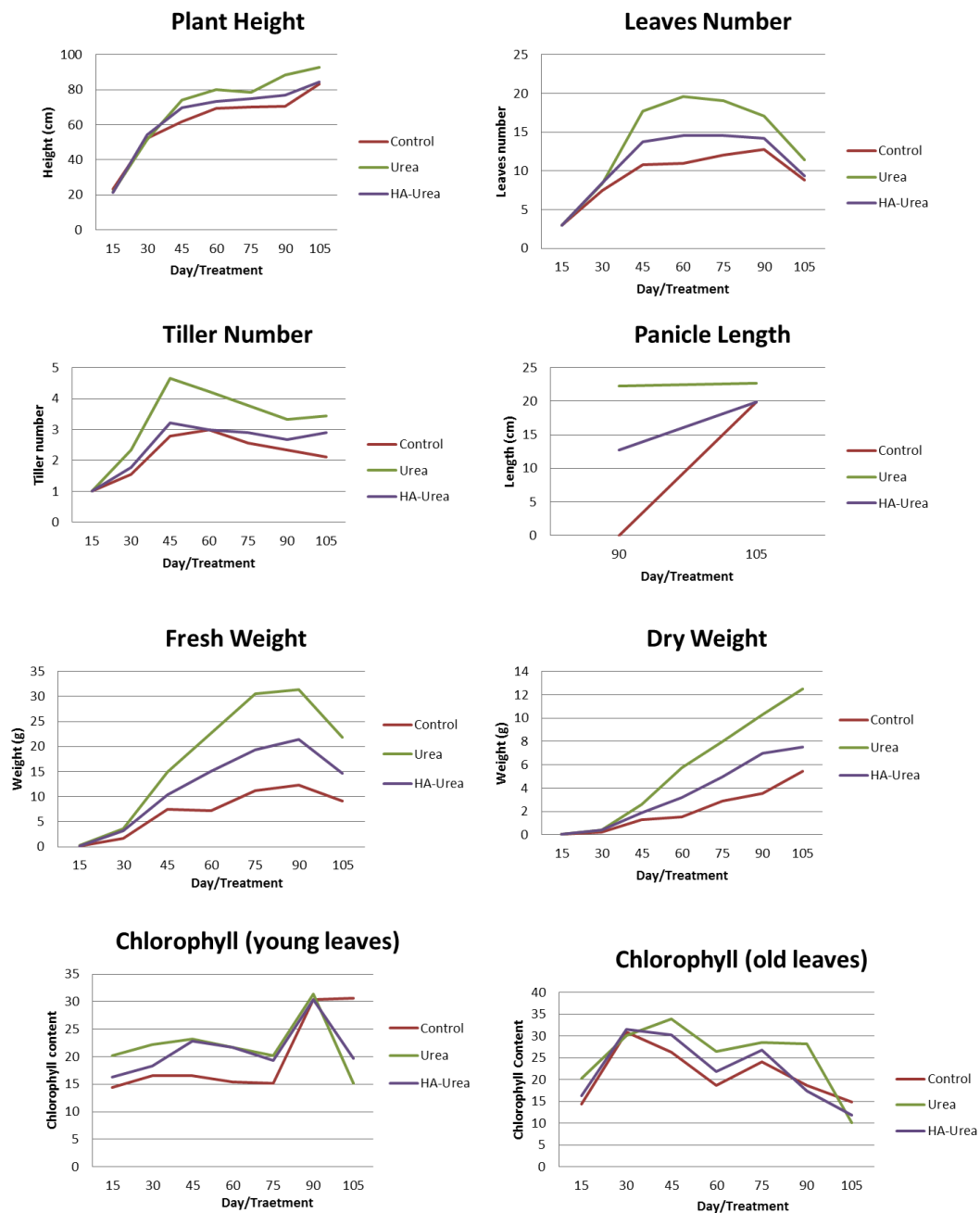


Figure 1: Physical analysis of paddy cultivation according to 105 days of treatment using urea. Modified urea-HA and control (no fertilizer).

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Improving the Fruiting Probabilities of Harumanis Mango through Architectural Approach

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Introduction

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae. Recently, Harumanis, registered as MA128 is one of the famous mango variety in Malaysia. Every year there is a high demand for Harumanis, but the production is still limited. Various approaches have been performed in order to sustain and increased the yield production of Harumanis mango. Increasing yield of mango production is commonly emphasized on fertilization and hormone induction. However, other agronomic practice such as pruning also plays an important role for continuous high yield production. This is because; pruning activities will rejuvenate the tree as well as provide optimum light penetration and improve air circulation. In addition, pruning can also stimulate the flower formation by increasing the shoot formation by 78% and the flower buds number of 31% (Kuswandi et al., 2013). Pruning activities require technical skill so that the branching pattern and plant architecture will be balanced and uniform. The shoot formation will be generated from either sequential or reiterated shoot. Sequential shoots are formed on the terminal parent shoot with dense pattern whereas reiterated shoots are formed on older branch with alternate branching pattern (Normand et al., 2009). These types of shoots vary in terms of number of shoots produce and affected plant density that is crucial for yield production. At present, effect of pruning in terms of shoot produce and plant density on yield production for mango is yet to be established. Therefore, collection of agronomic data of Harumanis mango such as crop load, branching sequence, number of shoots, number of flower panicles, number of fruit panicles, number of fruits and weight of fruit were observed and interpreted into correlation and stepwise regression model.

On the other hand, the statistical modelling approach can be employed whenever observation data on the yield response is available (Fukuda et al., 2013). According to Shinji in 2009, the statistical modelling approach is more useful for its simplicity and interpretability in extracting information from the observation data. The information retrieved from a statistical model can be tested using existing knowledge and then further incorporated into process-based models. Therefore, the use of statistical models can contribute to the accumulation of complex interactions between the yield response of a cultivar and environmental conditions, such as water supply (Shinji et al., 2013). As a conclusion, predicting yield of mango through statistical modelling approaches can be utilized by farmers to predict their yield and to sustain their farm productivity. This paper discusses the method on how the equation was developed to predict and estimate the shoot number required for fruit number targeted through statistical modelling approach.

Materials and Methods

An experiment was conducted at mango research plot located in MARDI Sintok, Kedah. Harumanis or MA128 was used as a sample due to high rated commercial mango variety in Malaysia. The observation was commenced at flowering stage during dry season in January 2015. Fourteen 12 years old Harumanis plants were selected based on the uniformity of branching pattern. All the selected plants consisted 6 branching sequence. The experimental trees received similar cultural practices including fertilization, irrigation, and pesticides and fungicides application. Several parameters of agronomic and fruit quality, consist of plant height, stem diameter, canopy spread, number of shoots,

number of branches at every branching sequence, SPAD value, number of flower panicles, number of fruit panicles, number of fruits, crop load, yield per tree, weight of peel and pulp, weight of seed and total soluble solid were taken. The data were subjected to analysis of variance (ANOVA) and Pearson correlation coefficient at $P \leq 0.05$ was used as a comparison to find the interaction among the parameters recorded. Then, it is further separated with the regression analysis with data of fruit numbers as a model to develop the equation for predicting the fruiting probabilities of mango. The model equation was divided into six branching sequence. Stepwise regression analysis was also done to develop the equation between the branching sequence and fruit number. Here, the fruit prediction table consisted of 6 branching sequences of Harumanis mango was developed. The table will predict the number of shoots required for the targeted fruit numbers for each branching sequences.

Results and Discussion

The representative data of 14 plants of Harumanis were assessed using Pearson's correlation coefficient (COR) to observe the interaction among the variables. From the data presented in Table 1, there is negative correlation between the number of shoot and the fruit number. It is due to the environment factor in MARDI Sintok which has long dry periods. This effects the development of fruit set which causes low yield for the majority of the sample for the season. However, there is a correlation between the number of shoot and number of flower panicle which is significant at the value of $r = 0.58803$. This condition has proven that, the interaction between the number of shoot and number of flower is related with the shoot development, which is important to support the flower initiation. Thus, flower development will reflect the number of fruit. As a close relationship, the number of shoot is important as it can influence the yield of mango trees. There is a study conducted in Bangladesh to observe the correlation effect on mango where the genetic association has found that, there is a significant positive correlation of plant height, shoot number, number of fruit set, inflorescence shoot towards the yield production of mango (Majumder et al., 2012). In reproductive parameters observation, there is a correlation between the number of fruit panicle and number of fruit, number of fruit harvested and weight of fruit harvested. This indicator shows that, the number of fruit panicle may influence and determine the yield of plant. Further analysis uses the parameters taken except for fruit quality data. This was analysed using stepwise regression analysis to develop the model equation for predict the shoot number required. As a fruit number at the 6th branch sequence become the model, there is an interaction between the number of flower panicle and the number of fruit at $p < 0.0001$. As a close relationship between shoot number and flower panicle number, the use of equation between numbers of flower panicle to the fruit is relevant. Poor fruit set incidence during the long dry period is the factor why the shoot number is not related through stepwise regression analysis. Thus, from Figure 1.5, an equation was formed for 6th branch sequence as $y = 1.30831x - 3.87831$ and the $r^2 = 0.9683$. Stepwise regression analysis is also done to the whole branching sequence and the equation was developed as stated in Figure 1.0, 1.1, 1.2, 1.3 and 1.4 according to the branching sequence. The model developed was used to expand the table of fruit number prediction by estimating the shoot number required. In the COR, it also reveals that, the correlation value between the primary, secondary and quaternary branching sequence with the number of shoot. Thus, stepwise regression analysis uses Figure 3 to determine the most significant parameter to the shoot number. Therefore, the equation develop will determine the predicted optimum shoot number at each branching sequences. The secondary branching sequence records significant effect with the model equation develop as $y = 56.1036x + 59.22072$. Table 2 shows the predicted table for recommended shoot number for yield targeted of Harumanis mango. It also shows the optimum shoot number that can be achieved according to each branching sequences using an equation $y = 59.1036x + 59.22072$ as Figure 1.6. The recommended shoot number which is required for achieving the targeted fruit number was determined by each model equation developed as stated in Table 2. The development of shoot number is reflected by the structure of the Harumanis tree. In order to achieve the number of shoot, there will be two types of recommended shoots formation of pruning can be used for maintaining and balancing the structure of trees. Sequential shoots can be formed by the above node pruning on the terminal parent shoot with dense pattern. While, reiterated shoots are formed by below node pruning on older branch

with alternate branching pattern (Normand et al., 2009). Thus, the combination of pruning method might be the guideline to match the shoot number required with the balance of tree structure. Balancing of tree structure is a crucial part that needs to be adopted to improve the light penetration in enhancing photosynthesis process on leaves and also to provide better air circulation. This architectural approach as a method of pruning may give an improved canopy structure that is convenient for maintenance.

Table 1: Correlation for all parameters taken on fourteen samples of Harumanis trees.

Var.	No. of branch at each branching sequences						Cropload	Shoot number	Flower panicle number	Fruit panicle number	Fruit number	Number fruit harvest	Weight fruit harvest	Peel weight	Pulp weight	Seed weight	TSS
	1	2	3	4	5	6											
1	1	0.9126 <0.0001	0.7876 0.0008	ns	0.5361 0.0481	ns	ns	0.5939 0.0251	ns	ns	ns	ns	ns	ns	ns	ns	ns
2		1	0.8058 0.0005	0.6769 0.0078	0.6515 0.0116	ns	ns	0.6155 0.0191	ns	ns	ns	ns	ns	ns	ns	ns	ns
3			1	0.6041 0.0221	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4				1	0.8042 0.0005	ns	ns	0.5375 0.0474	ns	ns	ns	ns	ns	ns	ns	ns	ns
5					1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6						1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cropload							1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Shoot number								1	0.58803 0.027	ns	ns	ns	ns	ns	ns	ns	ns
Flower Panicle Number									1	ns	ns	ns	ns	ns	ns	ns	ns
Fruit Panicle Number										1	0.984 <0.0001	0.9156 <0.0001	0.9166 <0.0001	ns	ns	ns	-0.71 0.0066
Fruit Number											1	0.9142 <0.0001	0.9104 <0.0001	ns	ns	ns	-0.652 0.0158
Number Fruit Harvest												1	0.9804 <0.0001	ns	ns	ns	-0.631 0.0208
Weight Fruit Harvest													1	ns	ns	ns	ns
Peel weight														1	ns	ns	0.6064 0.028
Pulp weight															1	ns	ns
Seed weight																1	ns
TSS																	1

Pearson Correlation Coefficients, $N = 14$; $Prob > |r|$ under H_0 : $Rho = 0$

Table 2: Predicted table for recommended shoot number for improve yield of Harumanis mango.

Yield targeted *fruit number	Primary branch	Secondary branch	Tertiary branch	Quarternary branch	5 th branch	6 th branch
	$y = 1.30086x + 0.02696$	$y = 1.29142x + 0.07541$	$y = 1.28328x + 0.18696$	$y = 1.3063x + 0.288961$	$y = 1.28015x + 0.62934$	$y = 1.30831x - 3.87831$
	BP	BP	BP	BP	BP	BP
5	10	10	9	9	9	17
10	19	19	19	19	18	27
20	39	39	39	38	38	46
40	77	78	78	77	77	84
60	116	117	117	115	117	123
80	155	156	157	154	156	161
100	193	195	196	192	195	200
120	232	234	235	231	235	238
140	271	273	274	269	274	277
160	310	312	314	308	313	315
180	348	351	353	346	353	354
200	387	390	392	385	392	392
220	426	429	431	423	431	431
240	464	468	470	462	471	469
260	503	507	510	501	510	508
280	542	546	549	539	549	546
300	581	585	588	578	589	585

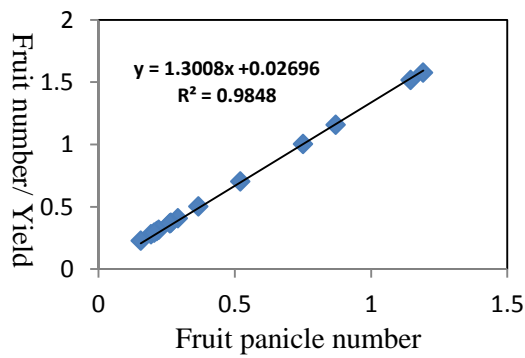


Figure 1.0: Regression for primary branching.

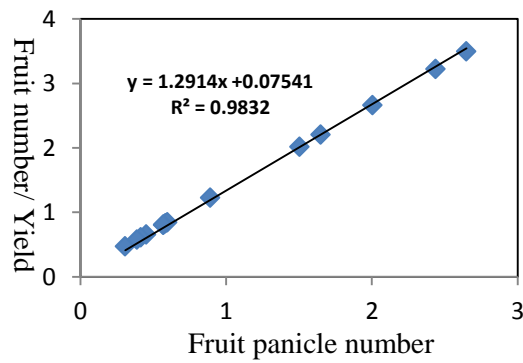


Figure 1.1: Regression for secondary branching.

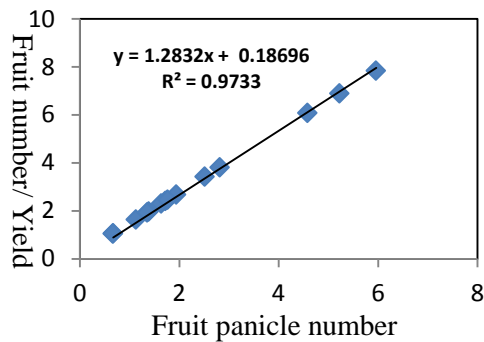


Figure 1.2: Regression for tertiary branching.

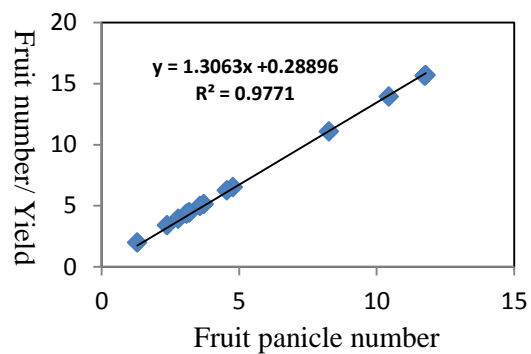


Figure 1.3: Regression for quarternary branching.

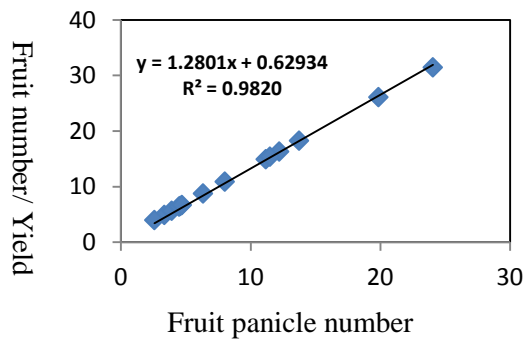


Figure 1.4: Regression for 5th branching.

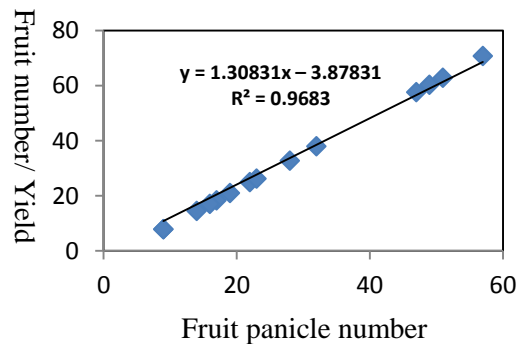


Figure 1.5: Regression for 6th branching.

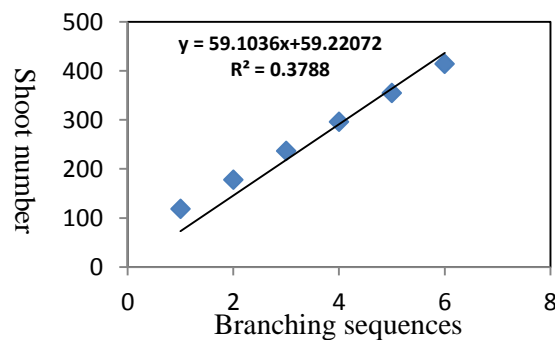


Figure 1.6: Regression between branching sequences and shoot number produced.

Conclusion

Positive correlation between shoot number as agronomic characteristics and number of flower panicle as reproductive characteristics is an important relation that can reflect to the yield (fruit number) of Harumanis trees. Pearson's correlation coefficient (COR) and further separation by stepwise analysis regression was the basic and significant tool that can be used to estimate and predicted the recommended shoot number required for achieving the targeted fruit number. Therefore, it is a proper guideline for Harumanis producers for structural maintenance to enhance potential yield.

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Chapter 2

Stress Biology

Effect of Water Irrigation Techniques on Growth and Yield of Rock Melon (*Cucumis melo* Linn cv. Glamour)

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Introduction

Water deficit has been one of the important issues in climate change. Drought crisis, pollution and desertification is becoming more serious and hence become a great threat to global food supply, human health and may also threatens global peace (Davtalabsabet et al., 2013; Davtalab et al., 2014). Crop production in many of Middle East and Asia regions is essentially dependant on at least supplemental irrigation systems or new techniques so as to increase the efficient use of available water. Effective use of limited water supply could be done in several approaches through irrigation technologies and proper irrigation schedule (Dry and Loveys, 1999).

One of the latest techniques introduced to manipulate crop water use is the regulated deficit irrigation (RDI) (English et al., 1990). In this technique, the irrigation input is removed or reduced for specific periods (Battilani, 2000), and is widely used in the horticultural industry to improve fruit quality (Turner, 2001). The technique, regulated deficit irrigation (RDI) was developed to overcome shortcoming of an earlier technique, partial root zone drying (PRD) where half of the root zone is irrigated, while the other half is allowed to dry out for a certain period of time (Loveys et al., 2000).

There is a need to study the effect of different water irrigation techniques using rock melon (*Cucumis melo* Linn cv. Glamour) as a trial crop which is have highly economic value. This paper will discuss the effectiveness of two irrigation techniques, regulated deficit irrigation (RDI) and partial root zone drying (PRD) in promoting growth and yield of rock melon as compared to well-watered (WW) plants.

Materials and Methods

Experimental site

This experiment was carried out in the rain shelter unit at Komplek Taman Pertanian Universiti, Universiti Putra Malaysia, Serdang, Malaysia with average maximum/minimum temperatures of 23.0–33.0 °C. Rock melon (*Cucumis melo* L. cv. Glamour) seeds were sown in germination trays filled with peat and was placed in a growth chamber under controlled conditions for one week. At 7 days after emergence, uniform seedlings were selected and gently removed from the trays and transplanted into two jointed poly bags (containers) measuring 27x23x50 cm (length x width x height) (1242 cm²). Plantlets with well-developed lateral root systems and two true leaves were transplanted to the center of the jointed poly bags (containers) respectively filled with 2 kg of coconut coir dust (CCD). Plants were manually irrigated twice a day and kept well watered for a week until the root system were well established and developed split equally into both parts of the poly bag. The plants were then subjected to three different regimes of water treatments:

- (a) Control (WW), both root compartments were watered manually at 100% substrate capacity (SC).
- (b) Regulated deficit irrigation (RDI), both sides of the root system received water at 50% SC.

- (c) Partial root drying (PRD), one compartment was watered at 50% SC and the other compartment was left to dry for 2 days. The two compartments were alternately irrigated once in every 2 days.

Results

Leaf area

At 31 days after transplanting (DAT), lowest leaf area of rock melon receiving RDI and WW watering treatment were 3226.10 and 4937.50 cm² respectively. At harvest, 76 DAT leaf area were significant between all treatments, with maximum of 5665.20 cm² in WW treatment followed by the RDI plants (3369.10 cm²) (Table 1).

Plant height

At 31 DAT, maximum and significant height was recorded from WW plants (182.24 cm) followed by RDI plants (132.48 cm). At 76 DAT, maximum but insignificant readings of 291.69 and 218.74 cm were from WW and RDI plants respectively (Table 1). Lowest plant height was recorded from the PRD plants at all three harvests.

Stem diameter

At 31 and 76 DAT stem diameter was significantly different between treatments, with maximum of 9.64 mm and 9.93 mm respectively recorded from the WW plants followed by the RDI plants, 8.03 mm and 8.46 mm respectively (Table 1).

Table 1: Effect of different watering treatments on total plant leaf area, plant height and stem diameter of rock melon at 31 and 76 days after transplanting.

Treatments	Days after transplanting					
	31	76	31	76	31	76
	Leaf area (cm ²)		Plant height (cm)		Stem diameter (mm)	
WW	4937.50a	5665.20a	182.24a	291.69a	9.64a	9.93a
RDI	3226.10b	3369.10b	132.48a	218.74b	8.03b	8.46b
PRD	477.90c	1582.50c	40.70b	163.12c	7.37c	7.36c
LSD _{0.05}	597.31	478.21	74.80	44.69	0.43	0.48
Source	-----F (Pr > F)-----					
Treatment	169.89 (<0.0001)	219.34 (<0.0001)	11.03 (0.0098)	24.93 (0.0012)	84.62 (<0.0001)	86.02 (<0.0001)

Means within a column followed by the same letter were not different at $P \leq 0.05$ by the Least Significant Difference (LSD) test. (WW = Well-Watered; RDI = Regulated Deficit Irrigation and PRD = Partial Root Drying).

Stem dry weight

Results obtained showed an increase in stem dry weight with increase in planting time (31 DAT to 76 DAT). Stem dry weight from WW plants i.e. 13.88 g was maximum and significant to PRD plants at 76 DAT. Result was not significant to RDI with 8.55 g also at 76 DAT (Table 2).

Leaf dry weight

Leaf dry weight also showed an increase with increase in plant growth, with maximum and significant results obtained from WW plants followed by RDI plants at the respective 31 DAT and 76 DAT (Table 2). Again PRD plants showed the least increase in leaf dry weight at all harvests.

Root dry weight

Root dry weight also showed an increase with increase in planting time (Table 2). At the respective 31 and 76 DAT, WW plants showed maximum and significant increase as compared to other treatments (0.38 g and 2.16 g, respectively). The RDI plants recorded 0.29 g and 1.19 g at the respective 31 and 76 DAT (Table 2). Again PRD showed minimum root dry weight of 0.73 g at the final harvest.

Table 2: Effect of different watering treatments on leaf dry weight, stem and root dry weight of rock melon at 31 and 76 days after transplanting.

Treatments	Days after transplanting					
	31	76	31	76	31	76
	Leaf dry weight (g)		Stem dry weight (g)		Root dry weight (g)	
WW	35.47a	46.62a	9.48a	13.88a	0.38a	2.16a
RDI	18.52b	29.97b	5.49b	8.55ab	0.29b	1.19b
PRD	1.35c	9.94c	0.86c	4.65b	0.05c	0.73b
LSD _{0.05}	8.42	7.43	0.55	7.29	0.07	0.70
Source	-----F (Pr > F)-----					
Treatment	49.13 (0.0002)	73.15 (<0.0001)	714.73 (<0.0001)	4.83 (0.0562)	58.91 (0.0001)	12.85 (0.0068)

Means within a column followed by the same letter were not different at $P \leq 0.05$ by the Least Significant Difference (LSD) test. (WW = Well-Watered; RDI = Regulated Deficit Irrigation and PRD = Partial Root Drying).

Biomass dry weight

The total of plant biomass dry weight also showed an increase with increase in plant growth, with maximum and significant results obtained from WW plants followed by RDI plants at the respective 31 DAT and 76 DAT (Table 3). Again PRD plants showed the least increase in plant biomass dry weight at all harvests.

Table 3: Effect of different watering treatments on total biomass of rock melon at 31 and 76 days after transplanting.

Treatments	Days after transplanting	
	31	76
	Biomass dry weight (g)	
WW	45.35a	62.68a
RDI	24.32b	39.73b
PRD	2.27c	15.34c
LSD 0.05	8.59	12.50
Source	-----F (Pr > F)-----	
Treatment	75.25 (<0.0001)	42.97 (0.0003)

Means within a column followed by the same letter were not different at $P \leq 0.05$ by the Least Significant Difference (LSD) test. (WW = Well-Watered; RDI = Regulated Deficit Irrigation and PRD = Partial Root Drying)

Yield of rock melon

At the final harvest (76 DAT), yield of melon from the WW plants was maximum for all fruit parameters compared to RDI or PRD treatments. The selective parameters and their respective individual readings recorded are shown in Table 4.

Table 4: Effect of different watering treatments on yield (fruit weight, fruit size and total soluble solid) of rock melon cv. Glamour at 76 days after transplanting.

Plant stages Reproductive (76 DAT)	Fruit fresh weight (g)	Fruit dry weight (g)	Fruit length (mm)	Fruit diameter (mm)	TSS (°Brix)	Flesh thickness (mm)	Rind thickness (mm)
Treatments							
WW	710.74a	75.37a	122.47a	109.64a	14.02a	25.70a	4.68
RDI	511.11b	62.01b	99.03b	98.55b	13.83a	25.98a	4.54
PRD	144.11c	13.16c	60.94c	66.07c	5.99b	12.32b	4.50
LSD _{0.05}	53.24	7.18	8.38	2.17	3.23	4.09	0.47
ANOVA	-----F(Pr>F)-----						
	348.85 (<0.0001)	248.65 (<0.0001)	164.46 (<0.0001)	1301.13 (<0.0001)	23.98 (0.0014)	43.58 (0.0003)	0.44 (0.6660)
Pr > F	<0.0001***	<0.0001***	<0.0001***	<0.0001***	0.0014***	0.0003***	0.666ns

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$ by the Least Significant Difference (LSD) test. Within column in each variable significantly different between different water levels at different stages are indicated by: ***significantly different at $P \leq 0.001$, **significantly different at $P \leq 0.01$, *significantly different at $P \leq 0.05$, ns – no significant difference.

Discussions

Effect of water deficit on vegetative growth of melon

Water is essential for crop production since plants need water for growth, tissue expansion and yield production. Growth and yield of rock melon was significantly affected by the watering system. Plants receiving full water capacity (WW) grew better at both vegetative and the reproductive stage. At 76 DAT, total leaf area of WW plants (5665.2 cm²) is 41 % higher than that of RDI plants (3369.1 cm²) and 72% higher than that of PRD plants (1582.5 cm²). Significant leaf area is strongly supported by production of massive root systems of WW plants, allowing more efficient uptake of nutrients by these plants. The RDI plants produce 50 % less roots followed by minimum root production by the PRD plants.

Melon (*C. melo*) is a plant with shallow root depth and requires soil water to be maintained at a minimum of 65 % capacity (Suat et al., 2007). The alternate drying and wetting of these roots over a two-day period under PRD watering system, significantly reduced its vegetative growth at 31 and 76 DAT as compared to controls (WW) or RDI treated plants. Water deficit has been shown to alter ratio of photosynthetic product between root and canopy (Cui et al., 2009). Effect of water deficit under the PRD system has significantly decreased fresh and dry biomass production of melons (Kusvuran, 2010) and hence a significant drop in yield (> 50%) when compared to the other two treatments. This clearly illustrates that the PRD plants is most water stressed especially as the plant aged. Hence the significant drop in fruit yield and quality.

Effect of water deficit on yield of melon

In case of fruit diameter, effects of the irrigation techniques were significant between treatments. It is obvious that WW and RDI plants have almost similar fruit diameter, with lowest diameter of 66.0 mm recorded from the PRD plants. Fresh fruit weight of 710.74 g was also significantly higher from the WW plants given full water capacity. Overall, the RDI treatment showed superior results as compared to PRD plants in increasing fruit size, unit weight and diameter of fruit. Fruit weight of RDI plants was only reduced by 29% while that of PRD plants showed a reduction of 72% (144.11 g). Results obtained contradicts fruit yield of apple and citrus, where PRD treatment did not affect fruit yields of these two crops (Hutton and Loveys, 2011). For RDI, water supply in deficit is usually applied during

the period of slow fruit growth when shoot growth is rapid. This is useful in reducing excessive vegetative vigour while minimizing irrigation and nutrient loss through leaching (Du et al., 2015). Hence the plants receive the benefit of full fertilization to support good growth of melon.

Conclusion

In conclusion, water stress strongly affects growth and yield of melon in soilless culture. Results obtained from the current study showed that both vegetative and reproductive growth of *C. melo* plants was strongly affected by levels of watering technique applied. Compared to the full watering capacity (control), alternating wet and dry watering capacity (PRD) significantly reduced dry matter production and hence fruit quality and yield of these plants. Maintenance of water supply even at 50% substrate capacity (RDI) throughout plant growth resulted in higher dry matter production and hence fruit quality and yield of melon, slightly less yield than the well watered plants (WW). Overall fruit yield at the final harvest in descending order is: WW (710.74 g) > RDI (511.11 g) > PRD (144.11 g). Total soluble solids in descending order is: WW (14.02 °Brix) > RDI (13.83 °Brix) > PRD (5.99 °Brix).

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The Effect of Water Stress on Vegetative Development of Tomatoes (*Solanum lycopersicum* L.) cv. Micro-Tom and MT1

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Introduction

Tomato (*Solanum lycopersicum* L.) is a climacteric fruit, where the fruit texture softens during ripening due to high ethylene production and respiration rate (Alexander and Grierson, 2002). In Malaysia, tomato is one of the important agricultural commodities, and tomato consumption by Malaysians was estimated about 139,000 tons per year (FAO, 2015). El Nino phenomenon that hits the country every year affects the production of crops consumed by Malaysians. These hot and dry phenomena lead to water stress in crops including tomatoes and also cause loss of yield.

In general, water deficit causes molecular, biochemical and physiological changes that influence plant response, which eventually will affect plant yield and quality. Plant response to water deficit is complex because it depends on species, genotype, developmental stage and organ, as well as the duration and severity of the stress (Bray, 1997; Skirycz and Inzé, 2010; Cramer et al., 2011). During drought stress, plants minimize stomatal aperture to reduce water loss through transpiration and also to maintain its water status. However, stomatal closure limits CO₂ diffusion and thus, will affect photosynthetic metabolism and causes oxidative stress (Chaves et al., 2009). In addition, plant limits its growth as a strategy to reduce water consumption during drought stress.

The aim of this study was to investigate the effect of water deficit on vegetative development of two tomato cultivars, namely Micro-Tom and MT1. Micro-Tom is a dwarf cultivar of tomato and is a preferred variety for molecular genetic research due to its small size, rapid growth and easy transformation (Marti et al., 2006). Meanwhile, MT1 is a local variety which was developed by Malaysian Agricultural Research and Development Institute (MARDI), and showed tolerance to bacterial wilt and heat stress (Rejab, 1986). Micro-Tom is a model plant system for fleshy fruits (Meissner et al., 1997). Therefore, understanding its response to drought stress is a fundamental part in producing stress-tolerant tomato. To date, there was no study reported on MT1 response under drought stress. Hence, this study may provide information on how water deficit affects vegetative stage of Micro-Tom and MT1.

In our study, the vegetative stage of both cultivars was treated with water stress for three weeks and rewatered for a week. To determine drought effects on both cultivars, we measured plant height, number of leaves, branches and flowers produced. We also examined relative water content (RWC) of both cultivars treated with drought. Then, comparison was made to determine their sensitivity to water deficit.

Materials and Methods

Plant materials and growth conditions

Tomato cv. Micro-Tom was obtained from Makmal Bioteknologi Tumbuhan (MBT), UKM and tomato cv. MT1 was provided by MARDI. The tomato seeds were sown in the pots using soils and vermiculites with the ratio of 3:1. The tomato seedlings were grown under natural light conditions in the glasshouse located in MBT. All plants were watered once a day to maintain the soil moisture

before stress treatment begins. Plants were grouped into two sets which are well-watered (control) and subjected to water stress treatment.

Water stress treatment

Water stress treatment was applied on four week-old tomato plants, where the plants were still in the vegetative stage and had yet reached maturity. Water withholding was applied on the plants for three weeks before they were rewatered at the fourth week.

Morphological analyses of tomato plants

All destructive and non-destructive measurements of three replicates were taken on the same day, and once a week. Plant growth measurements included counts of leaf, branch and flower numbers. Plant height was measured from the soil surface to the top of vegetative apex.

Relative water content (RWC) measurement

A leaf was cut and its fresh weight was determined. Then, the leaf was immersed in water overnight and blotted-dry with paper towel. The leaf was weighted to obtain its turgid weight. The same leaf was then dried in an oven overnight to acquire its dry weight. In this experiment, three replicates were used. Relative water content (RWC) was determined as following: $(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100\%$ (Smart and Bingham, 1974).

Results and Discussion

Plant growth changes after stress treatment

In this study, we determined the effect of water stress on the growth of both tomato plants. We found that the height of MT1 was significantly reduced under stress treatment. The MT1 height was reduced by 46% whereas 31% reduction was observed for Micro-Tom (Figure 1A). Relative to control plants, both cultivars that underwent water stress also showed reduced number of leaves and branches. The leaf number for both cultivars was obviously decreased after two weeks of stress treatment (Figure 1B). At the end of treatment, we observed that water stress significantly affected MT1 plants as its leaf number was decreased by 78%. On the contrary, the leaf number of Micro-Tom was only reduced by 48%. In addition, the number of MT1 and Micro-Tom branches was decreased by 49% and 16%, respectively (Figure 1C). Comparison with Micro-Tom showed that the number of leaves and branches of MT1 was greatly affected by water stress. Furthermore, MT1 was wilted and almost died, whereas Micro-Tom was slightly wilted and its leaves were rolled on the fourth week (Figure 2B and 2D). The large size of the MT1 plant might be the reason why it was severely affected by water deficit. In contrast, Micro-Tom is a small size tomato plant and can resist water withholding. Overall, reduced plant growth indicated that both cultivars may reduce water consumption under drought stress. Additionally, cell expansion and cell growth were suppressed due to low turgor pressure (Jaleel et al., 2009).

Flowers production under water stress

Another strategy of plants coping with adverse environment is by escaping from the stress condition. Plants may complete its life cycle by flowering earlier before the onset of severe drought (Meyre et al., 2001). In this study, we observed that Micro-Tom produced several flowers after four weeks, which was earlier than MT1. More flowers of Micro-Tom were produced under well-watered conditions compared to water deficit (Figure 3). However, MT1 did not produce any flower in both conditions. These results showed that after rewatering, Micro-Tom underwent slight recovery, but MT1 did not. Micro-Tom might escape prolonged drought stress by producing flowers, so new seeds can be

generated. It is also possible that the shorter life cycle of Micro-Tom caused its earlier transition from vegetative to reproductive stage.

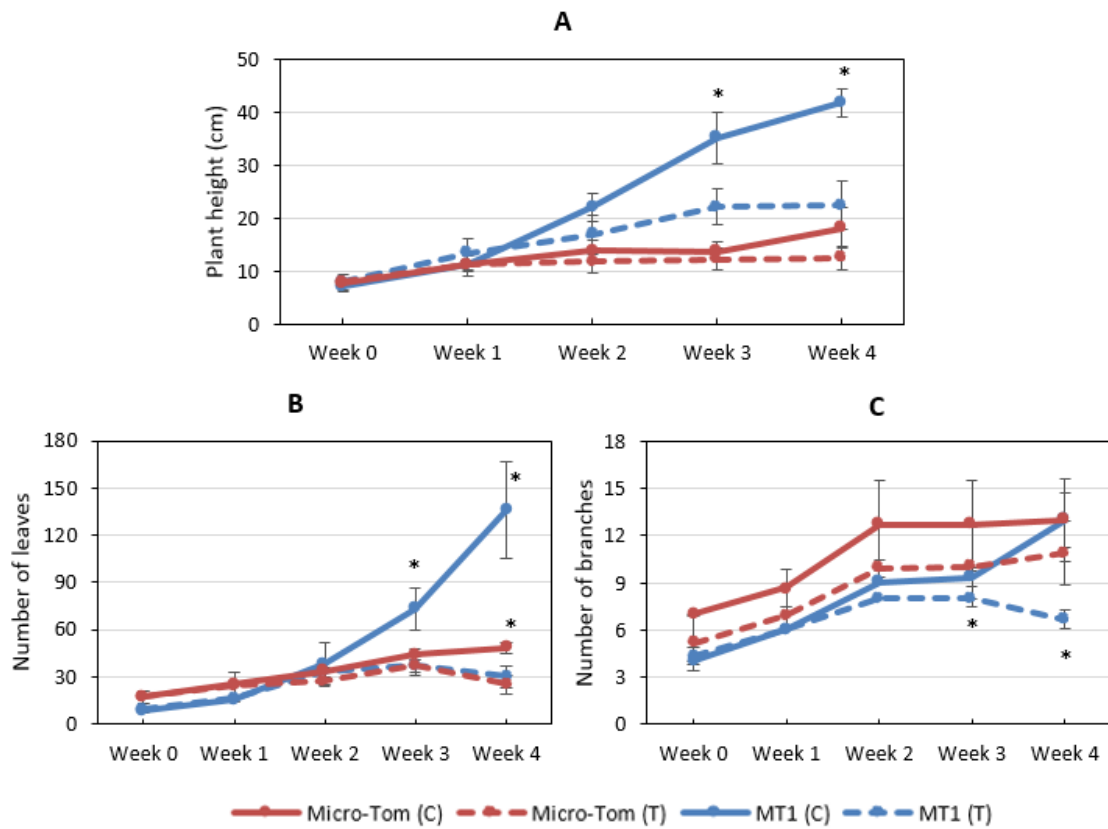


Figure 1: The number of leaves (A) and branches (B), and the height (C) of MT1 and Micro-Tom grown under well-watered conditions (C) and water stress (T). Asterisks indicate that the difference between control and treated plants was significant (T-test, p -value < 0.05), and error bars represent standard deviation ($n=3$).

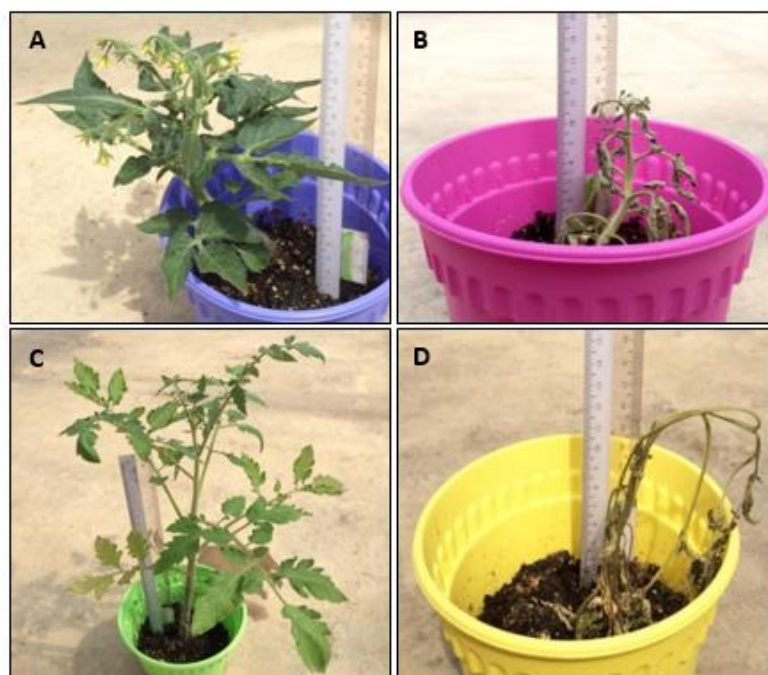


Figure 2: Tomato plants after four weeks of water stress treatment. A) Micro-Tom control, B) Micro-Tom under stress treatment, C) MT1 control and D) MT1 under stress treatment.

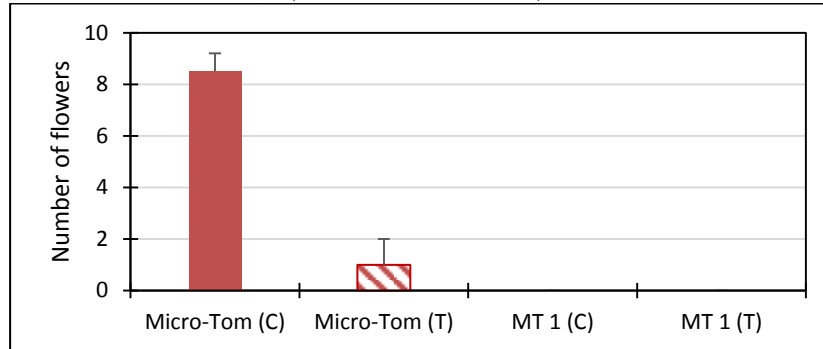


Figure 3: The number of flower produced by Micro-Tom and MT1 under well-watered (C) and water stress (T) conditions. Flowers were counted after four weeks of water stress treatment. Values are means ($n = 3$) and \pm standard deviation.

The water status of tomato cultivars under stress treatment

Relative water content (RWC) is a useful indicator of plant water status because it expresses the absolute amount of water that plant requires to reach artificial full saturation (Smart and Bingham, 1974). Water deficit reduces RWC values and hence, cause changes on plant metabolic and physiology. We found that the RWC of both tomato plants gradually decreased as water stress continues (Figure 4). It was unexpected that both cultivars under well-watered conditions had low RWC values ($<70\%$). This might have happened because of the leaves used for RWC determination were still young, small and not fully expanded. We also found that the RWC value of MT1 was lower than Micro-Tom after four weeks of stress treatment. This value corresponded to the phenotype shown by MT1 under stress conditions, where the stem was not well supported and the leaf lost its turgidity (Figure 2D). Overall, this result suggested that MT1 was severely affected by water deficit and did not recover after rewatering.

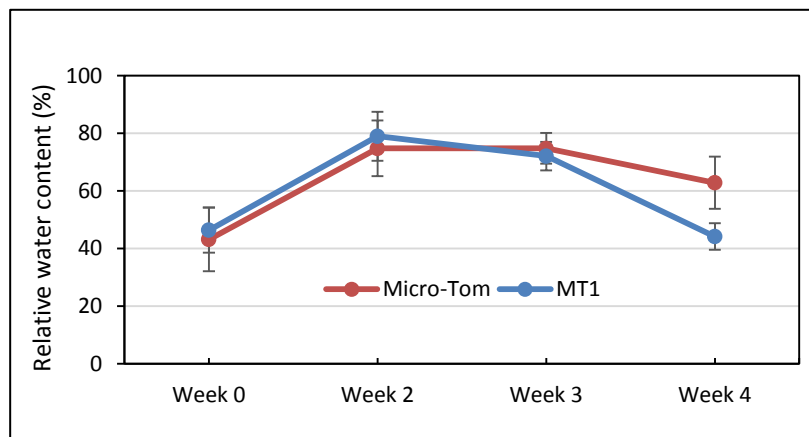


Figure 4: Relative water content (RWC) of Micro-Tom and MT1 under water stress conditions. Three replicates were used in RWC determination, and error bars represent standard deviation. Asterisk indicates that the difference between Micro-Tom and MT1 was significant (T-test, p -value < 0.05).

Conclusions

Our preliminary study suggested that MT1 was severely affected by water deficit compared to Micro-Tom. MT1 showed huge reductions in height, the number of leaves and branches, and relative water

content (RWC). In addition, MT1 did not produce flowers after rewatering and it did not recover as the plants were wilted at the end of this experiment. This indicated that MT1 was more sensitive to water deficit, whereas Micro-Tom had higher stress tolerance. Different size of cultivars may affect their response to water deficit. The large size of MT1 might be affected greatly by drought whereas the small traits of Micro-Tom might contribute to its better response to water stress. In conclusion, water deficit caused reduced vegetative growth of both cultivars, but more prominent changes can be seen in MT1.

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Wound Ethylene Evolution in RRIM 2025 and PB 350 during Low Yielding Period

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Introduction

Increased wound-ethylene production in response to mechanical stresses such as cutting, piercing, bruising, abrading, breaking or bending is common among higher plants (Wang et al., 2002; Druge, 2006; Che Husin et al., 2016). Mechanical wounding-induced endogenous ethylene production has led to xylem vessel plugging as a defence and healing response (van Doorn and Vaslier, 2002; Sun et al., 2007; Che Husin et al., 2013). Upon wounding, ethylene dependent physiological responses, including abscission and senescence have also occurred (Ichimura et al., 1998; Cameron and Reid, 2001; Jiang et al., 2002; Ella et al., 2003).

Exudation of latex upon tissue injuries is demonstrated as a defence trait in 10% of living angiosperm (Agrawal and Konno, 2009). While latex is not considered as medium for nutrient storage (Farrell et al., 1991; Lewinsohn, 1991; Hunter, 1994), it does contain a complex mixture of terpenoids, phenolics, proteins, and alkaloids, which are involved in plant defence mechanism (Langenheim, 2003). Previously, studies have denied latex flow at the tapping panel as a defensive mechanism, and the physiological and biochemical roles of ethylene have remained obscure (d'Auzac, 1989). Since 1984, endogenous ethylene concentration in excised bark from punctured, conventional tapped and spout wounds was examined in Hevea clones PB 235, RRIM 703, NAB 17, PR 261, RRIM 605 and PR 107 (Sivakumaran et al., 1984). It has been reported that wound ethylene was significantly higher in the wound-susceptible clones as compared to wound-resistant clones (Sivakumaran et al., 1984).

Mortex contains ethephon, 2-chloroethylphosphonic acid (CEPA) as an active ingredient. In plant system, ethylene is released upon uptake of ethephon by the cells (Warner and Leopold, 1969; Yang, 1969), and translocated to the phloem (Beaudry and Kays, 1988), where laticifers are located. In this study, we presume that Mortex stimulation and mechanical wounding by boring holes and tapping would induce wound ethylene evolution and latex flow in the mentioned Hevea clones, and thereby, promote plugging at the tapping panel as a defence mechanism of the species. The objective of this study was to evaluate the impact of mechanical wounding, i.e. mechanical boring and tapping following application of latex stimulant, Mortex, on the modern *Hevea brasiliensis* clones RRIM 2025 and PB 350.

Materials and Methods

Hevea clones RRIM 2025 and PB 350 were subjected to ½ spiral cut on panel BO-1 (original/virgin bark 1st panel) (Figure 1). Six treatments were evaluated, namely T1- mechanically bored trunk of untapped and unstimulated trees (control 1), T2 - Mechanically bored trunk of tapped and unstimulated trees (control 2), T3 - Mechanically bored, tapped and stimulated (BTS) trees with Mortex 0.75%, T4 - BTS trees with Mortex 2.5%, T5 - BTS trees with Mortex 5%, and T6 - BTS trees with 4 µL L⁻¹ (Ag⁺) Nanosilver (NS) as a control negative treatment (ethylene antagonist). Boring tree trunk was at 6 cm depth x 0.8 cm diameter using drill bit size 9 at 2 cm above tapping cut and 150 cm above ground (Figures 1.1 and 1.2) on day 0 of week 1.

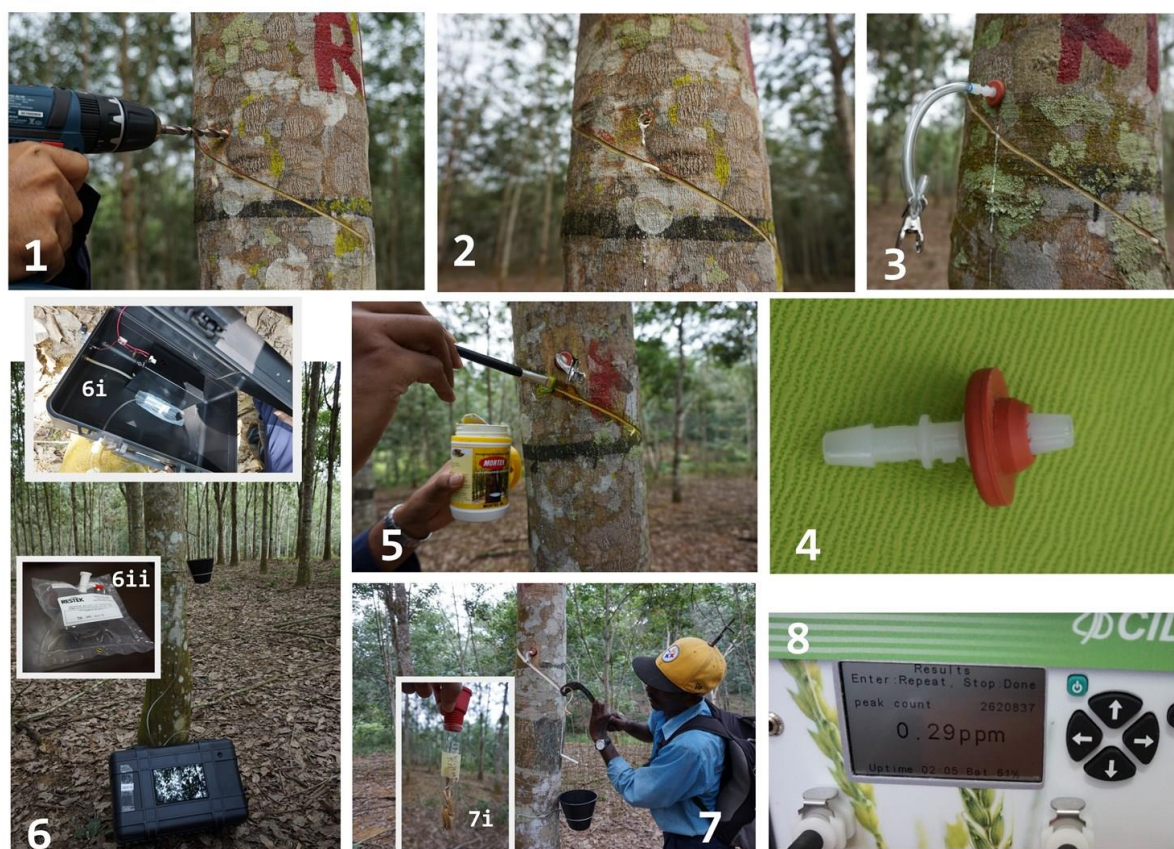


Figure 1: Collection of endogenous ethylene gas evolution in mechanically bored *Hevea* tree trunk and shaved bark, and measurement with portable ethylene analyser.

The hole was fitted with polyethylene straight connector (3/16) attached to rubber stopper and *ca.* 10 cm PVC tubing (5/32 x 7/32 x 1/32) (Figures 1.3 and 1.4) and then sealed with rubber sealant. The stimulation of T3 to T6 was by lace application (Figure 1.5) once a month for every monthly cycle. Prior to collection of endogenous ethylene gas, the holes were cleaned from any debris and coagulated latex using a pair of forceps. Incubation was for 24 h after tapping on every collection day. Prior to 24 h gas incubation in the bored holes, about 5 g of shaved bark after tapping was collected into a 50 mL glass tube enclosed with rubber sleeve cap (Figures 1.7 and 1.7i). During incubation, the tube end on the tress was clipped with paper clipper. The gas was then transferred into a 0.5 L ALTEF gas sampling bags equipped with 6"x6" w/polypropylene combo valve and septum (RESTEK. 110 Benner Circle, Bellefonte, PA 16823, US) (Figure 1.6ii) using vacuum procedure via *ca.* 1 m long disconnect PVC tubing attached to battery operated sampling pump vacuum bag sampler, model 1062 (Restex, USA) (Figures 1.6 and 1.6i). After every sampling day, the tube end on the trees was clipped with paper clipper to avoid perturbation by insects or water accumulation.

Gas and shaved bark samples were brought back to the laboratory in MRB Sungai Buloh for ethylene measurement with CI-900 Portable Ethylene Analyser (CID Bio-Science Inc. 1554 NE 3rd Ave, Camas, WA 98607, USA) equipped with GC injection port (using GC emulation menu) (Figure 1.8). For the accumulated gas in the holes, 15 mL gas from 0.5 L ALTEF gas sampling bags were analysed by direct injection into the portable ethylene analyser unit. The injection was performed using 25 mL Terumo® syringe with BD PrecisionGlide™ leur lock needles [21G, ½ TW (0.8mm x 38mm)]. Earlier, the portable ethylene analyser was calibrated with 15 mL standard ethylene at 0.15 $\mu\text{L L}^{-1}$ (BOC, Linde, Malaysia).

As for the bark sample, the sleeve cap of 50 mL glass tube containing shaved bark was detached from the glass tube and the tube was de-gassed (flushed) with a 18 V/DC diaphragm pump (GmbH, Fisher) or deflator battery air pump DC 12 V, 50W for *ca.* 1 min. Fresh ethylene gas was accumulated from equal volume of the sample in the same glass tube used for sampling for every experimental unit. The tube was then tightly enclosed with the rubber sleeve cap and incubated for 1 h in room temperature. 15 mL gas from the head space of the glass tube was then analysed by injection into a portable ethylene gas analyser following similar procedures aforementioned above.

Ethylene concentration was displayed on the monitor (Figure 1.8) and the calculation of actual endogenous ethylene production rates was determined as: ethylene production rate in (1) bored holes: $Er = [Ec/t]$ where Er = Ethylene production rate ($\mu\text{L L}^{-1} \text{h}^{-1}$), Ec = ethylene concentration in the ALTEF gas sampling bag read by the machine ($\mu\text{L L}^{-1}$), and, t = incubation period (h). (2) Wound ethylene production rate in shaved bark: $Er = [Ec \times Vf / Ws] / t$ where; Er = ethylene production rate ($\mu\text{L kg}^{-1} \text{h}^{-1}$), Ec = an ethylene concentration for the sample ($\mu\text{L L}^{-1}$), Vf = the free headspace volume of bottle/tube (L), Ws = fresh weight of shaved bark (kg) and t = incubation period (h).

Statistical analysis

Experimental design was CRBD and data were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) program of Statistical Analysis System (SAS®, SAS Institute Inc., Cary, NC, USA) release 9.2. Wherever the data was found significantly different by ANOVA, the means of treatment variables were compared by least significant difference (LSD) test at $P \leq 0.05$. Graphs were plotted using SigmaPlot 12.0.

Results and Discussion

Wound ethylene produced by the trees was accumulated in the bored holes (6 cm depth x 0.8 cm diameter) on the tree trunks of a total of 36 experimental trees for RRIM 2025 and PB 350 comprising six treatments in triplicates, each during low yielding period i.e. from March-May 2016. Stimulation was applied in week 3 of each month and sampling was carried out weekly giving 4 weeks recorded data for every month. The results show no significant difference of wound ethylene evolved from bored holes of RRIM 2025 and PB 350 (Table 1, Figures 2A and 2B). However, when compared between treatments, 2.5% Mortex ($0.0067 \mu\text{L L}^{-1} \text{h}^{-1}$) gave significantly higher mean wound ethylene production as compared to control 1 (untapped and unstimulated) ($0.0038 \mu\text{L L}^{-1} \text{h}^{-1}$). The results suggest that mechanical wounding by tapping and stimulation with 2.5% Mortex induced slight increase in wound ethylene production in the cambium of the trees. Meanwhile, the production of wound ethylene with other treatments was found not significantly affected by boring holes and/or tapping activity. There was no clear evidence of elevated ethylene produced prior to mechanically bored holes only, that was represented by lowest ethylene produced in control 1 (untapped and unstimulated). However, this outcome perhaps reflects on healing wall tissues of holes after six months of boring. No significant effect of ethylene antagonist was also observed (Table 1).

For bark sample, wound ethylene was accumulated from a shaved bark of ½ spiral tapped panel and incubated for one hour in 50 mL glass tube enclosed with rubber sleeve stopper. A total of 30 samples were analysed, but no sample was obtained from control 1. Generally, there were no significant differences between the clones tested. However, the results showed wound ethylene produced in the shaved bark of trees treated with 2.5% Mortex ($3.154 \mu\text{L kg}^{-1} \text{h}^{-1}$) was significantly lower than control 2 (tapped and unstimulated) ($3.568 \mu\text{L kg}^{-1} \text{h}^{-1}$), 5% Mortex ($3.556 \mu\text{L kg}^{-1} \text{h}^{-1}$) and $4 \mu\text{L L}^{-1}$ Nanosilver (inhibitor) ($3.560 \mu\text{L kg}^{-1} \text{h}^{-1}$) (Table 1). Thus, it appears that 2.5% Mortex contributed to lesser chemical stress that has led to less deterioration effect on wounded Hevea trees as compared to the 0.75% and 5% Mortex concentration.

Overall, wound ethylene was produced much higher in the barks as compared to the bored holes on the trunk (comprised of injured xylem) (Table 1 and Figure 2). Ethylene evolution in the holes and shaved bark was notably increased throughout the low yielding period from March to May 2016 (Figure 2). For the hole gas sampling in March 2016, evaluation started in week 3 giving only two weeks' data sets, where 4 $\mu\text{L L}^{-1}$ Nanosilver (0.0054 $\mu\text{L L}^{-1} \text{ h}^{-1}$) showed significant ($P=0.3261$) 4.9-folds higher wound ethylene evolution when compared to control 1 (0.0011 $\mu\text{L L}^{-1} \text{ h}^{-1}$). Meanwhile, all other treatments were not significantly affected as shown by the low level of wound ethylene as examined (Figures 2A and 2B). In the following month of April 2016, during week 1, control 2 showed significant ($P=0.3004$) 4.4-folds higher wound ethylene evolution (0.0040 $\mu\text{L L}^{-1} \text{ h}^{-1}$) as compared to control 2 (0.0009 $\mu\text{L L}^{-1} \text{ h}^{-1}$). All three Mortex treatments were not significant to each other in the whole month of April 2016. In week 1 of May 2016, treatment with 2.5% Mortex induced significantly ($P=0.1797$) higher wound ethylene (0.0089 $\mu\text{L L}^{-1} \text{ h}^{-1}$) as compared to both control 1 (0.0017 $\mu\text{L L}^{-1} \text{ h}^{-1}$) and control 2 (0.0097 $\mu\text{L L}^{-1} \text{ h}^{-1}$) (Figures 2A and 2B). All other treatments did not give significant effects of wound ethylene evolution in the following weeks of May 2016. Negative effects of ethylene antagonist, Nanosilver, was also pronounced. Nanosilver applied at a concentration of 4 $\mu\text{L L}^{-1}$ could be insufficient to inhibit wound ethylene evolution in Hevea. The distance of the hole from the panel cut where lace application of stimulants was applied was also a determining factor, where production of wound ethylene decreased with increased in distance.

Table 1: Mean wound ethylene evolution in Hevea clone RRIM 2025 and PB 350 after being stimulated with Mortex at three different concentrations of 0.75%, 2.5% and 5%, and NS (ethylene antagonist).

Clone	Mean ethylene evolution	
	Holes ($\mu\text{L L}^{-1} \text{ h}^{-1}$)	Barks ($\mu\text{L kg}^{-1} \text{ h}^{-1}$)
RRIM 2025	0.0056 ^a	3.616 ^a
PB 350	0.0053 ^a	3.303 ^a
<i>F-test probability</i>	0.487	0.006
<i>LSD</i> _{0.05 (n=180, 150)}	0.0024	0.738
Treatment		
Control 1 (untapped and unstimulated)	0.0038 ^b	nil
Control 2 (tapped and unstimulated)	0.0060 ^a	3.568 ^a
0.75% Mortex	0.0055 ^{ab}	3.457 ^{ab}
2.5% Mortex	0.0067 ^a	3.154 ^b
5% Mortex	0.0055 ^{ab}	3.556 ^a
NS (Control negative)	0.0053 ^{ab}	3.560 ^a
<i>F-test probability</i>	0.044	0.101
<i>LSD</i> _{0.05 (n = 60, 60)}	0.0017	0.738
<i>CV</i>	88.1	28.3

Values with a similar superscript letter are not significant at $P=0.05$

For the bark, in week 3 of March 2016, trees stimulated with 5% Mortex produced significantly elevated wound ethylene at an average of 4.729 $\mu\text{L kg}^{-1} \text{ h}^{-1}$, i.e. 2.3-folds higher than control 1 (2.074 $\mu\text{L kg}^{-1} \text{ h}^{-1}$) (Figures 2C and 2D). In week 2 of April, 1.8-folds of significantly ($P=0.0848$) higher wound ethylene evolution were recorded for the trees treated with 0.75% Mortex, which was at an average of 4.273 $\mu\text{L kg}^{-1} \text{ h}^{-1}$ as compared to an average of 2.525 $\mu\text{L kg}^{-1} \text{ h}^{-1}$ for control 2 (Figures 2C and 2D). In the following week 3, treatment with inhibitor, 4 $\mu\text{L L}^{-1}$ Nanosilver, induced 1.5-folds (5.251 $\mu\text{L kg}^{-1} \text{ h}^{-1}$) higher wound ethylene evolution in the bark of PB 350 as compared to treatment with 0.75% Mortex (3.416 $\mu\text{L kg}^{-1} \text{ h}^{-1}$) (Figure 2D). In general, results show no significant increment of wound ethylene evolution in week 3 for every month evaluation, i.e. the week when stimulations were applied. Higher wound ethylene in the weekly shaved bark, as compared to the accumulated gas in the holes, was attributed to fresh injury due to tapping activity during sampling. The bored holes on the trees were made six months earlier and were completely healed during the period of evaluation in this study.

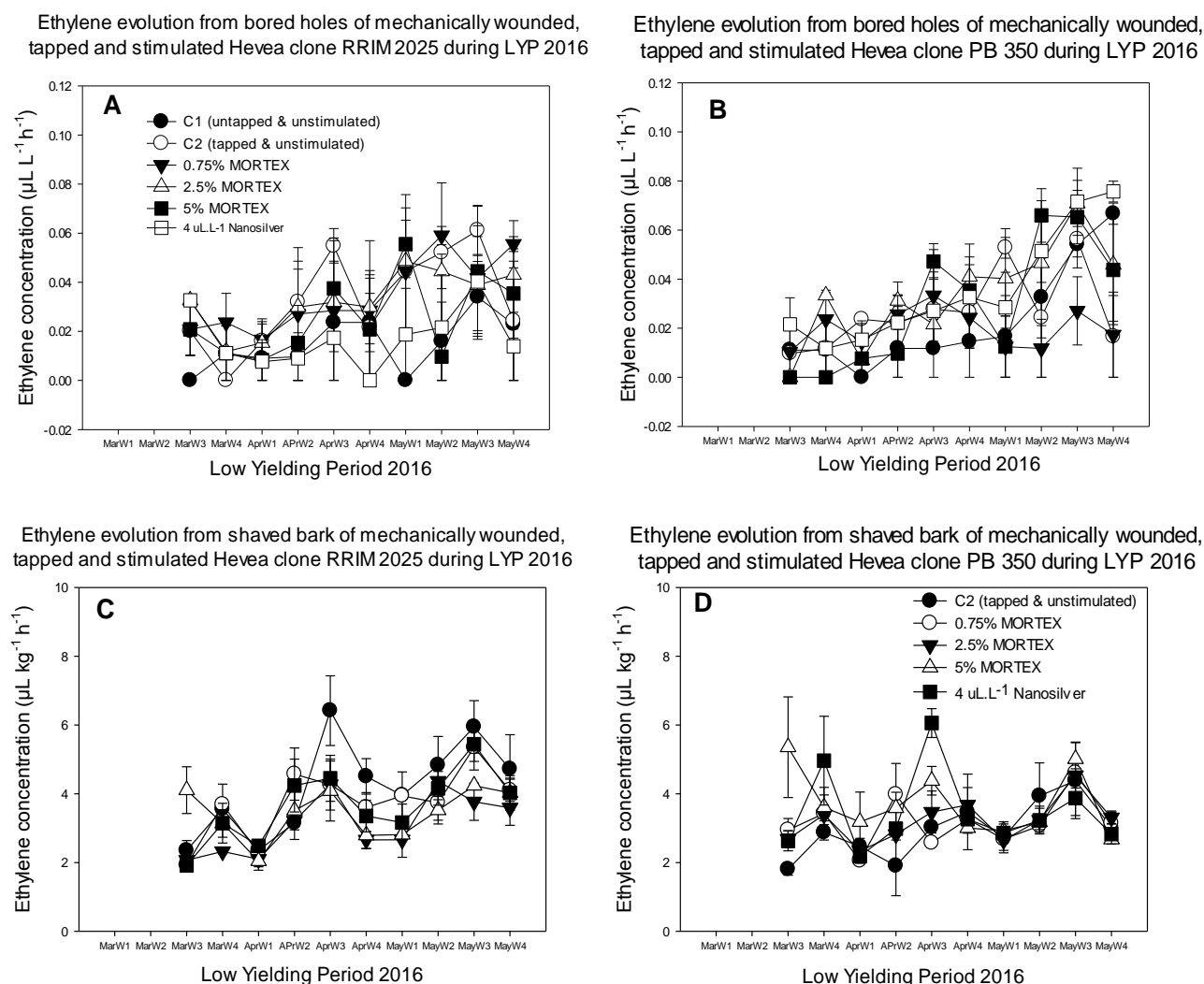


Figure 2: Wound (endogenous) ethylene production in RRIM 2025 and PB 350 after being mechanically wounded by bored holes, tapped and stimulated with Mortex.

We presume that exogenous ethylene treatment and mechanical wounding by boring holes and tapping activity would affect latex production and the physiological responses of *Hevea* trees including xylem vessels plugging, latex vessels plugging, panel dryness and healing mechanism.

Acknowledgements

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Responses of *Arabidopsis thaliana* in Acclimation to Decreases in Growth Irradiance

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Introduction

Due to the changing weather patterns, plants experience difficulties in surviving, so much so it could lead to stress conditions. Stress can be defined as an external factor that has detrimental influence on plants. Stress conditions can cause damage to plants and eventually can lead to death. To minimise the effects of stress, most plants are able to undergo a process named acclimation. A response mechanism to light irradiance is termed photoacclimation which is a phenotypic change in response to environmental adjustments in terms of light regime.

Work by Athanasiou et al. (2010) showed that *Arabidopsis* grown at a low light intensity ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) had the ability to photosynthetically acclimate when being transferred to a higher light intensity ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$). It was also found that the gene *At1g61800*, which encodes a Glucose-6-P/phosphate translocator (GPT2) is essential for this type of acclimation. GPT2 has a primary function of translocating sugar and phosphates across the chloroplast (Knappe et al., 2003). To confirm the impairment in acclimation of photosynthesis of *gpt2* mutants, mutants were complemented with a copy of the *gpt2* gene and it was shown that plants were able to acclimate. Therefore, it was concluded that GPT2 is important in dynamic acclimation to increased light in *Arabidopsis*. Previous studies also have shown that the *gpt2* gene is also induced during sugar-feeding and sugar-induced senescence (Gonzali et al., 2006; Li et al., 2006; Pourtau et al., 2006). In order to study the plant mechanism under fluctuating light, the direction of light changing was studied separately as from low to high light and high to low light. This is to give better insights to how plants respond to each of set of light condition. In contrast to the work of Athanasiou et al., (2010), this work was designed to understand the acclimation of *Arabidopsis* WS and WS-*gpt2* when plants were grown to maturity under high light condition and then transferred to a lower light condition. Plants were monitored by following the photosynthetic rate of low light plants upon transfer up to 9 days of acclimation. By doing the reverse acclimation, one question would be posed: Is this a simply the reverse of low to high light acclimation?

The amount of light used in high light and low light was determined by previous work so that it would not put the plants in a stress condition. Besides, the high light condition ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$) and low light condition ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$) are set where the acclimation is clearly seen and maximum photosynthesis (Pmax) rate is achieved at this growth light intensity.

Materials and Methods

Plant materials

Wild type seeds of Wassilewskija-2 (WS-2) and mutant seeds of WS-*gpt2* were sown onto soil and then placed at 4°C for two days before being transferred to 20 °C at low light ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$). The seedlings were left in low light for 7 days before being transferred to high light ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$). After germination, plants were grown in a growth cabinet (EJ Stiehl, Glasgow, UK) with light being provided by high frequency fluorescent lamps. The plants were put under high light condition (400

$\mu\text{mol m}^{-2} \text{s}^{-1}$) for six weeks. All plants were grown under eight hours light at $20 \pm 2^\circ\text{C}$ and sixteen hours dark at $16 \pm 2^\circ\text{C}$. After six weeks, half of the plants were transferred to low light ($100 \mu\text{mol m}^{-2} \text{s}^{-1}$). Control plants were kept at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Photosynthetic capacity measurement

The maximum capacity for photosynthesis was measured as the rate of photosynthesis at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ light and at 20°C . Measurements were carried out at 2000 ppm CO_2 . Immediately after the plant was removed from the growth cabinet, it was placed into a CIRAS 1 standard broad leaf chamber (area 2.5 cm^2). The plants were left in the chamber for 5 min until a steady-state of gas exchange level was reached. Afterwards, the plant was illuminated with an actinic light for 20 min, after which the value of photosynthetic capacity was recorded.

Chlorophyll fluorescence measurement

Simultaneous to the photosynthetic capacity measurements, chlorophyll fluorescence analysis was performed using a PAM 101 chlorophyll fluorometer (Walz, Effeltrich, Germany) to measure the chlorophyll fluorescence parameters of photosystem II efficiency (ΦPSII) and non-photochemical quenching (NPQ). Data were recorded on a PC using a National Instruments M series data acquisition card and running software written using Labview (National Instruments, Austin, US).

Prior to each chlorophyll fluorescence measurement, a plant was taken out of the growth cabinet and a full-size mature leaf was placed in the CIRAS 1 chamber while still attached to the plant. The leaf was left for 5 min in the chamber to equilibrate with the chamber environment. The fluorometer measuring beam was switched on to measure F_0 and the leaf was exposed to a saturating flash of $7500 \mu\text{mol m}^{-2} \text{s}^{-1}$ to determine the value of F_m . Afterwards, actinic light at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ was given for the next 20 minutes. During the 20 min interval, a saturating flash was given to the leaf every 120 sec to measure changes in F_m' over time.

The data from the fluorescence analysis was calculated for ΦPSII and NPQ using Equation 1 and 2.

$$\Phi\text{PSII} = \frac{(F_m' - F_t)}{F_m'} \quad (1)$$

$$\text{NPQ} = \frac{(F_m - F_m')}{F_m'} \quad (2)$$

Chlorophyll extraction

Following the measurements of photosynthesis, the same leaf was detached from the plant and the leaf area was measured by scanning using a Canon LiDE 20 scanner, with the leaf images being analysed using Scion Image (Scion Corp., Maryland, USA). The leaf was ground in a pestle and mortar in 80% (v/v) acetone. The extract was centrifuged using a microfuge (Progen) at full speed (16,000 g) for 5 minutes. The absorbance of the supernatant was measured using a USB2000 spectrophotometer (Ocean Optics, Dunedin, USA) and the absorbance value at 646.6 nm, 663.5 nm and 750 nm were recorded. The chlorophyll content was calculated according to Porra et al. (1989) as shown in Equation 3 and 4.

$$\text{Chl } a \text{ (ng / cm}^2\text{)} = \frac{13.71 (A_{663.5-750}) - 2.85 (A_{646.6-750}) \times 10\text{mL}}{\text{Leaf area (cm}^2\text{)}} \quad (3)$$

$$\text{Chl } b \text{ (ng / cm}^2\text{)} = \frac{22.39 (A_{646.6-750}) - 5.42 (A_{663.5-750}) \times 10\text{mL}}{\text{Leaf area (cm}^2\text{)}} \quad (4)$$

Statistical analysis

A data management software and statistics package SPSSv15 (IBM Inc. Chicago, Illinois, USA) was used to conduct a statistical analysis on the data. To test the data significance, a simple t-test and one-way ANOVA analysis where appropriate were carried out. The one-way ANOVA result was then followed with a Tukey's post hoc test with a significance level at 0.05.

Results and Discussions

Changes in maximum photosynthetic capacity during acclimation to low light

The photosynthetic capacity for *Arabidopsis* plants was measured after being acclimated to low light. WS and WS-*gpt2* plants were grown initially at $400 \mu\text{mol.m}^{-2}.\text{s}^{-1}$. "Low light" plants were moved to $100 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ for a period of 9 days. Photosynthetic capacity of these plants is then measured as the rate of photosynthesis with an actinic light at $1500 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ and CO_2 concentration at 2000 ppm. The photosynthetic capacity of both WS and WS-*gpt2* decreased during acclimation to low light (Figure 1A). When growing and measuring both WS and WS-*gpt2* in high light, the WS plants showed greater photosynthetic capacity than the mutant *gpt2* plants. In contrast, the treated plants of WS-*gpt2* had a higher photosynthetic rate under low light condition compared to the treated WS plants. This shows that the WS-*gpt2* plants acclimated less and the plants also had smaller photosynthetic capacity.

Changes in chlorophyll fluorescence parameters during acclimation to low light

Chlorophyll fluorescence analysis was performed mainly to investigate the quantum efficiency of PSII (ΦPSII) and the non-photochemical quenching (NPQ). ΦPSII measures the efficiency of PSII for photochemistry. In other words, it measures the proportion of light being absorbed by chlorophylls associated with PSII (Maxwell and Johnson, 2000). From the data (Figure 1 B), high light plants in both WS and WS-*gpt2* showed greater efficiency in PSII. However, low light plants had a much reduced efficiency in PSII. The PSII in low light plants might be too saturated with light absorption due to greater amount of chlorophylls.

Besides, NPQ was also measured to determine the ability of plants to dissipate excess excitation energy as heat. From the data (Figure 1 C), low light plants in both WS and WS-*gpt2* had lower value of NPQ. Theoretically, this tells low light plants quenched less energy from their system. Although the reverse effect was expected (low light plants quenched more energy), these factors might give an insight of the NPQ pattern seen here – ΔpH , zeaxanthin (xanthophyll cycle) and PsbS protein.

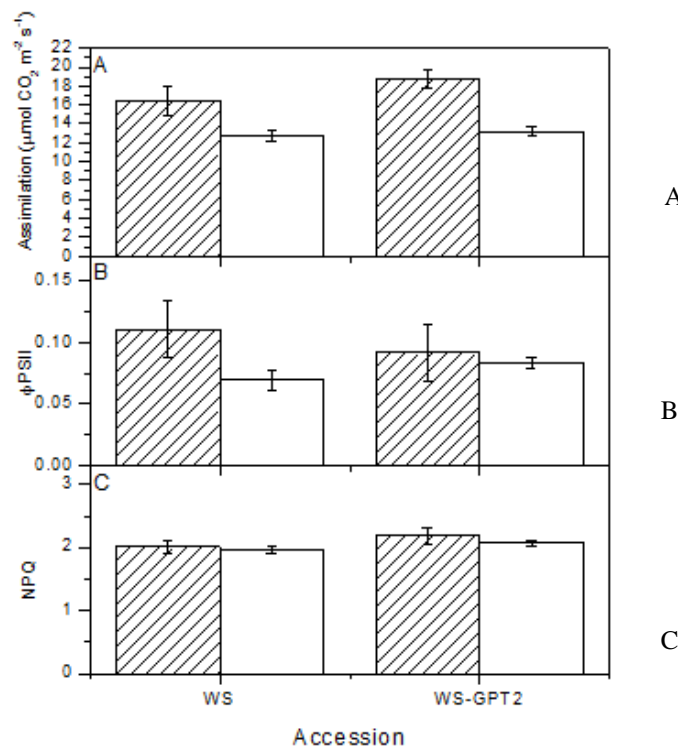


Figure 1: Acclimation responses of (A) maximum photosynthetic capacity, (B) PSII efficiency and (C) non-photochemical quenching (NPQ) on WS and WS-gpt2 plants following transfer to low light. Plants were grown at $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (High Light; HL; hatched bar) for six weeks and half were transferred to low light at $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Low Light; LL; white bar). Plants were measured at an actinic light of $1500 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ and CO_2 concentration at 2000ppm. All data are mean \pm SE for at least 3 biological replicates.

Changes in chlorophyll content during acclimation to low light

Chlorophyll analysis provides information on the light absorbing capacity of the photosynthetic apparatus and the proportion of chlorophyll bound to light harvesting complex II (LHCII). Therefore, chlorophyll content analysis was performed to calculate the total chlorophyll and chl a/b ratio (Porra et al., 1989).

From this data, there was a substantial difference between the plants in WS and WS-gpt2 throughout. The LL plants in both WS and WS-gpt2 had a higher content of total chlorophyll consisting of chlorophyll a and chlorophyll b (Figure 2 A).

In Figure 2 B, the chl a/b ratio showed a substantial decrease in LL plants in both WS and WS-gpt2. These data clearly show that the amount of chlorophyll b increased slightly upon transferring to low light condition for both WS and WS-gpt2. This shows that there is an increase in the proportion of light harvesting complexes in relation to the amount of reaction centres. It possible also reflects a change in the PSII to PSI ratio.

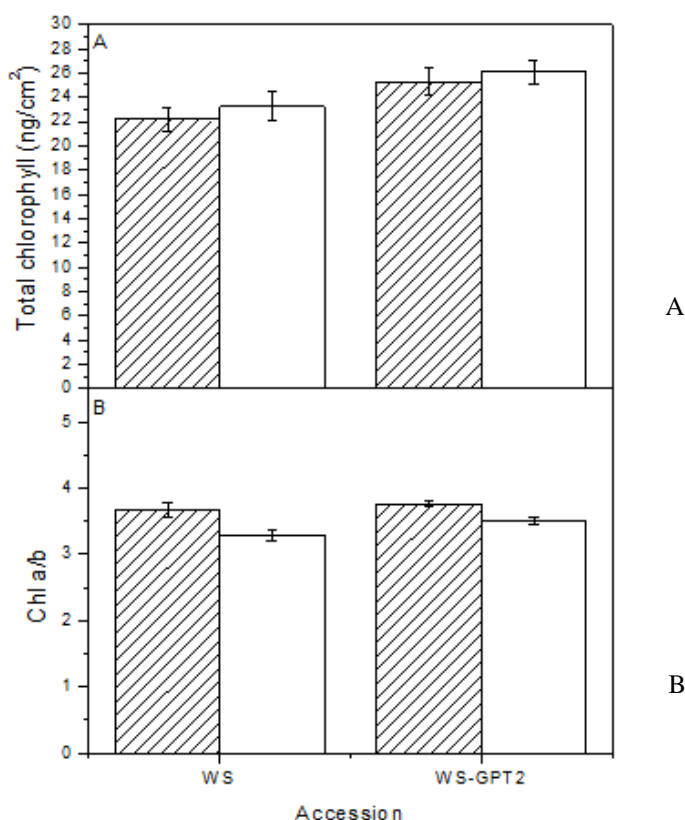


Figure 2: Acclimation responses of (A) total chlorophyll and (B) chl a/b on WS and WS-gpt2 plants following transfer to low light. Plants were grown at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (High Light; HL; hatched bar) for six weeks and half were transferred to low light at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Low Light; LL; white bar). The leaf used for maximum photosynthetic capacity measurement was used to estimate the chlorophyll content. The total chlorophyll content were calculated according to (Porra et al., 1989). All data are mean \pm SE for at least 3 biological replicates.

Photosynthetic capacity in WS using mature leaves was found to be decreased nearly 40% in low light plants (Figure 1A). Initially, plants were grown under high light by which sufficient light was available for conducting their photochemistry processes. However, when light becomes a limiting factor (under low light condition), there is not enough energy to drive the photochemistry processes at maximum potential rate. Since photosynthesis is a light-dependent reaction, insufficient light limits the overall rate of photosynthesis.

One of the fates of light absorbed by chlorophyll molecules is it can be re-emitted as light (chlorophyll fluorescence). ΦPSII is a parameter in chlorophyll fluorescence analysis that measures the quantum efficiency of PSII photochemistry. Both in WS and WS-gpt2 plants, low light plants had lower value than plants in high light at the saturating irradiance used to measure maximum photosynthesis. During long-term photoacclimation, many plants and algae change their antenna size associated with PSII and PSI as well as the ratio of PSI:PSII.

Since the low light plants have a larger antenna, they possess more chlorophylls per reaction centre so the rate at which light energy arrives at the reaction centre is faster at any given light intensity. This means that reaction centres work more efficiently at low light but they are more vulnerable to an oversaturation of PSII. When PSII is oversaturated, the electron transport will be less efficient. PSII will also be more vulnerable to photoinhibition. As a result, CO_2 fixation will be decreased. The observation (Figure 1 B) that ΦPSII is lower in low light acclimated plants is consistent with the idea

that the antenna size of PSII increases when plants acclimate to low light. There is however also a decrease in overall photosynthetic capacity at low light. Previously, Athanasiou et al (2010) did not observe consistent changes in Φ PSII during low to high light acclimation, suggesting that acclimation from high to low light is not simply the reverse of acclimation from low to high.

When plants are exposed to excess light, one of the short-term responses is non-photochemical quenching (NPQ) or feedback de-excitation. This response is switched on within seconds after the light exposure. When a low pH builds up in the thylakoids lumen, it switches the antenna into heat dissipation rather than trying to utilize the excess light (Kulheim et al., 2002). From the data (Figure 1C), it seems that the low light acclimated plants in both WS and WS-*gpt2* were less able to quench excitation energy than high light plants. This was unexpected as the low light plants absorbed more energy than they need and photosynthesis was more saturated. Previously, in the reverse acclimation to high light, no significant changes were found in terms of Φ PSII and NPQ (Athanasiou et al., 2010). Besides that, the total amount of chlorophyll (chlorophyll a and b) is seen to change during acclimation to low light. There was an increase in the total of chlorophyll in low light compared to high light (Figure 2A). There was no significant change in chlorophyll content upon acclimation from low to high light (Athanasiou et al., 2010).

In most species, depending on the light condition, differences in chl a/b ratio have frequently been reported (Moharekar et al., 2007; Pantaleoni et al., 2009). Hence, it has been taken as an indicator of a simple light acclimation response (Akoumianaki-Ioannidou et al., 2004). In this study, we found that the chl a/b ratio decreased in plants transferred from high to low light, compared to plants kept in high light (Figure 2 B). This is most likely due to an increase in the light harvesting complexes relative to reaction centre core. Reaction centre cores contain only chlorophyll a. Associated with the reaction centers are the light harvesting complex which contain both chlorophyll a and b. Thus, the expansion of the complexes results in an increase in chlorophyll b and decrease in the chl a/b ratio in low light plants. The ability of plants to change the amount of light harvesting complexes has been claimed to determine the plant's ability to change in response to light environment (Akoumianaki-Ioannidou et al., 2004). In contrast, acclimation from low to high light resulted in only very marginal changes in chl a/b, suggesting that this form of acclimation involved only small changes in antenna size. This is consistent with the observation that Φ PSII changes markedly during high to low but not low to high acclimation and reinforces the notion that these forms of acclimation are at least somewhat distinct processes.

Conclusion

In this study, we have measured the photosynthetic parameters – photosynthetic capacity, chlorophyll fluorescence (Φ PSII and NPQ) and chlorophyll composition (total chlorophyll and chl a/b ratio) which were affected during the acclimation from high to low light. In addition, we have shown that this type of acclimation (high to low light) is not simply a reversible process from the experiment done by Athanasiou et al., (2010). From the findings of this research, the farmers and plant growers could benefit from this information as it helps them on how to optimize crop production.

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***In vitro* Screening of Biological Control Agent of *Colletotrichum gloeosporioides* the Causal Agent of Mango Blossom Blight in Malaysia**

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Introduction

The mango tree is prone to be attacked by diseases such as anthracnose and stem-end rot. Anthracnose is recognized as the most important pre and post harvest fungal disease of mango worldwide caused by *Colletotrichum gloeosporioides* Penz. The post-harvest phase is the most damaging and economically significant phase of the disease worldwide. This phase is directly linked to the field phase where initial infection usually starts on young twigs and leaves and spreads to the flowers, causing blossom blight and destroying the inflorescences and even preventing fruit' set. Therefore, a control of blossom blight disease is crucial.

Fungicides, either as preharvest or postharvest treatments, form the main approach to reduce losses from anthracnose. However, their use is increasingly restricted due to public concerns of the accumulation of toxic compounds potentially hazardous to humans and the environment. It is therefore necessary to find an alternative to control the pathogen. Biological control seems to be a promising approach as an alternative to overcome the disease. Basically, biological control of plant diseases is a non-hazardous strategy that involves the use of an organism (or organisms) to inhibit the pathogen and reduce disease (Jaiswal et al., 2015). Few biological control agents (BCAs) have been reported against postharvest diseases of tropical fruit. Among the limited isolates, *Bacillus licheniformis* (Govender and Korsten, 2006), and preharvest application of the yeast *Rhodotorula minuta* (Patinovera et al., 2005) have been found effective for the control of postharvest diseases of mango.

Thus, the objective of the study is to evaluate the efficacy of BCA in controlling *C. gloeosporioides*, blossom blight disease in dual culture screening.

Materials and Methods

The pathogen causing blossom blight on mango plants, *C. gloeosporioides*, was isolated from infected mango flowers in the Malaysian Agriculture Research and Development Institute (MARDI) Sintok, Kedah. Five samples of diseased tissues were washed and dipped for 10 minutes in 10% clorox. Consequently, the tissue was re-washed with sterile distilled water for three times and subsequently dried. Then it was placed on potato dextrose agar (PDA). The isolates were identified by using the morphological characteristics and the pathogenicity of the fungus was confirmed through Koch's Postulate, inoculation of isolated fungus to healthy mango flowers (Plate 1). The fungus was then maintained in potato dextrose agar at room temperature.

For the biological control agents tested, designated B68, B85, B93 and B100 were isolated in fields of MARDI, Sintok. The bacterial strains designated S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 were obtained from Strategic Resource Research Centre, MARDI Headquarters, Serdang.

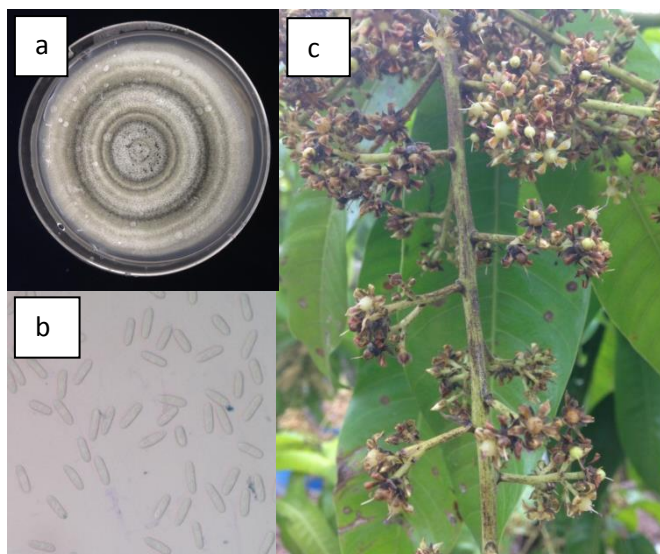


Plate 1: (a) *C. gloeosporioides*; Culture plate on PDA, (b) Spore under microscope observation (40 x) and (c) Koch Postulate on mango flowers.

Treatments

Screening was performed on PDA petri dishes by dual culture method (Zivkovic et al., 2010). A 5 mm of mycelia agar disc obtained from the 12 days old culture of *C. gloeosporioides* was placed 1.5 cm from the centre of the PDA petri dish. Then a loopful of bacteria was then streaked 3 cm away from the *C. gloeosporioides* isolate on the same petri dish. Control plates comprised only PDA with *C. gloeosporioides*. All the 15 treatments, bacterial strains designated B68, B85, B93, B100, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10 and control treatment was replicated five times and incubated under room temperature condition of $28 \pm ^\circ\text{C}$. The percentage growth inhibition (PGI) was calculated using the formula:

$$\text{PGI (\%)} = \frac{R1 - R2}{R1} \times 100$$

Where,

R1 = Growth of pathogen alone without antagonist (control)

R2 = Growth of pathogen along with the antagonist

Experimental design

The laboratory experiment consisted of 15 treatments including control with five replications was set up in completely randomized design (CRD) Data of the growth inhibition (in %) and inhibition zone were analysed using one-way ANOVA (SAS 9.3 TS Level 1M1). Differences within the means were compared by using Duncan's Multiple Range Test (DMRT).

Results and Discussion

The results showed that all the bacterial isolates significantly ($P < 0.05$) reduced the growth of *C. gloeosporioides* except for isolate S10, having a growth diameter of only 7.48 cm, grouping it significantly with the control treatments. Whereas, for isolate S8, growth of *C. gloeosporioides* was recorded with only 3.78 cm diameter growth. Results also stated that isolate S8 had the lowest growth of *C. gloeosporioides* amongst the other treatments.

Percentage growth inhibition (PGI) was taken into account to test the efficacy of the treatments. Results from the dual culture assay showed that these 14 bacterial isolates inhibited the mycelial growth of *C. gloeosporioides*, with varying efficiencies (Plate 2). The Duncan's test (Table 1) verified that isolate S8 exhibited the strongest antagonism against the *C. gloeosporioides* isolate with a high PGI mean value of 48.81%. While isolate S10 significantly showed no inhibition against *C. gloeosporioides*. PGI recorded for isolate S10 had -1.42%, indicating that the growth of *C. gloeosporioides* was faster in plates containing isolate S10 than the control treatments.

Plants are often colonized by many fungi and bacteria that do not cause any disease symptoms. Many of these may have beneficial effects on plant growth by providing essential nutrients to the plant (Harrison, 2005), indirectly making it less susceptible to pathogens, or directly protecting them through an antagonistic effect on pathogens (Wang et al., 2013). Ideally, a BCA that is able to provide and protect the plant is crucial.

Table 1: A comparison among the fifteen treatments on interaction between *C. gloeosporioides* and antagonistic bacterial strains.

Bacterial strains	Growth of <i>C. gloeosporioides</i> (cm)	Percentage of growth inhibition (PGI, %)
Control	7.38 a	N/A
S1	3.88 hi	47.39 ab
S2	4.00 ghi	45.76 abc
S3	4.66 def	36.81 def
S4	4.88 d	33.83 f
S5	4.75 de	35.59 ef
S6	4.06 ghi	44.95 abc
S7	6.78 b	8.14 h
S8	3.78 i	48.81 a
S9	4.70 def	36.27 def
S10	7.48 a	-1.42 i
B68	5.04 cd	31.66 fg
B85	4.36 efg	40.88 cde
B93	4.26 fgh	42.24 bcd
B100	5.32 c	27.86 g

Treatments with the same letters do not differ significantly ($P \geq 0.05$) according to the Duncan's Multiple Range Test.



Plate 2: Dual culture assay with *C. gloeosporioides* treatments: (a) Control, (b) isolate S8, (c) isolate S1, (d) isolate S2, (e) isolate S6, (f) isolate B93, (g) isolate B85, (h) isolate S3, (i) isolate S9, (j) isolate S5, (k) isolate S4, (l) isolate B68, (m) isolate B100, (n) isolate S7 and (o) isolate S10.

Conclusion

This study on *in vitro* screening has revealed that antagonistic strain S8, S1, S2 and S6 were promising biological control agents against the blossom blight disease. A serial screening is very important and necessary to get the best performance of BCAs. Thus, further research is needed to verify the efficiency of BCAs using life samples. A screening system involving the host plant, the pathogen and the antagonist is expected to give a more realistic picture on how the BCA will perform.

Acknowledgements

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Optimization of Starter Culture for Maximum Production of Dehydrogenases Enzyme Used for Fungicides Detection

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Introduction

Pesticides have been used extensively in agriculture for the removal of unwanted insects and pests and increasing farm production. They consist of insecticides, herbicides and fungicides which are considered to be one of the principal classes of environmental pollutants throughout the world. It was reported that excessive exposure to pesticides can lead to various diseases (Yadav et al., 2015). Despite the production of millions of tonnes of pesticides annually, they are very toxic compounds and responsible for ecological problems as well as damaging to human health, for example causing immunological and respiratory diseases (Carvalho, 2006). Dithiocarbamate fungicides have been extensively used in agriculture for many years to control a variety of diseases on vegetables, seeds and fruits (Mujawar et al., 2014). It is a group of organosulfur compounds that forms the most important class of fungicides which includes zineb, maneb, mancozeb, ziram and thiram. Table 1 shows example of dithiocarbamate active ingredients and products.

Table 1: Dithiocarbamate active ingredients and products.

Active constituent	Product
Mancozeb	Dithane [®] , Kencozeb [®] , Manzate [®] , Penncozeb [®]
Metiram	Polyram [®]
Propineb	Antracol [®]
Thiram	Vitavax [®] , Thiraflo [®] , Thiragranz [®]
Zineb	Zineb
Ziram	Ziram, Ziragranz [®]

Since dithiocarbamate group represents one of the largest consumed of fungicides in agriculture, it is very important to monitor and control their residue accumulations in vegetables and fruits especially during harvest (Rai et al., 2012). Although acute toxicity of dithiocarbamate compounds is low, these fungicides constitute a pesticide family of environmental concern since many reports suspected them of inducing neurological troubles resembling Parkinson disease (Soleo et al., 1996), carcinogenesis, teratogenesis, mutagenesis and goitrogenesis (Gupta, 2011). Furthermore, dithiocarbamate residues are easily transported in soils due to its solubility in water. According to Malaysian Food Act 1983 (2009 edition), maximum residue limits (MRLs) for dithiocarbamate in vegetables and fruits is 10 mg/L.

There is a need for rapid detection, simple procedure and on-site monitoring of dithiocarbamate residues in vegetables and fruits. Enzymatic determination of pesticides is most often based on inhibition of the activity of selected enzyme. Previous study has shown the capability of dehydrogenases enzyme secreted by *Bacillus* sp. in detecting dithiocarbamate compounds through inhibitory effects of enzyme in the presence of dithiocarbamate functional groups (Azima et al., 2014). In this study, we focused on starter culture optimization for maximum production of dehydrogenases enzyme from the bacteria which included the optimization of standard inoculums and the starter culture volume.

Materials and Methods

Optimisation of standard inoculums

A single colony of *Bacillus* sp. was cultured and grown overnight in special broth obtained from Taiwan at 37 °C. Bacterial cultures were then centrifuged at 5000 rpm. Supernatant was discarded. The cell pellet was then dissolved with 0.85% (w/v) NaCl. The optical density (OD) of the starter culture was optimized in the range of 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0. A two mL of each standard inoculum was transferred into 100 mL of broth and cultivated at 37°C for 16 hours. The colony forming units (CFU/mL) were counted using spread plate method to obtain the highest bacterial cells. Bacterial culture was diluted until the dilution of 10⁶. Experiment was performed in triplicates.

Optimisation of starter culture volume

The starter culture volume was carried out in different range (0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0 and 4.0 mL per 50 mL of broth using the optimized standard inoculums O.D of 0.6. The experiment was started by inoculating a single colony of *Bacillus* sp. in broth culture. The bacteria was grown overnight at 37 °C. Bacterial cultures were then centrifuged at 5000 rpm. The cell pellet was then dissolved with 0.85% (w/v) NaCl. The standard inoculum was set at O.D of 0.6. Each volume of starter culture bacteria was put into 50 mL of broth and incubated for 16 h at 37 °C. The colony forming units (CFU/mL) were counted using spread plate method. Bacterial culture was diluted until the dilution of 10⁶. Experiment was performed in triplicates.

Results and Discussions

Dehydrogenases enzyme secreted by *Bacillus* sp. has a potential to use in the detection of dithiocarbamate residues in vegetables using enzyme inhibition method. In order to detect the dehydrogenases enzyme produced by the bacteria, triphenyltetrazolium chloride or known as TTC was incorporated using method described by Bitton and Dutka (1986) as shown in Figure 1.

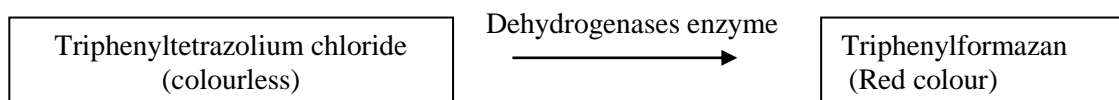


Figure 1: Reaction of Triphenyltetrazolium chloride in the presence of the *Bacillus* sp. Dehydrogenase enzymes.

In this study, starter culture was optimized in order to obtain large amount of bacterial cells for high production of dehydrogenase enzyme. Currently, the starter culture has been optimized as the following:

Optimisation of standard inoculum.

The optical density of starter culture was optimized in the range of 0.5 – 1.0 in which the colony forming units (CFU/mL) were counted to obtain the highest bacterial cells. Figure 2 shows the colony forming unit of various standard inoculums which was optimized at OD 0.6 and produced bacterial cells of 2.18 x 10¹⁰ CFU/mL. Subsequently, the amount of bacterial cells started to decrease at O.D of 0.7 and exhibited the lowest bacterial colony formed at O.D 1.0.

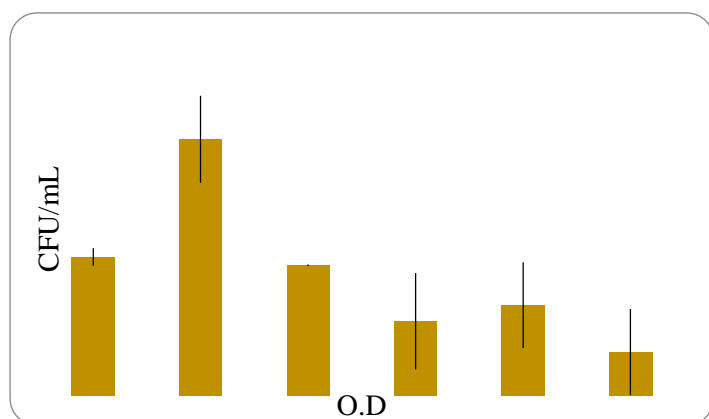


Figure 2: Optimization of optical density of the standard inoculums (mL)

Optimisation of starter culture volume

Optimisation of starter culture volume is carried out in the range of 0.5 mL to 4 mL per 50 mL of broth using optimized standard inoculum of 0.6. The results (Figure 3) showed that 0.5 mL was the optimize volume of starter culture to be added in 50 mL broth in order to grow maximum bacterial cells. The starter culture volume of 0.5 mL has exhibited the highest colony forming unit of 4.2×10^{10} CFU/mL of bacterial cells. Bacterial colonies start to increase at starter culture volume of 0.2 mL until 0.5 mL. At volume of 1.0 mL, the colonies formation starts to decrease and became plateau.

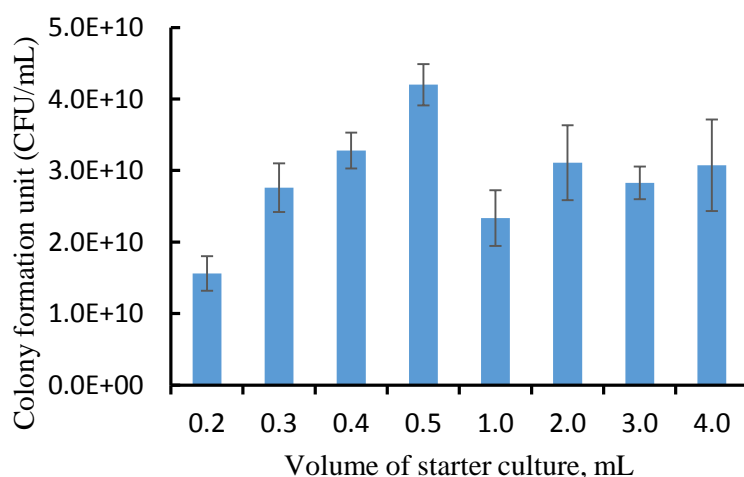


Figure 3: Optimization of starter culture

Conclusion

The optimized standard inoculum was achieved at O.D 0.6 which showed the highest bacterial cells growth of 2.18×10^{10} CFU/mL while the optimized volume of starter culture was at 0.5 mL in 50 mL broth culture. These optimum starter culture values will be further used for large scale of enzyme production.

Acknowledgements

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Towards the Production of Genetically Modified Rice with Resistance to Sheath Blight Disease

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Introduction

Rice sheath blight disease, or Penyakit Hawar Seludang Padi, is one of the major rice crop diseases. It is caused by a soil-borne pathogenic fungus, *Rhizoctonia solani* (*R. solani*) and has impacted heavy yield loss in rice production every year (Srinivasachary et al., 2010). Currently, the Rice and Industrial Crop Research Centre (RI) at MARDI is actively conducting conventional breeding for the development of a new, high quality rice hybrid which is resistant to the major pest and diseases to ensure consistent productivity and high yield per acre. However, breeding for sheath blight resistant rice variety is facing a lot of setbacks due to lack of identified resistant donors in the cultivated varieties. To date, no rice variety has been found to be completely immune to *R. solani*, although cultivars with varying levels of resistant have been reported (Yu-xiang et al., 2011). Alas, chemical protection remains as the major controlling method for this disease (Zhao et al., 2008). The continuous application and over-use of chemical fungicides not only cause environmental problems, but also increase the fungicide resistance in pathogens. Hence, an alternative method to produce a new hybrid with enhanced resistance against sheath blight disease is proposed by using genetic engineering approach.

Our previous findings showed there were significantly different gene expression profiles of several pathogenesis-related (PR) proteins involved in defense upon *R. solani* pathogen challenge between tolerant (Tetep) and susceptible (IR64) rice varieties by using quantitative PCR approach. Therefore, in this project, we would like to validate and understand the function of two candidate genes (*Chitinase* and *Thaumatin-like protein*) in MR219 rice during sheath blight infection. Over-expression of PR proteins, including chitinase (PR3) and thaumatin-like protein (PR5) in transgenic rice cultivars has shown an enhanced resistance against *R. solani* (Sridevi et al., 2008; Naseri et al., 2012). Chitinase is an efficient enzyme in the lysis of chitin of the fungal cell wall whereas thaumatin-like protein has potential to change the permeability of fungal membrane, which implies both genes have a positive anti-fungal activity (Kalpana et al., 2006).

Hence, this project involves genetic engineering method where resistant genes from Tetep rice will be isolated, characterized and transformed into local commercial cultivar (MR219) to generate transgenic rice resistant to sheath blight disease. Upon obtaining the transgenic lines, the plants will be challenged with *R. solani* in the controlled and contained environment under transgenic glasshouse condition to evaluate the degree of resistance.

Materials and Methods

Gene cassettes development

Two candidate genes *Thaumatin-like protein* and *Chitinase* related to plant defense mechanism against sheath blight disease which had been characterized previously were used in this study. The full length of both genes were first cloned into pGEM-T[®] easy vector (Promega) according to manufacturer's protocol before they were sub-cloned into pCAMBA 1305.2 plant transformation vector. This pCAMBIA 1305.2 vector has *nptII* (neomycin phosphotransferase) and *hptII* (hygromycin

phosphotransferase) genes as the selectable markers for bacteria and plants respectively. The positive gene cassettes were finally transformed into *Agrobacterium tumefaciens* strain EHA 105.

Induction of embryogenic callus

Embryogenic callus of rice cultures were initiated from mature embryos of MR219 rice variety. To induce callus formation, the mature embryos were cultured on the callus induction medium and grown at 25°C in the dark for one month. Callus induction media was prepared using Gamborg's B5 basal salts containing all vitamins supplemented with 10 g/L maltose, 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 10 mg/L naphthalene acetic acid (NAA), gelled with 4.0 g/L gelrite and pH adjusted to 5.8 before autoclaving.

Rice transformation

Embryogenic callus of rice obtained from mature seed embryos were transformed with pCAMBIA 1305.2 vector harboring individual above-mentioned candidate gene using *Agrobacterium*-mediated transformation method. Selection of putative transformed calli was carried out on selection media containing antibiotic hygromycin. The selection process was carried for a total of four months. The surviving calli on the selection media were individually transferred onto the regeneration media for further growth into the plantlets.

Results and Discussions

Isolation, characterization and construct development of Thaumatin-like protein and Chitinase

Two defense related genes that had potential against sheath blight disease obtained from previous study (Rohaiza et al., 2015) was selected for gene construction. The genes are *Thaumatin-like protein* (Pr5) and *Chitinase* (RCh2) belong to PR5 and PR3 family, respectively. Both genes were isolated from a tolerant rice variety (Tetep) using PCR based method. The full length of both genes was first cloned into pGEM-T[®] easy vector (Promega) according to manufacturer's protocol and transformed into *Escherichia coli* TOP10 competent cells. Subsequently bacterial colony PCR was performed to screen for successful transformants. Plasmid DNA was isolated from the identified positive clones. Restriction enzyme digestion was carried out to confirm the insert fragment and followed by DNA sequencing. Based on the results, the positive clones were then sub-cloned in sense orientation into plant binary vector pCAMBIA 1305.2. This vector has the *hptII* gene in the T-DNA region which is driven by the CaMV35S promoter and NOS terminator and confers resistance to the antibiotic hygromycin as a plant selection marker. There are two different gene cassettes were constructed. The first cassette harbouring both Pr5 and RCh2 gene fragments while the second gene cassette contains only either Pr5 or RCh2 gene fragment in one construct. The orientation and the reading frame were checked both by PCR and sequencing analysis.

Based on PCR validation for single construct, both Pr5 and RCh2 gave the correct amplification size. For pCAMBIA1305.2- Pr5-RCh2 stacked-gene construct, gradient PCR amplification at 56-60°C were conducted to investigate the optimum Ta for Pr5 and RCh2 genes inside the stacked-gene construct. Amplicons for RCh2 were detected for all five Ta tested. However, No Pr5 respective amplicon (552 bp) were detected. pCAMBIA1305.2-Pr5-RCh2 stacked-gene construct was sent for sequencing validation and based on the results it showed that both genes were detected in spite of the absence of Pr5 amplicon in PCR validation. The positive clones were then mobilized into *Agrobacterium tumefaciens* strain EHA 105 and were used in *Agrobacterium*-mediated transformation experiment.

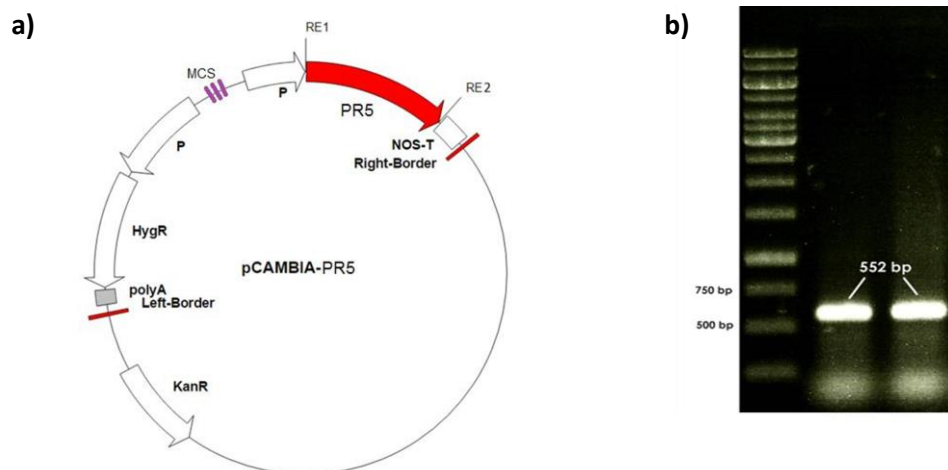


Figure 1: a) Map of pCambia- Pr5 (10336 bp). The gene of interest (552 bp) is controlled by 35S promoter and NOS terminator, and this gene replaces GUS gene.; b) PCR validation of insert in two pCambia1305.2-Pr5 clones. Pr5 specific amplicons were produced at Ta 58°C.

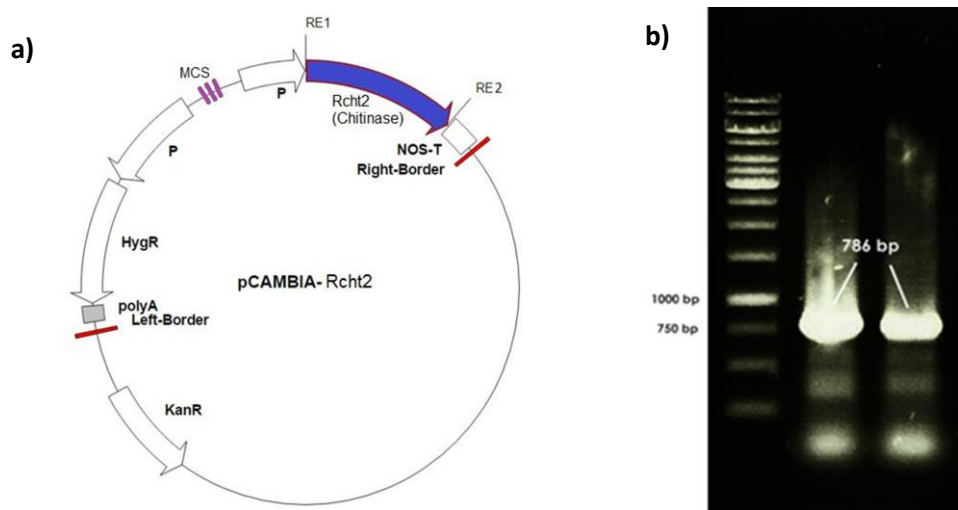


Figure 2: a) Map of pCambia-Rcht2 (10674 bp). The gene of interest (786 bp) is controlled by 35S promoter and NOS terminator, and this gene replaces GUS gene; and b) PCR validation of insert in two pCambia1305.2-Rcht2 clones. Rcht2-specific amplicons were produced at Ta 58°C.

Embryogenic callus induction and Agrobacterium-mediated transformation

Mature seeds of MR219 rice variety were sourced from MARDI, Seberang Prai, Penang, Malaysia. The mature seeds were de-husked and surface sterilized before culturing into callus induction media. Callus formations were observed within 2 weeks of culturing and more than 98% of the seeds cultured produced embryogenic callus after one month. The callus obtained was friable, granular and yellow in colour. One month-old embryogenic callus were used in *Agrobacterium*-mediated transformation experiment. For *Agrobacterium* infection, the density of the bacteria was adjusted to OD600 = 0.2. Embryogenic rice calli were immersed in a bacterial suspension for 30 min, and excess bacteria were removed by blotting the calli on sterile filter paper before transfer to co-cultivation media. Currently 1,200 and 1000 embryogenic calli have been transformed with pCambia- Pr5 and pCambia1305.2-Rcht2, respectively. While for pCambia1305.2-Pr5-Rcht2 stacked-gene construct, the

transformation process will be carried out once the embryogenic calli is ready. The transformed calli are still under selection process on hygromycin media and the selection process will be carried out for a total of four months. After 1 week of selection more than 50% of the calli started to turn brown. During the selection process, the calli were kept in the dark at 25 ± 2 °C and will be transferred onto fresh media every two weeks. Transformation of more embryogenic calli will be carried out periodically.

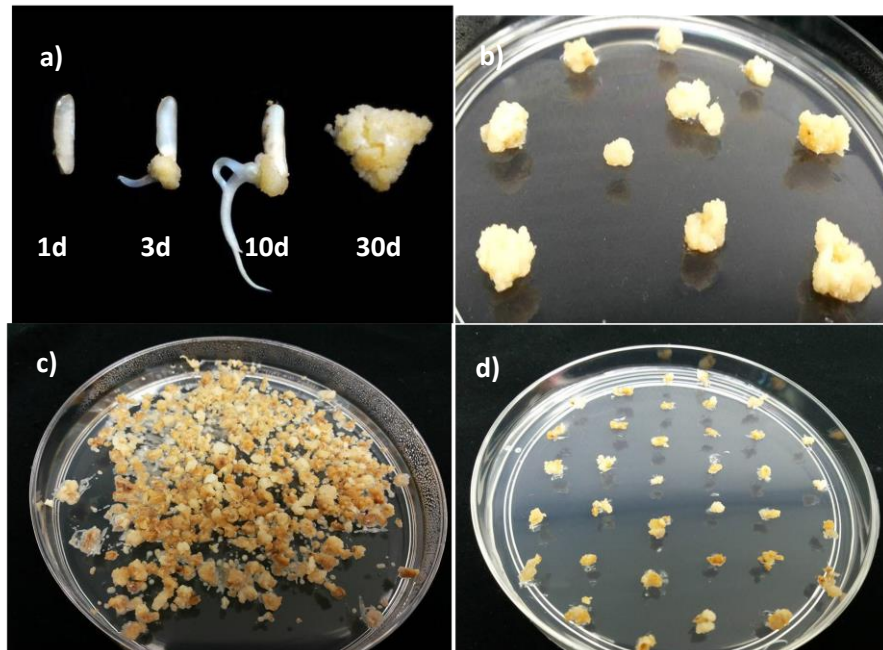


Figure 3: *Agrobacterium*-mediated transformation of embryogenic rice callus. a) Different stages of embryogenic callus induction; b) Embryogenic calli used in transformation; c) Agro-infection of rice calli and d) Transformed calli on selection media.

Conclusion

The current study indicates that 30% of the transformed calli are still survived after 3 months cultured on the selection media. The putative transformed calli will be transferred into regeneration media to get full plantlets.

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Acclimatization of Superior Clone *Labisia pumila* var. *alata* (KFeFRIM01) in Different Potting Media and Shade

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Introduction

Labisia pumila is an herbal plant from Primulaceae family. The plant is usually found in the tropical forest of Asean countries. This plant favors shady area and humus rich soil for growth. *L. pumila* is very synonym in treating women health and due to that a lot of *L. pumila* based products are sold in the market. *L. pumila* is commonly propagated through cuttings but for large scale production of planting materials, tissue culture technique using temporary immersion system (RITA™) is preferable. The process of tissue culture technique involved surface sterilization, culture initiation, shoots multiplication, *in vitro* or *ex vitro* rooting and acclimatization. The acclimatization process is a prerequisite in many species grown *in vitro* to ensure high plant survival and vigorous growth when transferred to soil. This is because, in the lab, the tissue culture plantlets were developed within culture vessels under low level of light, high humidity, sufficient sugar and nutrients for heterotrophic growth. Therefore, a step by step of acclimatization process is required for the plantlets to prevent transfer shock.

To date, there is lack of study reported on the acclimatization of *L. pumila* to *ex vitro* condition. Therefore, this study was conducted with the objective to identify the suitable potting media and percentage of shade for acclimatization of superior clone of *L. pumila* var. *alata* (KFeFRIM01). This superior clone of *L. pumila* var. *alata* (KFeFRIM01) was obtained from clonal trial study conducted previously by Farah Fazwa et al. (2014). The findings from this study can be used as a guideline for mass production of *L. pumila*.

Materials and Methods

In vitro rooted KFeFRIM01 plantlets were used in this study. The plantlets with the height of 2-5 cm were taken out from RITA™ and washed under running tap water to remove the traces of medium from the plantlets surface. After that, the plantlets were dipped into Thiram solution (fungicide) for few seconds to disinfect the plants. About 120 plantlets of KFeFRIM01 were planted in two different potting medium; 100% jiffy and 100% sand within three replicates (Figure 1). The plantlets were kept in a transparent plastic chamber (1.5 m x 1.0 m) with varying percentage of shade (0%, 50% and 70%) (Figure 2). The plantlets were watered once per day for 30 seconds to maintain the humidity. The survival rates and growth such as stem height, number of leaves, and leaf length and leaf width of the plants were recorded before and after acclimatization process. The environmental data for example temperature and humidity in each acclimatization bed were also monitored. The acclimatization process for KFeFRIM01 was conducted for a month period.



Figure 1: Different potting media used during acclimatization of KFeFRIM01 (a) Plantlets of KFeFRIM01 in the acclimatization chamber (b) 100% jiffy (c) 100% sand



Figure 2: Transparent plastic chamber with varying percentage of shade (a) 0% (no shade); (b) 50% shade; (c) 70% shade for acclimatization of KFeFRIM01 plantlets.

Results and Discussion

Effects of different potting media on the growth of KFeFRIM01 during acclimatization

After one month of acclimatization in transparent plastic chamber, it was observed that plantlets in 100% jiffy had 94% survival rate while 100% sand recorded 88.3% (Figure 3). In other study, Muhammad Fuad et al. (2015) reported that the usage of 100% jiffy during acclimatization of *Eurycoma longifolia* (tongkat ali) gave 100% of survival rate while 100 sand gave 67% of survival rate. The ability of 100% jiffy in retaining moisture compared to 100% sand could be the factor of high survivality. This is because during acclimatization, minimal watering is provided in order to harden the plants. In terms of growth, the analysis of variance (ANOVA) shows there was significant difference between two potting media (100% sand and 100% jiffy) with the growth of KFeFRIM01 except for leaf length variable. Based on Figure 4, plantlets in 100% sand had higher stem height (3.76 ± 0.07 cm) and number of leaves (5.81 ± 0.11) compared to 100% jiffy. In contrast, the leaf width of KFeFRIM01 was found greater in 100% Jiffy (1.40 ± 0.03) than 100% sand. While the leaf length of KFeFRIM01 in 100% jiffy and 100% sand was 2.21 ± 0.05 cm and 2.20 ± 0.05 cm respectively. The findings from this study, suggest that 100% jiffy was the suitable potting media for *L. pumila* var. *alata* (KFeFRIM01) during acclimatization.

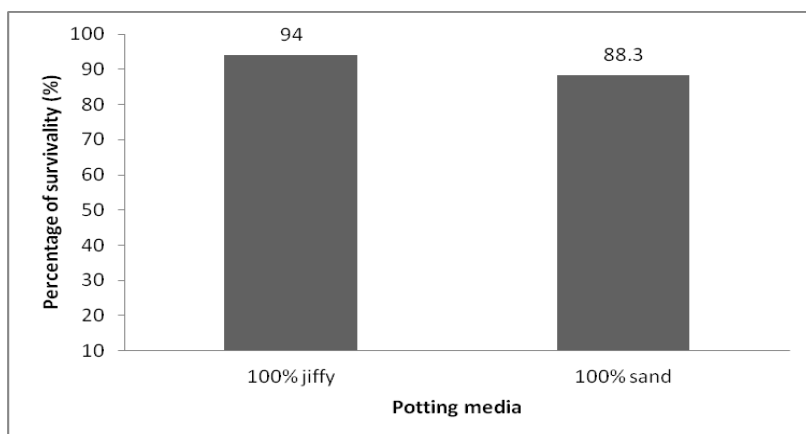


Figure 3: Percentage of KFeFRIM01 survivality in 100% jiffy and 100% sand.

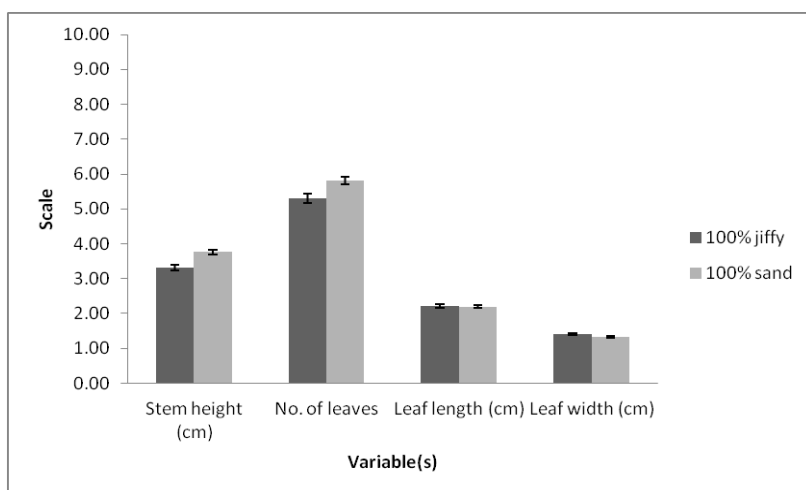


Figure 4: Growth of KFeFRIM01 in 100% jiffy and 100% sand.

Effects of different shade percentage on the growth of KFeFRIM01 during acclimatization

The plantlets of KFeFRIM01 were acclimatized in different percentage of shade for a month. The survivality of the plantlets was recorded 100% in 0% (no shade) followed by 50% shade (94%) and 70% shade (80%) (Figure 6). The ANOVA test was conducted to determine the effects of different shade percentage on the growth of KFeFRIM01. The result shows that there was significant difference between different percentage of shade with the growth of KFeFRIM01 (Figure 7). Plantlets of KFeFRIM01 grown in 0% (no shade) recorded the highest growth for all variables measured followed by 50% shade and 70% shade. The findings revealed that *L. pumila* var. *alata* (KFeFRIM01) can be acclimatize in transparent plastic chamber without shade (0%) and increasing the percentage of shade from 50% to 70% may contribute to mortality effect and slow growth. This finding is in line with Ginting et al. (2015) where the higher the percentage of shades, the lower the number of tiller in upland rice plants.

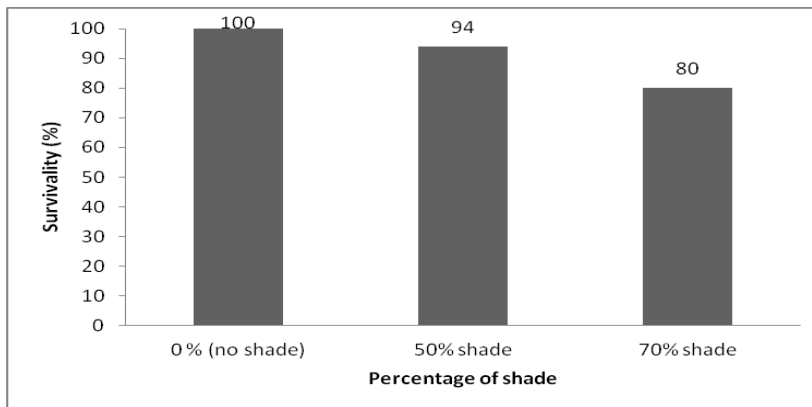


Figure 5: Percentage of KFeFRIM01 survivality in different percentage of shade.

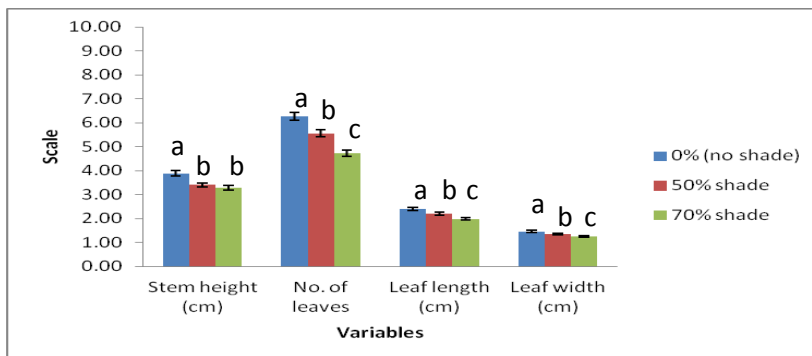


Figure 6: Growth of KFeFRIM01 in different percentage of shade.

Conclusion

The results from this study revealed that 100% jiffy is the best potting media and 0% (no shade) is the suitable shade requirement for acclimatization of superior clone *L. pumila* var. *alata* (KFeFRIM01).

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Expression Responses of NAC Transcription Factor Family Genes to Virus in Susceptible and Resistant Near-isogenic Rice Plants

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Introduction

The NAC gene family was named after three transcription factors: NAM (no apical meristem, *Petunia*), ATAF1–2 (*Arabidopsis thaliana* activating factor), and CUC2 (cup-shaped cotyledon, *Arabidopsis*), which share the same DNA-binding domain (Aida et al., 1997). Rice tungro disease (RTD) is a serious constraint in the production of rice in South and Southeast Asia. Rice plants affected by RTD show symptoms such as stunting and yellow to orange discoloration of leaves (Hibino, 1983). RTD is caused by two viruses, *rice tungro spherical virus* (RTSV) and *rice tungro bacilliform virus* (RTBV). These two viruses are transmitted mainly by green leafhoppers (GLH, *Nephotettix virescens*). RTBV can be transmitted by GLH only in the presence of RTSV (Hibino, 1983). RTBV is primarily responsible for causing the disease symptoms, whereas RTSV plays as a helper virus for insect transmission of RTBV, and also enhances the disease symptoms affected by RTBV (Hibino, 1983). RTSV has a single-stranded polyadenylated plus-sense RNA genome of around 12 kb encapsidated in polyhedral particles (Hull, 1996). RTSV alone generally does not cause evident symptoms in rice, except mild stunting (Hibino, 1983). Utri Merah is an Indonesian rice cultivar resistant to RTSV and RTBV (Encabo et al., 2009). Lee et al. (2010) reported that RTSV resistance in Utri Merah is controlled by a single recessive locus (*tsv1*) mapped around 22.1 Mb of chromosome 7. The *tsv1* locus contains a gene encoding translation initiation factor 4 gamma (eIF4G), one of the key components of translation initiation in rice (Lee et al., 2010). It is suggested that the mutations in the eIF4G gene in Utri Merah are associated with resistance to RTSV.

In this study, we investigated the expression of NAC family genes in the seedlings of two near-isogenic rice plants susceptible (TN1) and resistant (TW16) to RTSV using a customized whole genome oligoarray system to profile the transcriptomes of *OsNAC* genes during RTSV infection. The result indicated that several NAC genes or subgroups of this gene family are associated with a host defense system which is activated in RTSV-susceptible TN1 by the propagation of the virus, but not in resistant TW16. We also examined conserved *cis*-elements in the 2-kb region upstream of the promoters of differentially expressed genes (DEGs) during virus infection. We selected some NAC genes and confirmed our array data by RT-PCR analysis. Taken together, these results offer a solid basis for future functional genomic research of *OsNAC* genes.

Materials and Methods

Plant materials and virus accumulation

TW16 is a backcross line (BC5F7–8) developed from donor cultivar Utri Merah (International Rice Germplasm Collection accession number 16682) and recurrent parent Taichung Native 1 (TN1) (Lee et al., 2010). TN1 is susceptible to RTSV, whereas TW16 is resistant to in RTSV (Encabo et al.,

2009). The RTSV accumulation in plants was estimated by ELISA as reported by Shibata et al. (2007). All the tests were performed triple times (biological replications).

Preparation of RNA

Preparation of RNA was described previously by Nuruzzaman et al. (2010).

Microarray analysis

Cyanine-3 (Cy3) and cyanine-5 (Cy5)-labeled target complementary RNA (cRNA) samples were prepared from 850 ng total RNA using the low-input RNA labeling kit (Agilent Technologies, USA) in accordance with the manufacturer's instructions. Transcriptome profiles specific to infected plants were examined by the direct comparison of transcription activities between RTSV-infected and mock-inoculated plants on the customized oligoarray. Hybridization solution was prepared containing 825 ng of each of the Cy3- and Cy5-labeled cRNA preparations using an *in situ* Hybridization Kit Plus (Agilent Technologies, USA). The fragmented cRNAs were added to the hybridization buffer, applied to the microarray, and hybridized for 17-h at 60 °C. The microarray experiments were performed in triplicate with independent samples. The Cy3 and Cy5 signal intensities were normalized using rank-consistency filtering and the LOWESS method, processed by Feature Extraction version 9.5 (Agilent Technologies, USA). Expression patterns of all samples were transformed into log₂-based numbers and normalized using EXPANDER version 4.1 (Shamir et al., 2005) according to the quantile method for standardization of array slides. Differentially expressed gene (DEG) was defined as a gene whose log₂ (mean expression intensity in infected plant/in control plant) was ≥ 0.585 or ≤ -0.585 , and the difference in the gene expression change between infected and control plants was significant by a paired t-test ($\alpha = 0.05$, permutations, all possible combinations; FDR collection, adjusted Bonferroni method). Data processing was performed with MeV version 4.4 (Saeed et al., 2006). Gene expression data used in this study (GSE16142) is available at NCBI Gene Expression Omnibus GEO (<http://www.ncbi.nlm.nih.gov/geo/info/linking.htm>).

Cis-element analysis in the promoter sequence of up-regulated genes

This analysis was performed by Nuruzzaman et al. (2010).

RT-PCR analysis

DEGs were analyzed using RT-PCR and methods of these PCR were described by Nuruzzaman et al. (2010).

Results

RTD disease symptoms caused by RTSV and RTBV infections

In this study, we focused on RTSV virus infection mentioned in methods. RTD is caused by two viruses RTSV and RTBV. Rice plants affected by RTSV and RTBV at different dpi showed disease symptoms such as stunting and yelloworange discoloration of leaves (Figure 1).

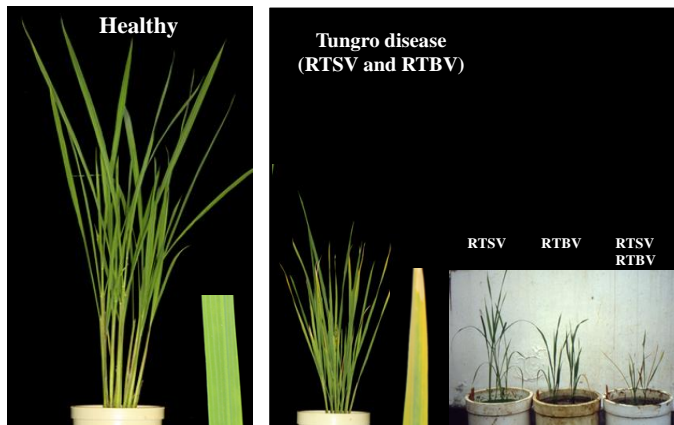


Figure 1: Tungro disease phenotype in infected rice plants by RTSV and RTBV.

Expression of NAC genes in susceptible and resistant plants during RTSV infection

To gain insight into the comprehensive roles of the *OsNAC* gene family members in response to RTSV, their expression patterns were investigated in infected rice seedlings by microarray analysis. Only the genes whose expression change was at least 1.5 folds (increased or decreased) were considered to have responded to RTSV (Figure 2). Out of 151 *OsNAC* genes identified in the rice genome, (Nuruzzaman et al., 2010) 112 were examined by our customized microarray, and 52 genes were found to be differentially expressed. About 46% (52) of the 112 differentially expressed *OsNAC* genes were detected commonly in both TN1 and TW16 (Figure 2). The *OsNAC* genes had been previously classified into 16 subgroups (Nuruzzaman et al., 2010). With the exception of *Os04g52810* and *Os11g31380*, DEGs belonging to subgroups NAC1 (60%) and ONAC1 (50%) were down-regulated in both TN1 and TW16 after RTSV infection. Many DEGs in subgroups ONAC2 (50%), ONAC3 (56%), and ONAC7 (80%) were more highly regulated in TW16 than in TN1 after RTSV infection (Fig. 2). Five genes *Os05g34310*, *Os07g37920*, *Os11g03300/SNAC10*, *Os11g08210/OsNAC5*, and *Os12g03040* belonging to the SNAC subgroup were up-regulated after RTSV infection in TW16, whereas the SNAC genes appeared to be predominantly suppressed in TN1 (Figure 2).

The greatest number of up-regulated genes (29) was found in resistant rice TW16 at 6 dpi. The lowest number of up-regulated genes (3) was observed in susceptible TN1 at 9 dpi (Figure 3). The greatest number of down-regulated genes (9) was found in TW16 at 9 dpi, whereas the lowest number (5) was found in TN1 and TW16 at 9 and 15 dpi, respectively (Figure 3). Overall, the *OsNAC* genes appeared to be more highly activated in TW16 than in TN1 after RTSV infection (Figure 3).

Selection of the most promising putative RTSV infection responsive candidate genes and subgroups

To identify putative candidate genes that are responsible for virus infection responses in the seedlings, this study focused on genes that exhibited high levels of expression in infected plants compared to control plants. Four genes *Os01g15640* (TIP), *Os08g23880* (ONAC2), *Os09g12380* (ONAC3), and *Os01g64310* (ONAC7) were found to be continuously up-regulated in TW16 by RTSV during the observation (Figure 2). In the rice seedlings, six genes *Os10g42130* (TIP), *Os04g52810* (NAC1), *Os11g08210/OsNAC5* (SNAC), *Os08g23880* (ONAC2), *Os09g12380* (ONAC3), and *Os01g64310* (ONAC7) exhibited higher expression levels (≥ 2 folds) in TW16 after RTSV infection (Figure 2). In addition, in the seedlings during RTSV infection, we noted that most of the genes (80%) assigned to the ONAC7 subgroup were highly expressed when compared with control (Figure 2).

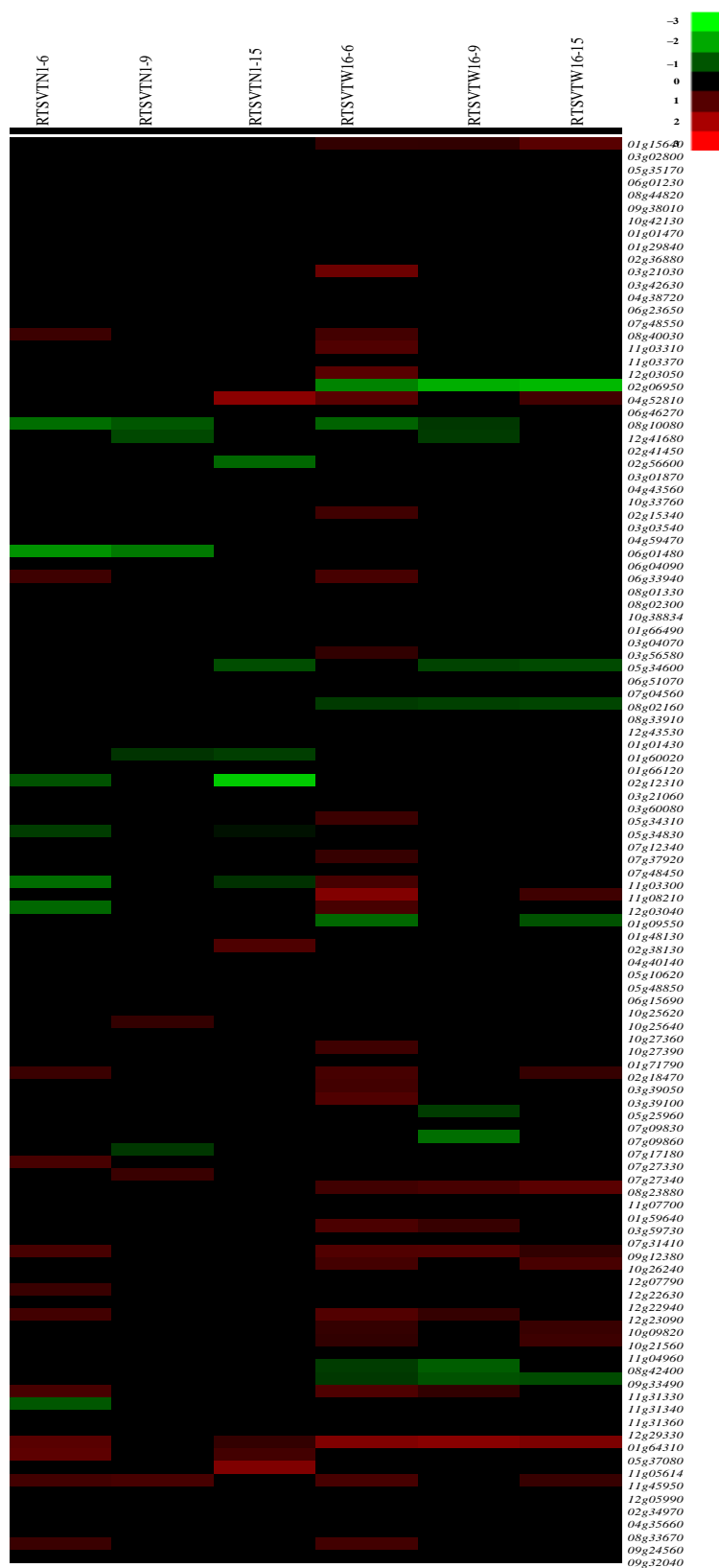


Figure 2: Differential expression of *OsNAC* genes under RTSV infection (\log_2 ratio); color bar at top shows level of expression. Red indicates expressed genes and green indicates unexpressed ones.

Consensus *cis*-regulatory elements for selected genes

The *cis*-regulatory DNA sequences control gene responses in different tissues and constitute the essential functional linkage between gene regulatory networks. We found that *cis*-motifs matching to RNFG1, ABRE, GT-1, GCN4, GluB-1, BPBF, WRKY, TATAboxIII, ARF, and MYC were the most abundant *cis*-elements in the selected *OsNAC* genes that were differentially expressed during RTSV infection. Several elements (RYREPEATVFLEB4, ARFAT, MYBGAHV, MYCATRD22, PYRIMIDINEBOXHVEPB1, ACGTABREMOTIFA2OSEM, ABREOSRAB21, ACGTABREMOTIFA2OSEM, P-box, WBBXPCWRKY1, BOXIINTPATPB, and GT1CORE) were reported to be linked to gene expression associated with tissue specificities, and responses to SA (Eulgem et al., 2000), and auxin (Goda et al., 2004). We found that most of the selected up-regulated *OsNAC* genes with tissue-specific expression profiles contained at least one of these above *cis*-elements, but uncommon *cis*-elements were found in the down-regulated *OsNAC* genes in the RTSV susceptible near-isogenic rice plants (data not presented).

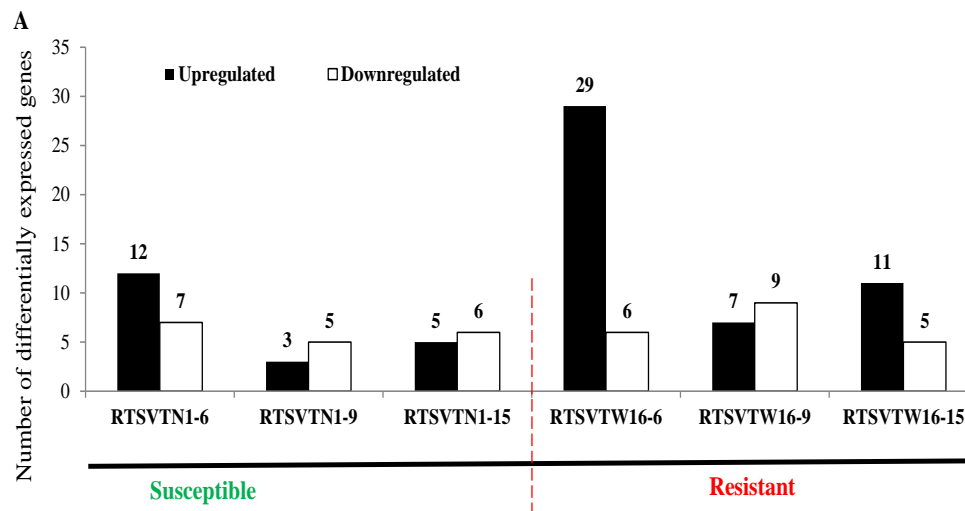


Figure 3: Number of differentially expressed genes (DEGs) in susceptible and resistant near-isogenic rice plants (TN1 and TW16). Infected with RTSV at different dpi. Y-axis represents the number of DEGs and treatments RTSV are indicated on the X-axis.

9 dpi		15 dpi		Gene name	Gene regulation	Primer sequences (5'-3')
TW16	Mock	TW16	Mock			
				<i>Os02g06950</i>	Downregulated	Forward: GCATTGGAGGGAAGGAATGG Reverse: CTACTCTCTACAGTCTTACTA
				<i>Os02g02160</i>	Downregulated	Forward: ATGGGGCACAGTGGTGGCGTTG Reverse: CTACTGCTGCTGATACCGCGTC
				<i>Os01g15640</i>	Upregulated	Forward: CGTGACATGGTCTCCACCAG Reverse: GGCTCCACCTATATATGTTCTG
				<i>Os08g23880</i>	Upregulated	Forward: ACGAGGAGGACGCCTACTTCC Reverse: CTCTCGTGTCCGAGTTGATG
				<i>Os01g64310</i>	Upregulated	Forward: GATATGCTTCTACCTCCGAAC Reverse: GTCGAGAGTCTCTAAGTGGTG
				<i>Os09g12380</i>	Upregulated	Forward: CTGATGGCTGGAGGAGTACAC Reverse: CTCTCGAGGCCGCTTAGTAGTA
				<i>Os11g06390</i>	Actin	Forward: AGTGCTCCTCGTCGTCGT Reverse: GAGCTGCAGGAGAAGTCAT

Figure 4: Evaluation for the expression levels of selected DEGs by RT-PCR under *rice tungro spherical virus* virus infection.

Expression analysis by RT-PCR

In Figure 4, gene primers are shown and rice *actin* gene (*LOC_Os11g06390*) was used as an internal control, whose expression remained nearly constant compare to microarray data under all experimental conditions.

Discussions

Viruses are a major agricultural constraint, thus, understanding the responses of crops such as rice, to virus infection is important for agricultural production. To establish infection in plants, viruses need host factors for their replication and for cell-to-cell and long-distance movement. Our goals in this study were to (i) know the expression patterns of members of the *OsNAC* gene family in plants susceptible and resistant to RTSV, (iii) select the best putative candidate genes for further functional analysis, and (iv) predict the important *cis*-elements in the promoter regions, which in turn may aid to elucidate gene functions and gene networks. Some *OsNAC* gene family members showed a strong response to RTSV infection in both resistant and susceptible plants, indicating their association with responses to RTSV infection.

Expression of the OsNAC gene family in response to RTSV

Virus infection affects plant growth development and morphogenesis processes, and the disturbance of gene expression by virus infection may lead to the development of disease symptoms such as dwarfism and yelloworange on leaves (Hibino, 1983). In this gene family, 52 (46%) non-redundant genes were up-regulated during RTSV infection (Figure 2). We observed that the number of genes up-regulated was highest at 6 dpi in TW16 during RTSV infection, followed by 15 and 9 dpi (Figure 3). The number of *OsNAC* genes with up-regulated expression was higher in TW16 than TN1 (Figures 2 and 3), which points that defense systems were activated in RTSV infection. One of the host defense systems against virus infection is the gene silencing system. The expression of genes involved in the gene silencing system is often activated by viral infection (Diaz-Pendo and Ding, 2008). Changes in gene expression in large numbers of *OsNAC* genes coincide with symptoms induced by RTSV infection of rice tested in this study, leading us to hint that members of this family contribute heavily to the plant response to virus infection. Among the *OsNAC* genes responding during RTSV infection, for example *Os01g15640*, *Os08g23880*, *Os11g08210/OsNAC5*, and *Os11g03300/SNAC10* were continuously activated preferentially in RTSV-resistant TW16. Moreover, six genes such as *Os10g42130* (TIP) and *Os04g52810* (NAC1) exhibited higher expression levels (≥ 2 folds) in TW16 after RTSV infection (Figure 2). Therefore, it is possible that these genes are involved in the regulation of rice seedling growth and development.

Role of different subgroups of OsNAC gene family

OsNAC genes play crucial roles in various developmental processes, including signaling, stress responses and plant defenses. About 35% of *SNAC* genes were up-regulated in resistant TW16 during RTSV infection (Figure 2). From our microarray analysis, we observed that *OsNAC* genes (*Os11g08210/OsNAC5* and *Os11g03300/SNAC10*) that were up-regulated during virus infection include those previously reported to be induced by abiotic stress such as cold temperature, drought, submergence, and different hormonal treatments (Nuruzzaman et al., 2010; Nuruzzaman et al., 2013). Around 33% genes of the NAM/CUC3 subgroup were induced during RTSV infection. Many genes of subgroups ONAC3 (55%), ONAC5 (67%), and ONAC7 (80%) were up-regulated during RTSV infection (Figure 2). In our study, six genes *Os10g15640* (TIP), *Os04g52810* (NAC1), *Os03g01870* (NAC22), *Os11g08210/OsNAC5* (SNAC), *Os08g23880* (ONAC2), *Os09g12380* (ONAC3), and *Os01g64310* (ONAC7) appeared to highly activated in TW16 than in TN1 after RTSV infection (Figure 2). Together, these results reported here suggest that up-regulation of the subgroups ONAC3, ONAC5, and ONAC7 genes or specific candidate genes may be involved in regulating the seedling

development and in the response to RTSV infection. While, many genes in the NAC subgroup NAC1 were down-regulated for RTSV virus infection. These *OsNAC* genes might be related to the health stage maintenance of the host plants. Therefore, through monitoring the changes of *NAC* genes' transcriptional data, it might be possible to discriminate the functional roles of host *NAC* genes after virus infection. There is high homology with known genes and tight clustering of members in each subgroup reported by Nuruzzaman et al. (2010). It is assumed that members of this subgroup may be involved in morphogenesis related to virus infection, plant growth and development processes. Although phylogenetic analysis provides important support for candidate gene selection, it alone cannot precisely indicate gene function but this study can help to understand the function and relationships of *NAC* transcription factors.

Responses of OsNAC genes during different treatments

We found that RTSV-responsive *OsNAC* genes were among those we previously reported to be activated by at least one of the treatments with naphthalene acetic acid NAA, GA3, SA, ABA or JA, and abiotic treatments (cold temperature, drought, and submergence) in rice seedlings (Nuruzzaman et al., 2010). In this study, *Os11g31380* (ONAC1), *Os10g09820* (ONAC5), *Os05g37080* (ONAC7), and *Os11g08210/OsNAC5* (SNAC) genes were induced after RTSV infection (Figure 2) and these genes were reported to be also activated by NAA, KT, ABA, and SA treatments (Nuruzzaman et al., 2010). Therefore, these key genes (*Os11g31380* and *Os10g09820*) might be involved in the defense system during RTSV infection and abiotic stress conditions. Before, we revealed that *Os03g21030* gene was induced in the leaf under severe and mild drought conditions and ABA treatments (Nuruzzaman et al., 2010), and we observed the same genes (*Os03g21030* and *Os11g08210/OsNAC5*) responding to infection by RTSV in this study. By these results, we suppose that there are *OsNAC* genes which function in ABA signaling pathways that are involved in the defensive response against virus infection.

The defense mechanism of OsNAC genes in plants

Gene expression patterns have been published for genes encoding proteins containing protein kinase, leucine-rich, NB-ARC, and EF-hand domains, which might function in signal transduction for defense systems (Tameling and Baulcombe, 2007; Li et al., 2009). *Os11g31380*, *Os10g09820*, and *Os05g37080* genes belonging to ONAC1, ONAC5, and ONAC7 subgroups, respectively, were reported to be involved in tissue specificities, and the responses to ABA, GA3, SA, auxin, and light (Nuruzzaman et al., 2010) and they contain *cis*-elements such as RNFG1OS, RYREPEATVFLEB4, ARFAT, and PYRIMIDINEBOXHVEPB1, or *cis*-motifs (e.g., ABRE, GT-1, GCN4, GluB-1, and BPBF) in their upstream regions. Some reports suggested that CaNAC1, BnNACs, and OsNAC6 members of subgroup SNAC, share common functions in the plant induction response to virus/pathogen infection and abiotic stresses (Nuruzzaman et al., 2010; Nuruzzaman et al., 2013). With the help of bioinformational analysis, it is significant to identify target genes for transcription factors involved in stress responses and to do comparative analysis of gene expression profiles during RTSV infection to determine the functional role of *OsNAC* genes in the growth of the plant and its response to virus infection.

Conclusion

The application of a comprehensive 44K oligoarray platform with different near-isogenic rice plants enabled us to determine gene expression profiles during infection of rice seedlings under RTSV infection. Interestingly, many genes in the *NAC* subgroups NAC1 and ONAC1 were down-regulated at all days tested during RTSV infection, while the genes in subgroups ONAC2, ONAC3, and ONAC7 were more highly expressed in TW16 compare to TN1 during RTSV infection. Some subgroups showed a high level of expression in virus infection, suggesting that they might have undergone functional divergence. Together with a number of putative *cis*-elements which were identified, may

help to clarify the function of these key genes in network pathways. Present work to function a number of selected genes through overexpression and mutant analyses is underway in our laboratory towards the optimization of molecular breeding schemes for the *OsNAC* gene family in rice.

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Chapter 3

Biotechnology

Regeneration of Latex Timber Clone RRIM 3001 via Somatic Embryogenesis

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Introduction

The Malaysian Rubber Board (MRB) has produced a number of *Hevea brasiliensis* clones to cater for the high demand for planting materials. In the current strategic plan, modern clones are developed for dual purposes i.e. latex and timber known as Latex Timber Clones (LTC). The development of *Hevea* as an attractive forest plantation programme caters for rubberwood industry, particularly for furniture, medium density fibreboard and moulding materials. This sub-sector of rubber plantation industry has contributed to the growth of 66.9% of export value to Malaysia in 2000 to 2010 (Mohd-Akbar and Ang, 2011). In 2013, MRB recommended selective clonal materials that were classified under two groups namely Group 1 and Group 2 based on their performance (mean yield). The clones under Group 1 are RRIM 928, RRIM 2001, RRIM 2002, PB 260 and PB 350, while RRIM 2007, RRIM 2023, RRIM 2024 and RRIM 3001 are classified as Group 2. Mean yield of Group 1 and Group 2 in large scale clone trial is 1,500 kg/ha/yr and 1,800 kg/ha/yr, respectively. In order to serve as a timber species, the trees should achieve a minimum clear bole volume of 0.3 m³/tree (Nurmi-Rohayu et al., 2015). RRIM 3001, reportedly produced 2,276 kg/ha/yr yield based on 3 years tapping on panel BO-1 (virgin bark panel 1), and recorded the highest girth increment after the 6th year of planting at 56.3 cm i.e. annual girth increment at an average of 10.6 cm/yr. The tree trunk of RRIM 3001 is straight with minimum branches at the crown part. At the age of 21 years, its clear bole volume can achieve 0.6 m³/tree, which is the highest among all the recommended clones (Nurmi-Rohayu et al., 2015).

Somatic embryogenesis is another tool to propagate *Hevea* clones, besides serving as a platform for genetic engineering. In the previous report, the presence of zeatin and kinetin has maintained somatic embryogenesis and plantlets regeneration of RRIM 2025 (Nor Mayati, 2015). Zeatin added at 0.5 mg/L in differentiation media enriched with IBA, AgNO₃ or NAA and BA showed a synergistic effect on embryogenesis and plantlets regeneration of this clone. Meanwhile, kinetin at 0.3 mg/L was found to have synergistic effects in enhancing embryogenesis and induced plantlets recovery while applied in the media containing IBA and BA (Nor Mayati, 2015). However, the study has suggested that medium composition was not the determinant factor. Embryogenesis of RRIM 2025 was reported largely dependent on the seasonal and origin of explant (Nor Mayati, 2015; Srichuay et al., 2014).

This study aims to develop specific media to enhance callogenesis and embryogenesis of *Hevea* clones particularly RRIM 3001. It is hypothesized that, an improved media containing cytokinin; zeatin and kinetin would enable the complete regeneration of somatic embryogenesis of this clone.

Materials and Methods

Anther explant for RRIM 3001 was collected from matured trees planted in Johor i.e. in Kota Tinggi Experimental Station (SPKT), Penawar Division, Malaysian Rubber Board. The initiation media used were; Murashige and Skoog (1962) with zeatin [MS(ID)]Z (Indian modification), Gamborg et al. (1968) (B5), and Schenk & Hildebrandt 1972 (SH). Callogenesis was induced for 40 to 45 days at 27 °C in the dark. After completion of the initiation process, callus was sub-cultured for 1 to 3 months onto solid differentiation media for an induction of embryogenesis. They are including; (i) Control: RRIM differentiation media 1 (RD1) containing 4.0 mg/L IBA + 0.5 mg/L zeatin + 0.5 mg/L AgNO₃ + 7% sucrose, (ii) RD1-C1 containing 4.0 mg/L IBA + 0.5 mg/L zeatin + 0.5 mg/L AgNO₃ + 0.2 mg/L

NAA + 1 mg/L BA + 7% sucrose, (iii) RD1-D1 containing + 0.4 mg/L IBA + 0.5 mg/L zeatin + 1.0 mg/L BA + 7% sucrose, (iv) RD1-E1 containing 0.4 mg/L IBA + 0.3 mg/L kinetin + 1.0 mg/L BA + 7% sucrose, and (v) RD1-E2 containing + 1.0 mg/L IBA + 0.8 mg/L kinetin + 1.5 mg/L BA + 7% sucrose. Differentiation was performed in the dark at 27°C for another three months. After the completion of embryogenesis stage, all the embryogenic calli were then desiccated for ten days followed by transferring onto differentiation media RD2 and RD3 for 1 month consecutively. The plantlets obtained were then transferred onto developmental media DM94 for further growth before undergo acclimatization in a light room under phytotron equipped with TLD 36W/54-765 cool daylight fluorescent tubes (Philips, Lifemax) for 12 hour photoperiod at *ca.* 25-27°C, RH at 50-70%, UV solar radiation = 12 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Wat/m²), Quantum (PAR) = 60-75 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

Results and Discussion

A total of 11,120 anther explants of RRIM 3001 that were cultured, from which the average callogenesis achieved was 33.93% (Table 1). Embryogenesis of this clone was quite promising i.e. at range from 1.44% to 8.96% given an average embryogenesis at 4.73% (Table 1). Vigorous calli and embryos were formed (Figure 1A and B), and about 1.13% of the embryos were rooted (Table 1, Fig. 1C). Calli induced onto B5 and SH initiation media successfully generated seven and four rooted embryos respectively. Embryogenic calli (Figure 1B) were mostly obtained after being differentiated onto either control RD1 containing 0.5 mg/L zeatin, RD1-E1 containing 0.3 mg/L kinetin, and (v) RD1-E2 containing 0.8 mg/L kinetin. Therefore an addition of kinetin at 0.3 mg/L and 0.8 mg/L would probably work for this clone (Table 1). Two plantlets regenerated were from anther initiated onto B5 that were differentiated in RD1-E1 (Table 1, Figure 2), suggested RD1-E1 containing 0.3 mg/L kinetin could serve as a potential media for complete regeneration of RRIM 3001 (Table 1).

Table 1: Regenerations of anther culture explant of Latex Timber Clone RRIM 3001.

Initiation media	Total explant cultured	Callogenesis (%)		Development (DM94)	
		Callogenesis	Embryogenesis	Rooted embryo/s	Plantlet/s
MS(ID)Z	3,450	18.17	RD1	5.26	
			RD1 C1	3.47	1*
			RD1 E1	1.63	
			RD1 E2		
B5	3,075	40.23	RD1	4.76	2
			RD1 C1		
			RD1 E1	1.44	2
			RD1 E2	3	2
SH	4,595	41.55	RD1	8.96	1
			RD1 C1	2.94	
			RD1 E1	5.43	2
			RD1 E2	8.71	1
Total/Average	11,120	33.93		4.73	11
					3

Interestingly, one plantlet was also successfully derived from “popcorn” callus differentiated onto MS(ID)Z media (Table 1- mark with an asterisk, Figure 2D). This type of callus in some cases produces plantlet by surpassing normal rooting and shooting inducement during the embryogenesis stage. This occurrence could be due to delayed transfer of callus while in differentiation media. An exceeding 45 days during the initiation stage, created stress to the culture, possibly due to the decreased of nutrients content in the media. It is believed that, the nutrient deficiency contributed to the absence of tap roots for this type of plantlet, and thereby, warrant for further research.

Regenerated plantlets were then transferred onto DM94 media with reduced glucose content from 7% to 3.5% for acclimatization for the subsequent three months under the lights at TLD 36W/54-765 cool daylight fluorescent tubes (Philips, Lifemax) for 12 h photoperiod at *ca.* 25-27 °C. The hardened

plantlets were well developed with fully young expanded leaves (Figure 2) and transplanted into the polybags containing soil and kept under the similar condition until transfer to the nursery. During acclimatization, light intensity was increased gradually to *ca.* 60 $\mu\text{mol/s}$, to create semi-autotrophic conditions, i.e. to increase chloroplast content and leaves number. The Semi-autotrophic condition will also induce hardening, a crucial trait of many perennial plants to improve successful transplant in soil. This technique will also reduce possible wilting and diseases that might affect the newly formed *in vitro* plantlets (Guido Bongi-pers. comm). Recently, lighting systems Philips GreenPower TLED with three light spectrums i.e. White (W), Deep red/white (DR/W) and Deep red/white/far red (DR/W/FR) with a photon flux ranging from 21 to 25 $\mu\text{mol/s}$ were also tested to further improve acclimatisation.

This experiment is being repeated to further improve regeneration of this clone. However, tissue culture of woody *H. brasiliensis* proves challenging. Tissue culture of this species is very much dependent on the origin of anther explant, where the juvenility and physiological ages of mother plant is an important element (Nor-Mayati, 2015). Besides, microclimate changes could also affect the quality of inflorescence, where rainfall, humidity, temperature and lights received by the trees have been shown to affect photosynthesis (Srichuay et al., 2014). High contamination during handling process in the laboratory is also a crucial determinant.

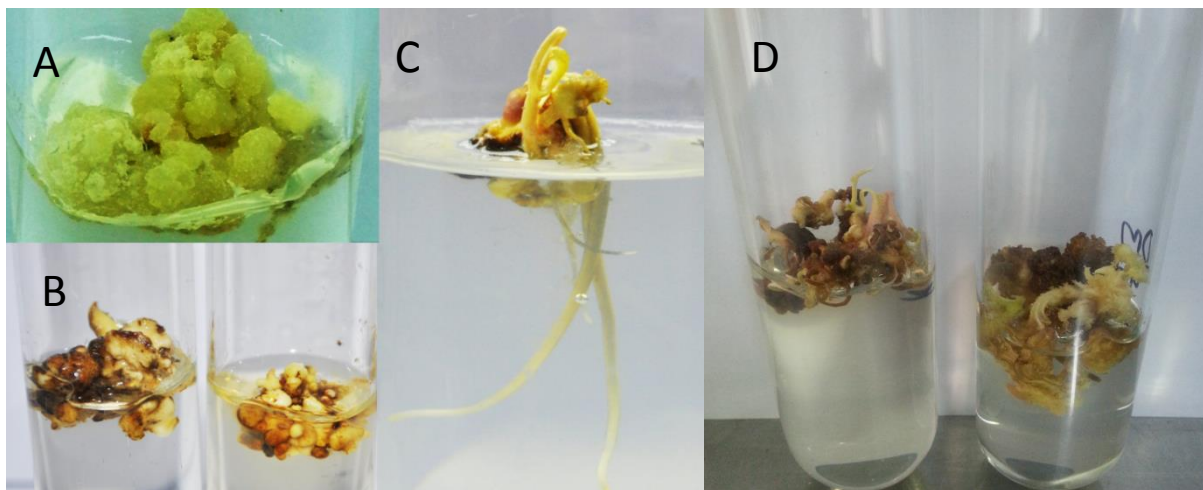


Figure 1: (A) Calli, (B) embryos, and (C) rooted embryoids obtained for anther culture explants of Latex Timber Clone RRIM 3001. (D) “Popcorn” callus that potentially developed into plantlet.



Figure 2: The plantlets obtained from anther culture of Latex Timber Clone RRIM 3001 that are survived during acclimatization.

Conclusion

The regeneration of anther culture of RRIM 3001 showed initial success. Calli induced onto B5 and SH media more readily produced rooted embryos compared to calli induced onto MS(ID)Z. Control RD1 appeared as the most suitable differentiation media in this study, and hence the use of kinetin at 0.3 mg/L could be potentially manipulated for successful regeneration of RRIM 3001 clones.

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Evaluation of the Performance of MD2 Pineapple Clone on Peat Soil

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Introduction

Pineapple (*Ananas comosus* L.) is the most important fruit crop of the Bromeliaceae family and is widely cultivated in the tropical and subtropical regions for local consumption and international export (Van de Poel et al., 2009). The Food and Agriculture Organization of the United Nation has highlighted pineapple as one of the most economically important tropical fruits (Duval et al., 2001). It is one of the most important fruit crops and ranked among the top ten fruits in Malaysia. Currently, the pineapple plantation areas in the country totalled 10,319 ha with total production of 412,720 metric tons per year (Anon, 2015). There are many pineapple cultivars with various colours, shapes, sizes, odours and flavours. However, MD2 is the most preferred pineapple variety in Malaysia due to its sweeter taste, higher vitamin C and longer shelf life. It also has less fibrous, less acidic and brighter gold colour than other pineapple varieties. MD2 pineapple possesses a high production capacity and good fruit quality characteristics as compared to 'Smooth Cayenne' cultivar (Chan et al., 2003). MD2 pineapple is advantageous for growers due to its uniformity and consistency in size and ripeness. The fruit weight ranges 1.3-2 kg with large and flat eyes, while the leaves are spineless.

Shortage of planting materials has limited the production of this cultivar in Malaysia. Generally, MD2 only carries one or two suckers at most, and they are left for production of the ratoon crop; hence the only source left for planting materials is the slips. Large quantities of plant materials are needed to fulfil the market demand which could not be obtained from the conventional vegetative propagation method. Hence, *in vitro* procedure can be used as an alternative method to improve the multiplication rate of MD2. Tissue culture techniques are widely used for commercial production of plants as well as for plant research. For instance, this technique allows an efficient and rapid production of selected elite pineapple varieties. It creates a large number of clones from a single seed or explant. Tissue culture involves the production of true-to-type plantlets; eliminating pathogens (through meristem culture) and is considered as an effective approach for mass propagation. However, genetic integrity of the regenerated plants must be taken into consideration (Rani and Raina, 2000). Genetic changes in *in-vitro* culture of plant cells, organs and tissues have been reported in numerous studies, such as cabbage (*Brassica oleracea*) (Leroy et al., 2000), tomato (*Solanum lycopersicum*) (Soniya et al., 2001), rice (*Oryza sativa*) (Abeyaratne et al., 2004), kiwifruit (*Achnidia deliciosa*) (Palombi and Damiano, 2002) and pineapple (*A. comosus*) (Santos et al., 2008). The altered traits in variant lines should be stable during vegetative propagation and grown in different environments.

Thus, the objective of the study is to determine the performance of somaclonal MD2 pineapple on peat soil in order to evaluate the pure line of planting materials.

Materials and Methods

The experiment was conducted on a peat area at the MARDI station, Pontian, Johor. A total of 2200 planting materials were planted in November 2013 and arranged in a randomised complete block design (RCBD). There were 200 plants grown in each double-row beds. The spacing was 30 cm x 60 cm between plants and 90 cm between rows. All plants were treated with standard cultural practices. Planting MD2 is similar to planting other pineapple cultivars in term of plot preparation, agronomy practices, final harvesting and propagation. However, there are some differences in relation to disease

control and hormone application. All planting materials treated with fungicide (benomyl) before planting to decrease disease severity. Flower induction was done in July 2014 with hormone spray application; a mixture of 60 mL ethrel, 180 g urea and 18 L water to prevent delayed or uneven flowering. Before harvesting, plant characters namely plant height, leaf length, leaf width, peduncle length, number of suckers and slips were measured and recorded. After harvesting, fruit characteristics i.e. crown length, crown width, crown weight, fruit weight, fruit length and fruit width were recorded. Other fruit quality traits recorded were fruit shape, core diameter, total soluble solids (TSS) and total acid content (TTA). TSS was recorded using a digital refractometer (0-30 °Brix) while acid content was determined by titration method. Leaf was visually scored from spiny, marginal and piping-leaf. Disease severity also observed for each plant. The data were recorded from all sample plants in the plot. In this experiment, 50 plants were used for each treatment and each treatment was replicated at least four times. The field blocks were arranged in a RCBD. All data were statistically analyzed using SAS analysis of variance and the means were ranked using Fisher's least significant difference test.

Results and Discussion

The analysis of variance of seven plant characters and ten fruit quality traits are shown in Table 1. The mean squares of plant height, leaf length, leaf width, peduncle length, spiny leaf, crown weight, fruit width, fruit shape, core diameter and total titration acid (TTA) were not significantly different in each row but significantly different between rows (treatment). There was variation in each row but no significantly different between rows for character of number of slips. However, variation occurs in each row and between rows for characteristics of number of suckers, crown length, crown width, fruit weight, fruit length, and total of soluble solids (TSS). This indicates that the somaclonal MD2 were significantly different from each other in terms of all plant and fruit traits based on F value obtained. The mean values of the 17 phenotypes for somaclonal MD2 are shown in Table 2. Coefficient of variation (CV) analysis showed that certain plant and fruit traits had high variability, with CV values ranging from 12.12% for leaf length to 361.86% for number of slips. The CV value for plant characteristics ranged from 12.12% to 361.86% while the quality traits ranged from 13.53% to 79.63%.

Thus, these statistics indicated that number of slips was highly variable as compared to number of suckers (103.70%), spiny leaf (36.58%), fruit weight (44.20%) and fruit shape (79.63%). Except for crown weight, although does not have CV value more than 30% but it has high value of standard deviation (88.00). The wide range of crown weight starting from 12.9 g to 1.64 kg contributed to high standard deviation. Crown weight more than 1 kg caused by double, triple or multiple crown. From data observation less than 3% of somaclonal MD2 produced varied crown shape. Fruit size of the somaclonal MD2 is usually considered medium-large as reported by Amar Ahmadi et al. (2015) with range between 1.5 kg and 2.5 kg. However in this trial, the fruit weight was considerably mediocre with mean value 1.12 kg although ranged from 0.04 kg to 2.80 kg. There are about 25% of population produced smaller fruits of less than 500 g/fruit. Fruit shape obtained high CV value with ranged from 1 to 6 categories. Data analyses showed that 33% of fruit shape is not cylindrical with 16.3% of them produced tapered out of 2060 total fruits recorded. The mean core diameter was 2.08 cm with the range of 0.5-3.2 cm. Beside of core size, this variety is crunchy in core texture. Chan and Lee (1999) reported that it is quite acceptable when the fruit are consumed fresh. It also had high sugars with mean value of TSS was 14.8° Brix, well-balanced with high acid with mean of 0.59% for good aroma and taste (Chan and Lee, 1995). However it did not produce much suckers and slips due to mean value (1.13 and 0.13) respectively with wide range of suckers number from 0 to 15 and number of slips from 0 to 12. It showed that most of somaclonal MD2 produced suckers than slip, fluctuating around two suckers per plant. CV of spiny leaf for MD2 population is slightly lowest (36.58%) as compared to other characteristics with high CV value because this trait was divided to three categories and mostly are marginal type of spiny. Furthermore 'piping-leaf' genotype of somaclonal MD2 showed the presence of this desirable trait with 5% which could be used for future breeding purposes.

Table 1. Mean squares from ANOVA for plant and fruit quality traits of MD2 pineapple clones.

Source	df	Plant traits							df	Fruit quality traits									
		Plant height	Leaf length	Leaf width	Peduncle length	No. of suckers	No. of slips	Spiny leaf		Crown length	Crown width	Crown weight	Fruit weight	Fruit length	Fruit width	Fruit shape	Core diameter	TSS	TTA
Rep	3	803.3 ^{ns}	439.3 ^{ns}	0.2 ^{ns}	24.6 ^{ns}	11.8 ^{**}	1.8 ^{**}	3.3 ^{ns}	3	278.3 ^{**}	133.0 ^{**}	38976.7 ^{ns}	2.3 ^{**}	183.8 ^{**}	15.7 ^{ns}	4.6 ^{ns}	0.1 ^{ns}	80.1 ^{**}	0.03 ^{ns}
Treat	10	16338.3 ^{**}	13037.7 ^{**}	21.5 ^{**}	219.6 ^{**}	31.2 ^{**}	0.4 ^{ns}	15.6 ^{**}	10	131.0 ^{**}	57.3 ^{**}	50053.7 ^{**}	1.7 ^{**}	44.4 ^{**}	10.1 ^{**}	14.4 ^{**}	0.7 ^{**}	30.7 ^{**}	0.21 ^{**}
Error	2151	121.1	75.7	0.6	8.7	1.3	0.2	0.6	2046	21.8	7.7	7744.9	0.2	7.1	2.3	2.1	0.1	5.8	0.02
Total	2164								2059										

^{ns} Not significant; ^{**} Significantly different at $p < 0.01$

Table 2. Mean, standard deviation, coefficient of variation, range and F value of plant and fruit quality traits of MD2 pineapple clones.

No	Character	Mean	Standard Deviation	Coefficient of Variation	Range	F Value
Plant traits						
1.	Plant height (cm)	83.01	11.01	13.26	32 - 180	105.26 ^{**}
2.	Leaf length (cm)	71.81	8.70	12.12	24 - 105	133.79 ^{**}
3.	Leaf width (cm)	5.19	0.79	15.25	2 - 9	26.53 ^{**}
4.	Peduncle length (cm)	12.46	2.96	23.76	4 - 30	19.91 ^{**}
5.	Number of suckers	1.13	1.18	103.70	0 - 15	19.40 ^{**}
6.	Number of slips	0.13	0.47	361.86	0 - 12	3.50 ^{**}
7.	Spiny leaf	2.16	0.79	36.58	1 - 3	20.64 ^{**}
Fruit quality traits						
1.	Crown length (cm)	26.00	4.67	17.95	8.5 - 52	7.57 ^{**}
2.	Crown width (cm)	15.12	2.77	18.33	6 - 35	9.73 ^{**}
3.	Crown weight (kg)	352.66	88.00	24.95	0.01 - 1.64	6.13 ^{**}
4.	Fruit weight (kg)	1.12	0.50	44.20	0.04 - 2.8	7.65 ^{**}
5.	Fruit length (cm)	13.08	2.67	20.39	4 - 26	10.76 ^{**}
6.	Fruit width (cm)	11.15	1.51	13.53	3.7 - 23.4	4.98 ^{**}
7.	Fruit shape	1.83	1.46	79.63	1 - 6	5.70 ^{**}
8.	Core diameter (cm)	2.08	0.35	17.04	0.5 - 3.2	4.23 ^{**}
9.	TSS (% Brix)	14.78	2.41	16.28	1.5 - 24.5	7.27 ^{**}
10.	TTA (%)	0.59	0.16	26.19	0.09 - 1.05	7.00 ^{**}

^{**} Significantly different at $p < 0.01$

The main objective of micropropagation is to produce clones i.e. plants which are phenotypically and genetically identical to the mother plants. While, direct systems of regeneration through the culture of organized meristems usually guarantee the production of true-to-type plants, variations in the progenies have been widely reported (Govinden-Soulange et al., 2010). One potential problem utilizing somaclonal planting material which some variants is epigenetic effects and result in unstable phenotypes (Kaeppeler and Phillip, 1993) not suitable for cultivar development. Several strategies have been adopted to detect somaclonal variation in *in vitro*. These include phenotypic observation and molecular works have been used complementarily along with traditional methods of assessing somaclonal variation for example in micropropagated *H. Sabdariffa* (Govinden-Soulange et al., 2010). However, this technology involved high method expenses. To evaluate the stability of the variant lines, they were grown in replicated field tests and observed for at least two years/cycles with some being grown at multiple locations with various climate conditions (Li et al., 2010). Measurement of the selected somaclonal variant lines was carried out together with the control (MD2 plant with pure characteristics as reported by LPNM/DOA) especially for fruit shape, fruit size, crown shape, TSS and spiny leaf.

Conclusion

The pineapple variety MD2 has become the preferred variety for the fresh pineapple market because of its exceptional sweetness, flavour, golden colour, shape and life span. Tissue culture process provides for rapid production of pineapple plants with improved yields and fruit characteristics. Results from this trial indicated that there have variants in the somaclonal MD2 although it was the second cycle of evaluation. Planting materials from tissue culture should be selected pure line to obtain uniformity and authentically. As the main objective of micropropagation is to produce clones, the occurrence of any type of variation in regenerated plantlets needs to be closely investigated as it could be heritable. Appropriately the altered traits in these lines remained the same during vegetative propagation and when grown in different environments, suggesting that the morphological changes in these variants are stable and could be employed in plant propagation efforts.

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Expression and Purification of Antifungal Protein (endo- β -1,3-1,4-glucanase) from *Bacillus* SP 289

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Introduction

Recombinant DNA technology has created a new breakthrough in treatment of human diseases which further increase in life expectancy of patients. The first product of human insulin through recombinant protein has opened a new pharmaceutical market with high demand and global sale. Recombinant DNA technology also contributed high benefits in agricultural industry through recombinant DNA technology in enzyme (Ferrer-Mirallis et al., 2015). Most of the enzyme used in brewing industry and animal feed production has been produced in large scale by using recombinant protein (Luo et al., 2010).

Production of a specific interest protein in large quantity is difficult to obtain from the original organism. Thus, heterologous protein production using protein recombinant and expression has been increasingly attracting the interest of researchers due to the ability of this technology to produce the desired recombinant protein in large volume. Next, it will allow for the protein's biochemical characterization, its use in industrial processes and for commercial product development. Tagged recombinant proteins are commonly produced which is more convenient for detection during expression and purification. Expression systems that have been developed include bacteria, yeast, fungi, plants and mammalian cells (Faridah, 2015, Gellissen et al., 2005).

There are many factors that should be considered when selecting the expression system. These include the characteristics of the cell growth, level of expression, intracellular or extracellular protein expression, post-translational modification, the desired protein biological activity and also the regulatory issues in the therapeutic proteins production. Besides, a cost breakdown is also essential factor that should be considered for expression system selection (Faridah, 2015). *E. coli* is one of the popular bacteria used as the heterologous expression host in the production of protein recombinant (Pacheco et al., 2012). *E. coli* is easy to culture, has very rapid cell growth and grows to high densities on simple and inexpensive culture media (Faridah, 2015). For the expression, it can be easily induced using isopropyl- β -D-thiogalactoside (IPTG). Protein purification steps in prokaryotic expression systems are also quite simple compare to others.

Glucanases are enzymes that function to break down a glucan, a polysaccharide made of several glucose subunits. As they perform hydrolysis of the glucosidic bond, they are hydrolases and classified as glycoside hydrolases. In the literature, antifungal activity was mainly observed in β -1,3-glucanases. The enzymes have been isolated from various sources including plants, fungi and bacteria. Previous studies also reported several number of β -1,3-1,4-glucanases which have been isolated and purified especially from bacteria. However, not much is known about the antimicrobial activity of β -1,3-1,4-glucanase since there are not many researchers who made reports regarding the antifungal activity of the enzyme (Britto et al., 2013, Luo et al., 2010).

This study is aimed to clone, express and purify potential antifungal protein gene of endo- β -1,3-1,4-glucanase from an antagonist bacteria against Rice Sheath Blight pathogen, *Bacillus* SP 289. The bacterium was isolated from rhizosphere soil paddy field in our previous study. We believed that the

enzyme has involved as one of the antifungal compound which responsible in suppressing the growth of sheath blight pathogen, *Rhizoctonia solani*. Since purification of the enzyme through conventional method has been difficult, this recombinant protein technique was used as an alternative in obtaining the purified endo- β -1,3-1,4-glucanase.

Materials and Methods

PCR amplification of endo- β -1,3-1,4-glucanase from Bacillus SP 289

Primers for PCR were designed based on the complete sequence of endo- β -1,3-1,4- glucanase gene from two *Bacillus subtilis* registered in the GenBank database with accession number BSU60830 and EU082110 respectively. *Bacillus* SP 289 Genomic DNA was isolated using GenElute Bacterial Genomic DNA Extraction Kit (Sigma-Aldrich, USA) according to protocol provided by the manufacturer. PCR amplification was carried out in 25 μ L reaction for 30 cycles using thermostable DyNAzyme™ EXT DNA polymerase (Finnzymes, Finland) in PTC-200 thermal cycler (MJ Research, USA). The reaction mixture consisted of 1x PCR buffer; 2.0 mM of $MgCl_2$, 0.2 mM of each dNTPs, 2 μ M of forward and reverse primers, 100 ng of *Bacillus* SP 289 genomic DNA as a template and 2.5 U of the enzyme mix. PCR product was resolved using 1% of agarose gel and purified using QIA quick gel extraction kit (QIAGEN, Germany).

Cloning of enzyme into cloning vector

The purified PCR product was cloned into TOPO® TA Cloning® Kit of PCR 2.1 TA cloning vector (Invitrogen, USA). A mixture consisting of 4 μ L of fresh PCR product, 1 μ L of vector and 1 μ L of salt solution were gently mixed and incubated at room temperature for 15 min. The ligated products were then transformed into competent cells, *E. coli* Top 10. Solution mixture was incubated for 30 min on ice, given a heat shocked at 42 °C for 30 s and was then transferred on ice immediately. A 250 μ L of SOC medium was added into the mixture. The mixture was then shaken (150 rpm) for 1 hour at 37 °C. LB agar plates were prepared with ampicillin 100 μ g/mL. The transformation medium was then plated onto LB agar plates. Plates were incubated at 37 °C. The positive clones were screened after 18 hours. Plasmid DNA of recombinant clones were isolated using QIAprep Spin miniprep kit (QIAGEN, Germany) and sent for automated DNA sequencing service (Research Biolabs Technologies, Singapore).

Subcloning of endo- β -1,3-1,4-glucanase into expression vector

The 720 bp of endo- β -1,3-1,4-glucanase fragment was isolated from TOPO 2.1/ *β glu* plasmid by digestion with *Bam*HI and *Xho*I and was extracted using QIA Quick Gel Extraction Kit (Qiagen, Germany) by following the manufacturer's procedures. The fragment was then ligated into the pRSET A Expression Vector (Invitrogen, USA). The 10 μ L of ligation mixture consisted of vector and insert with ratio 1:1, 2 x of ligation buffer and 1 Weiss unit of T4 DNA ligase. The reaction mixture was incubated overnight at 4 °C. The ligation mixture (5 μ L) was added into 50 μ L of *E. coli* BL21 (DE3) pLysS in microcentrifuge tube. The mixture was then incubated on ice for 20 min, followed by heat shock at 42 °C for 45 s and then chilled on ice for 2 min. A 250 μ L of LB broth was then added into the tube aseptically. The tube was incubated at 37 °C for 1 hour. Finally, the transformation mixture (50 to 100 μ L) was spread on LB agar that contained 100 μ g/mL ampicillin. The plates were put in the position of inverted and incubated at temperature of 37 °C overnight. The positive transformants were selected and inoculated into 5mL of LB broth containing 50 μ g/mL ampicillin. The culture media was grown for 16 to 18 hours at 37 °C with shaken at 200 rpm. Plasmids were then extracted using QIAprep Spin Miniprep Kit (Qiagen, Germany) by following the protocols provided in the kit.

Expression of endo- β -1,3-1,4-glucanase in E. coli

E. coli BL21 harbouring plasmid pRSET A/ β glu was inoculated in 5 mL of LB broth that was supplemented with 50 μ g/mL ampicillin as seed culture. The culture was grown overnight at 37 °C with 180 rpm in a shaking incubator. After 16 hours of incubation, 0.2 mL culture was added into 10 mL of LB broth containing 50 μ g/mL ampicillin. The new inoculated culture was grown again with vigorous shaking at 37 °C until the culture reached an Abs_{600nm} of 0.6 (mid-log phase of bacterial cells). Then, 1 mL aliquots of cells were taken from the culture and centrifuged at 9,306 x g for 10 min. Pellet obtained was frozen at -20 °C. This pellet was marked as zero time point sample. Protein expression was induced for another 9 mL culture left using isopropyl- β -D-thiogalactoside (IPTG) as inducer. IPTG was added into the culture medium to a final concentration of 1 mM. The *E. coli* BL21 with pRSET A / β glu cultivation was continued for 1, 2, 3, 4, 5 and 16 hours. Bacterial strain harbouring pRSET A vector without insert was also induced with IPTG as control. Levels of protein expression were assessed and analyzed based on the total protein on SDS-PAGE, western blotting and enzym assay.

Scale-up of expression

A single colony of recombinant *E. coli* BL21 was cultured into 5 mL LB broth containing 50 μ g/mL ampicillin. The broth culture was grown at temperature of 37 °C overnight by using 200 rpm speed agitation rate. The overnight culture (1 mL) was then inoculated into 50 mL of new LB broth. The culture was grown again at 37 °C with the same agitation rate until the cells in mid-log phase when Abs_{600nm} reached 0.6. A 50 μ L of 1M IPTG stock was added into the 50 mL culture to a final concentration of 1 mM and was left grown at 37 °C until the optimal time point was reached. The cells culture was then centrifuged at 9,306 x g for 10 min at 4 °C. Pellet obtained was stored at -80 °C for future use or purified immediately.

Purification of recombinant endo- β -1,3-1,4-glucanase

The cells pellet was resuspended in 4 ml of Bug Buster Protein Extract Reagent (Novagen, USA) and lysed gently for 20 min at room temperature. The Bug Buster Extract Reagent also contained 1 μ L of 25 U/ μ L benzonase (Novagen, USA) and 7.5 KU rlysozyme (Novagen, USA). After 20 min, the lysed cells were centrifuged at 18,547 x g for 5 min. The supernatant was then mixed with 1 mL of 50 % Ni-NTA His Bind Resin (Novagen, USA) and pre-washed with Ni-NTA bind buffer (Novagen, USA). The mixture was incubated for 60 min at temperature of 4 °C before being loaded into the empty column (Novagen, USA). The column was washed for 6 times with 4 mL of Ni-NTA washing buffer (Novagen, USA). Lastly, the recombinant of endo- β -1,3-1,4-glucanase was eluted with 4 x 250 μ L of Ni-NTA elute buffer (Novagen, USA).

Quantitative determination of endo- β -1,3-1,4-glucanase activity

A colorimetric method as described by Miller (1959) was used to quantify the endo- β -1,3-1,4-glucanase activity. The reducing sugar released from lichenan (MP Biomedicals, USA) was measured. The assay solution contained of 90 μ L of 5 mg/mL lichenan dissolved in 0.1 M sodium acetate buffer pH 5 and 10 μ L of enzyme solution. Mixture was then incubated with gentle shaking at 40 °C. After 10 min of incubation, the mixture was boiled for 5 min to stop the reaction. A 200 μ L of 1 % dinitrosalicyclate and sodium acetate buffer were added into the mixture. The mixture was boiled again for 5 min and immediately placed in ice bath. A 900 μ L of sterile distilled water was added. Lastly, the assay was read at Abs 540 nm. Standard curve was prepared using glucose. The total of reducing sugar release was calculated from the standard curve. The standard curve of glucose was plotted using glucose standard solutions. Glucose stock solution (3mg/mL) was prepared by dissolving 15 mg of glucose (Sigma, USA) with 15 mL of sterile distilled water. Glucose stock

solution was then diluted with sterile distilled water in order to prepare different glucose concentrations range from 0-1.5 mg/mL.

Determination of protein concentration

Determination of protein concentration was assayed using Bicinchoninic acid (BCA) protein assay. This assay was first described in 1985 by Smith. For the standard, bovine serum albumin (BSA) (Biorad, Australia) was used in different concentrations range from 0 to 2.5 mg/mL. Stock (2mg/mL) was diluted using 0.01M phosphate buffer saline pH 7.4. BCA working reagents A and B (Pierce, USA) were mixed in ratio 9:1. Mixed reagent (200 μ L) was added to 25 μ L of enzyme sample. The mixtures were then incubated for 30 min at 37 $^{\circ}$ C and read at Abs 560 nm using spectrophotometer.

Results and Discussion

pRSET A (Invitrogen, USA) was chosen as expression vector for cloning of endo- β -1,3-1,4-glucanase. The 720 bp fragment encoding *Bacillus* SP 289 endo- β -1,3-1,4-glucanase was isolated from the TOPO 2.1/ *βglu* plasmid by digestion with *Bam*HI and *Xho*I and ligated into the *Bam*HI and *Xho*I-digested pRSET A vector in frame to the T7 promoter and Histidine tag at the N-terminal. The pRSET A are pUC-derived expression vectors that were designed from cloned genes in *E. coli* for high level of protein expression and purification. The presence of T7 promoter enables high levels expression of protein sequences that cloned into the pRSET A vector. Additionally, DNA inserts were placed downstream and in frame with a sequence that encodes an N-terminal fusion peptide. This sequence includes an ATG translation initiation codon, a polyhistidine tag that functions as a metal binding domain in the translated protein, a transcript stabilizing sequence from gene 10 of phage T7, the Xpress™ epitope, and the enterokinase cleavage recognition sequence. Digestion analysis result showed the formation of two DNA fragments with the expected size, ~2890 bp and ~720 bp that refers to the size of the pRSET A expression vector and the endo- β -1,3-1,4-glucanase respectively (Figure 1).

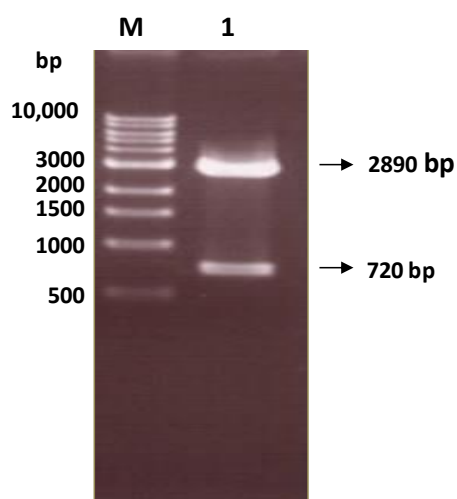


Figure 1: Enzyme restriction analysis of PCR amplified fragment of endo- β -1,3-1,4-glucanase into pRSET A expression vector.

The endo- β -1,3-1,4-glucanase expression from pRSET A is controlled by the strong phage T7 promoter that drives expression of gene 10. In this study, the expression of the T7 RNA polymerase was induced by IPTG. The *E. coli* BL21 carries the DE3 bacteriophage lambda lysogen. This lambda lysogen contains the *lacI* gene, the T7 RNA polymerase gene under control of the *lacUV5* promoter and a small portion of the *lacZ* gene. The *lac* repressor represses expression of T7 RNA polymerase.

Addition of IPTG allows expression of T7 RNA polymerase. One hour is the best time for *E. coli* growth after induction with IPTG to obtain the highest expression of the recombinant enzyme. After 16 hours, the recombinant endo- β -1,3-1,4-glucanase was degraded. Figure 2 shows the Western blot analysis of endo- β -1,3-1,4-glucanase expression.

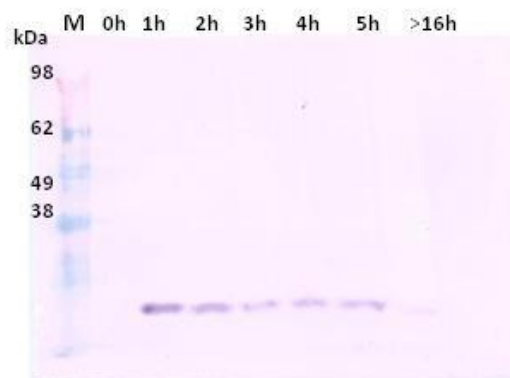


Figure 2: Time course of recombinant endo- β -1,3-1,4-glucanase expression in *E. coli* BL21 analyzed by Western blot. M: standard protein marker (SeeBlue Plus2); Lane 1: 0h; Lane 2: 2h; Lane 3: 3h; Lane 4: 4h; Lane 5: 5h; Lane 6: >16h

Purification of recombinant endo- β -1,3-1,4-glucanase process started with gentle disruption of *E. coli* BL21 cell wall with use of BugBuster® Protein Extraction Reagent (Novagen, USA). Disruption of the cells through sonication was found not suitable since it caused denaturing of the recombinant protein. The eluted fraction of purified recombinant protein of endo- β -1,3-1,4-glucanase was analyzed in SDS-PAGE and shown in figure 3. The yield was about 37.38%. The protein content of the purified recombinant enzyme was around 0.278 mg/mL with a specific activity of 26.007 U/mg. The low yield of purified endo- β -1,3-1,4-glucanase showed that there was a loss of the enzyme during the purification steps.

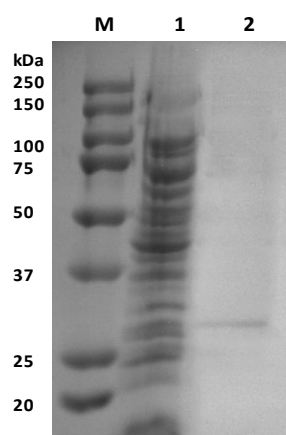


Figure 3: SDS-PAGE of His-tagged recombinant endo- β -1,3-1,4-glucanase purified through Ni-NTA His Bind Resin Affinity Column. Lane M: standard protein ladder which contain recombinant protein bands (250, 150, 100, 75, 37, 50, 25, 20 & 15 kDa); Lane 1: Crude lysate; Lane 2: Purified His- tagged recombinant enzyme

Conclusion

Potential antifungal protein gene of endo- β -1,3-1,4-glucanase from an antagonist bacteria against Rice Sheath Blight pathogen, *Bacillus* SP 289 was successfully cloned, expressed and purified. The recombinant protein method is a good alternative technology in isolating and obtaining specific interest of protein in large quantity.

Acknowledgements

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Effect of Stirring Time with Various Percentage of Hydroxyapatite (HA) as Nano Carrier for the Development of Nano-Fertilizer

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Introduction

In order to sustain crop yields, fertilizers have to be applied to soils to provide plants with essential nutrients. Conservative estimates show that 30 to 50% of crop yields are attributed to natural or synthetic commercial fertilizers. As modern agriculture relies increasingly on non-renewable fertilizer resources, future related minerals are likely to yield lower quality at higher prices. Some of the nutrients in these non-renewable fertilizers are not absorbed by plants and therefore, leaches into groundwater or surface water, imposing great risk to the ecosystem. To improve fertilizer quality and protect the environment and the ecosystem, there has been increasing research towards developing new technologies for delivering plant nutrients in a slow- or controlled-manner in the water or soil.

Approximately 50 to 70% of the nitrogen applied using conventional method with particle size greater than 100nm is lost to the soil due to leaching. As a result, nitrogen utilization efficiency (NUE) by plant is low due to this problem. Therefore, additional cost is needed to get nitrogen to the plant. Leaching of nitrogen can happen by way of water-soluble nitrates, emission as ammonia and nitrogen oxides and soil microorganism.

A smart-carrier fertilizer is the most technically advanced way of supplying nutrients to crops and can improve fertilizer use efficiency. Compared to conventional fertilizers, their gradual pattern of nutrient intake meets plant needs and minimizes leaching. A nano strategy involving a smart carrier fertilizer based on modification of fertilizer using hydroxyapatite (HA) nanoparticles was studied. HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) nanoparticles are rated as one of the prominent candidates in agricultural applications, which can provide phosphorus nutrient.

The proposed fertilizer composition may maximize the fertilizer efficiency while minimizing the adverse effects to the environment due to use of large quantities of fertilizer in agriculture.

Materials and Methods

All materials unless stated were of analytical purity. Chemical analysis was done using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), Fourier Transform Infra-Red (FTIR) and Scanning Electron microscope (SEM)

Incorporation of urea to hydroxyapatite

In previous study, HA nanoparticles were synthesized using aqueous solutions (wet chemical precipitation method) of calcium hydroxide ($\text{Ca}(\text{OH})_2$) and orthophosphoric acid (H_3PO_4). With the presence of hydroxyl group (OH) as binding site for the incorporation of urea as shown in Figure 1.

In order to study the optimization of stirring time with various percentage of urea, HA nanoparticles were stirred vigorously at room temperature according to various concentrations ranging from 50 to 90 percent using aqueous solutions (wet chemical precipitation method) of HA and urea. The solution was stirred for a period of 1 to 2 days. The solid thus obtained was dried at 100°C for 2 hours.

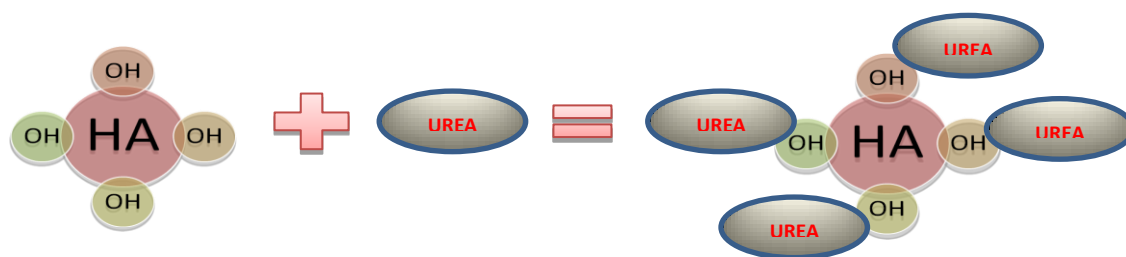


Figure 1: Schematic diagram for the incorporation of HA nanoparticles with various concentrations of urea.

Results and Discussion

Through our observation, it was found that urea stirred for 1 day with 50% urea concentration was the highest value which was 7.08, 14.14 and 25.81 for nitrogen, phosphorus and calcium respectively compared to other concentrations. Therefore, the optimum point of the series was the concentration marked with red line as presented in Figure 2. The lowest range of nitrogen content was in the range of about 80 to 90 % of urea. This may be caused by the high concentration of urea that might cause electrolyte or element movement to be slow leading to limitation of binding between urea and HA.

Even though nitrogen content at 80% with stirring for 2 days had the highest value of 9.18, but the phosphorus and calcium levels were not as high as for 50% urea which were 9.03 and 17.05 respectively. From the data obtained, we can conclude that the concentration and time for the optimum condition mentioned above.

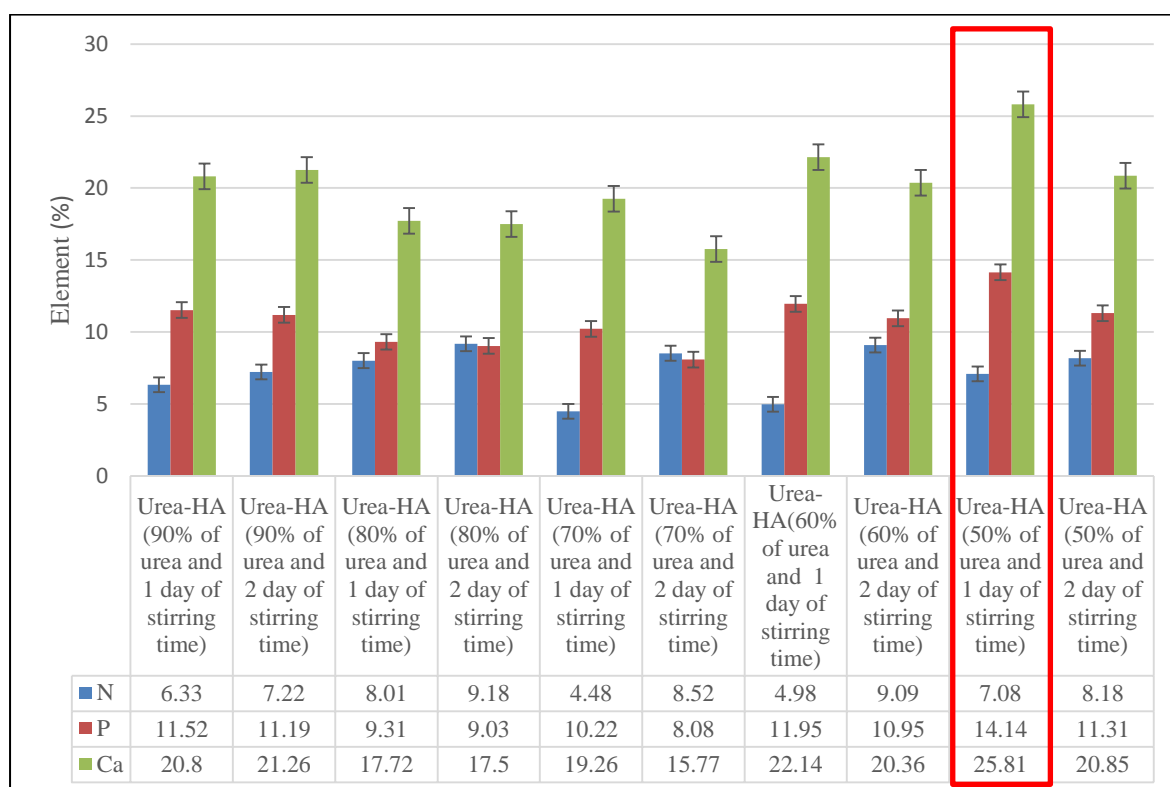


Figure 2: Effect of stirring time with various concentrations of urea.

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Chapter 4

Post-Harvest Technology and Quality Planting Material

Postharvest Quality of Sweet Corn is Affected by Hydro-cooling and Packaging Type

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Introduction

Sweet corn or scientifically known as *Zea mays* is a very common horticultural crop in Malaysia. It is among the top ten cash crops planted among growers in Malaysia. The taste and quality of sweet corn depends heavily upon its sugar content. Sweet corn has high respiration rate (Kader, 2002) with rate of 30-51 and 282-435 mg CO₂ kg⁻¹h⁻¹ at 0 and 25 °C, respectively (Gross et al., 2004). High respiration causes sugar content in the sweet corn to reduce by about 6 and 60% in a single day at 0 and 30 °C, respectively (Brecht, 2004). So, the quality of sweet corn will rapidly degrade if not handled properly. Lowering internal commodity temperature following harvest is critical in extending postharvest life. This can be done in two-stage process starting with precooling and low temperature storage. Precooling using water as cooling medium (known as hydro-cooling) is recommended to remove field heat and extend postharvest life for sweet corn (Vigneault et al., 2007). However, lack of proper packaging during storage can cause serious deterioration and loss of tenderness and sweetness of sweet corn (Risse and McDonald, 1990). Thus, proper packaging during storage is essential to retain sweet corn quality. However, there was no literature on the effect of hydro-cooled sweet corn followed by packaging.

Therefore, this study was conducted to examine the quality of sweet corn after hydro-cooled and packed using cling wrap and polyethylene bag.

Materials and Methods

Freshly harvested sweet corns that were uniform in size and color without any defects and disease were taken from Batang Berjuntai, Selangor. Upon arriving at Postharvest Laboratory, UPM, 40 cobs of the corns were placed in a hydro-cooler with water temperature of 4 °C. 20 cobs of sweet corn were removed from cold water when the core temperature of corns reached 13 °C or achieved its 1/2 cooling time in the pre-cooling process. The remaining pieces of corn were removed from cold water when the core reached 5.2 °C or achieved its 7/8 cooling time. For control, 20 cobs of sweet corn without underwent hydro-cooling process were used.

All the corns were minimally trimmed by removing its shank, silk and some husks to expose few rows of kernels. The 20 pieces of sweet corn from respective treatment were then divided into two lots of 10 cobs/lot. One of the lots were packed using polyethylene bag (20 cm x 30 cm with 0.04 mm thickness) with 2 cobs in a bag. Another lots of sweet corn were packed using cling film (0.02 mm thick) with 2 cobs in a pack. The packed corns were then stored in a chamber of 7 °C with 70% relative humidity. These sweet corn were analyzed for its quality index (QI), weight loss and soluble solids concentration (SSC) at day 0, 2, 4, 6 and 8. QI of sweet corn was determined using a 9-point scale based on husk, silk and kernel freshness, turgidity and colour (Vigneault et al., 2004).

The experimental design used was a randomized complete block design with three factorial arrangement (cooling time x type of packaging x days of storage). The experiment was repeated for three times. Data was analyzed using analysis of variance (ANOVA). When ANOVA gives the significant F-value at 5% level, Duncan's multiple range test (DMRT) was used to separate the means using Statistical Analysis System (SAS Institute, 1989).

Results and Discussion

The QI and SSC of sweet corn was not affected by interaction between hydro-cooling time x packaging type, hydro-cooling time x storage day, packaging type x storage day and hydro-cooling time x packaging type x storage day (Table 1). However, water loss of sweet corn was affected by interaction between packaging type x storage day.

There was significant different in sweet corn QI between control and corn that achieved 7/8 cooling time (Table 1). However, there were no differences in QI between control and corn that achieved ½ cooling time, and also between the two cooling times used. This indicated that 7/8 cooling time is preferable in retaining packed sweet corn quality. Both type of packaging used in this study did not affect QI of sweet corn. As expected, the QI of sweet corn decreased as storage day progressed, from excellent (score 9) to average (score 4). This indicated that after 8 days of storage at 7°C with 70% relative humidity the husk of sweet corn appeared as pale green and slightly dry while silks showed light browning and little drying. The kernels were dull but not dented and no major defects were found on the cobs. The increase of storage duration caused the produce color change which was due to the action of the light, temperature, oxygen, metal ions, and endogenous enzymes (Stintzing and Carle, 2004). This could explain the degradation of color in sweet corn husk and silk.

Table 1: Main and interaction effects between hydro-cooling time, packaging type and storage day on quality index, water loss and soluble solids concentration of sweet corn during 8 days of storage at 11 °C.

Factors	Quality index	Water loss (%)	Soluble solids Concentration (%SSC)
Hydro-cooling time (CT)			
0	6.73 b ^z	1.63 a	13.40 a
½	7.27 ab	1.72 a	12.82 a
7/8	7.53 a	1.63 a	13.22 a
Packaging type (PT)			
Polyethylene bag	7.13 a	1.38 b	13.35 a
Cling film	7.22 a	1.98 a	12.95 a
Storage day (SD)			
0	9.00 a	0.00 e	14.08 a
2	8.67 a	0.86 d	14.07 a
4	7.67 b	1.69 c	14.20 a
6	6.11 c	2.55 b	12.68 b
8	4.44 d	3.32 a	10.72 c
Interaction			
CT x PT	ns	ns	ns
CT x SD	ns	ns	ns
PT x SD	ns	*	ns
CT x PT x SD	ns	ns	ns

^zFor each treatments, means within a column and factor followed by the same letter are not significantly different by DMRT at $P \leq 0.05$. ns, *, Non-significant or significant at significant different by DMRT at $P \leq 0.05$.

The water loss of sweet corn was significantly affected by interaction between packaging type x storage day (Table 1). At the initial stage of storage, there was no difference between type of packaging (Figure 1). After storage day 6, the sweet corn packed using cling film showed significant

higher of water loss than corn packed in polyethylene bag. This indicated cling film is not a good packaging material as compared to polyethylene bag in storing hydro-cooled sweet corn at low temperature.

The SSC of sweet corn was not affected by hydro-cooling and packaging type (Table 1). However, storage day had an effect on SSC where SSC of sweet corn decreased by 9.94% after 8 days of storage. Initially, there was no difference in SSC from day 0 until 4, then it dropped significantly after day 6. A similar finding was also reported by Vigneault et al. (2007) where increasing storage days caused decreasing SSC in sweet corns.

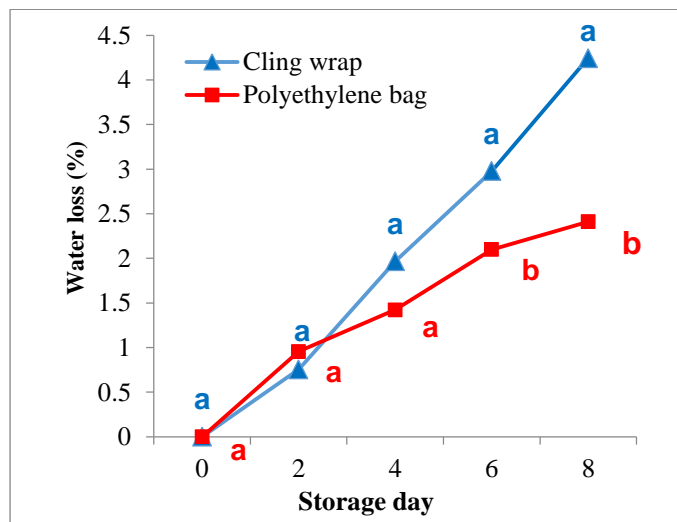


Figure 1: Water loss of hydro-cooled sweet corn packed using cling wrap film and polyethylene bag during storage. Means followed by same letters are not significantly different ($P \leq 0.05$) within the same storage day.

Conclusion

As conclusion, quality of sweet corn can be retained by hydro-cooling and packing but decreased as storage duration advanced. Thus, it is essential to reduce temperature of sweet corn as fast as possible after harvest and packed with proper material to retain its quality during low temperature storage.

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Selection of Half Sib Families in *Shorea leprosula* (meranti tembaga)

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Introduction

Shorea leprosula Miq. belongs to the family of Dipterocarpaceae. This species is widely distributed in Peninsular Malaysia, Sumatra, Borneo, and the neighboring islands (Symington, 1974). It has the fastest growth rate among the dipterocarp species (Symington, 1943; Barnard, 1954; Appanah and Weinland, 1993). According to Appanah and Weinland (1993), this species is amenable to a 40 to 50-years rotation under plantation conditions and should be considered as one of the species for forest plantations in this country. With the recent development in wood processing technology that enables the utilizations of small diameter logs, *S. leprosula* plantations could possibly be managed on a 30 to 40-years rotation.

This species is one of the main sources of light red meranti and has been classified as light hardwood with an average air-dry density of about 545 kg m⁻³. The density ranges from 425-685 kg m⁻³. As a general utility timber, it is commonly used for joinery, utility furniture, shops and office fittings, showcases, counter tops, paneling, ceiling, shelving, cabin fittings, fence, boxes, fabricated coffins, light-duty flooring and interior partitions. It is also used for plywood manufacture (Choo and Lim 1983). All of the seeds for this trial came from the seed stands or from the phenotypically plus trees, which are selected solely based on the phenotypic value. The genetic values of neither the stands nor the plus trees are known and only assumed to be good.

It is a well-known fact that phenotypic variance (σ^2_P) is made up of genotypic variance (σ^2_G) and environment variance (σ^2_e). Genetic variance comprises additive variance (σ^2_A) and non-additive variance (σ^2_{NA}). Cumulative effects of alleles at all gene loci influencing a trait are called the additive variance. Non-additive variance is made up of two components i.e. dominance variance (σ^2_D) and epistasis variance (σ^2_E). Dominance variance is due to interaction between alleles of the same gene locus while the epistasis variance is due to interaction among the loci. The only way of evaluating the breeding values (additive value) of selected individuals is through the tests of progeny or clones (Toda, 1969).

Total height and diameter at breast height are important morphological features for tree breeding as well as wood utilization and consequently for silvicultural purposes. It is believed that the variations observed within these characters are heritable or genetically controlled, but evidence for appreciable additive variation is not available. It is desirable for a tree breeder to know the degree to which various aspects of total height and diameter at breast height are inherited. Such information is needed in planning for selection programs to help in allocating priorities to different traits and to predict genetic gains. Generally, the aim of the study was to evaluate the variation of growth performances between families based on the parameters such as height and diameter at breast height. The information collected through this study is very useful for the future breeding strategies of *S. leprosula*.

Materials and Methods

A total of 40 phenotypically plus trees of *S. leprosula* were selected from five forest reserves in Peninsular Malaysia namely Sungai Menyala Forest Reserve (FR), Bukit Rengit FR, Bangi FR, Gombak FR and Trantum FR (Table 1). The selection was based on a good stem form, total height, diameter at breast height (DBH) [DBH was taken at the height 1.3 m from the soil surface] and

branching habit. The progeny test was established under *Acacia mangium* plantation at the Ulu Sedili FR, Johor using open-pollinated seeds from each of the 40 half sib families (plus trees). The randomized complete block design (RCBD) was used and each half sib family plot consisted of 32 progenies which was replicated eight times. The spacing used was 4 m x 4 m and covered an area of 3.5 hectares. Starting from year one, the total height and diameter at breast height were measured annually until the age of six years.

The data were statistically analysed by the analysis of variance (ANOVA) to determine the height increment variations among half sib families and by Duncan's multiple range test (DMRT) to determine the significant differences of means among progenies (SAS Institute Inc. 1985). The data were analyzed using PROC GLM by SAS as per following model:

$$Y_{ijk} = \mu + b_i + f_j + (bf)_{ij} + \epsilon_{ijk}$$

Where;

Y_{ijk} : the observation of j^{th} family in i^{th} block,

μ : the population mean,

b_i : effect of the i^{th} block (fixed),

f_j : effect of the j^{th} family (random),

$(bf)_{ij}$: the effect of interaction between i^{th} block with j^{th} family (random),

ϵ_{ijk} : the random error associated with the i^{th} block in j^{th} family.

Table 1: Details of the 40 half sib families of *S. leprosula*.

Half sib family	Provenance	Latitude and longitude
F1	Sungai Menyala FR	02° 48'N 101° 91'E
F2	Sungai Menyala FR	02° 48'N 101° 91'E
F3	Sungai Menyala FR	02° 48'N 101° 91'E
F4	Sungai Menyala FR	02° 48'N 101° 91'E
F5	Sungai Menyala FR	02° 48'N 101° 91'E
F6	Sungai Menyala FR	02° 48'N 101° 91'E
F7	Sungai Menyala FR	02° 48'N 101° 91'E
F8	Sungai Menyala FR	02° 48'N 101° 91'E
F9	Bukit Rengit FR	03° 45'N 102° 19'E
F10	Bukit Rengit FR	03° 45'N 102° 19'E
F11	Bangi FR	02° 55'N 101° 47'E
F12	Bangi FR	02° 55'N 101° 47'E
F13	Bangi FR	02° 55'N 101° 47'E
F14	Bangi FR	02° 55'N 101° 47'E
F15	Bangi FR	02° 55'N 101° 47'E
F16	Bangi FR	02° 55'N 101° 47'E
F17	Gombak FR	03° 17'N 101° 46'E
F18	Gombak FR	03° 17'N 101° 46'E
F19	Gombak FR	03° 17'N 101° 46'E
F20	Gombak FR	03° 17'N 101° 46'E
F21	Gombak FR	03° 17'N 101° 46'E
F22	Gombak FR	03° 17'N 101° 46'E
F23	Gombak FR	03° 17'N 101° 46'E
F24	Gombak FR	03° 17'N 101° 46'E
F25	Gombak FR	03° 17'N 101° 46'E
F26	Gombak FR	03° 17'N 101° 46'E
F27	Gombak FR	03° 17'N 101° 46'E
F28	Gombak FR	03° 17'N 101° 46'E
F29	Gombak FR	03° 17'N 101° 46'E
F30	Gombak FR	03° 17'N 101° 46'E
F31	Gombak FR	03° 17'N 101° 46'E
F32	Gombak FR	03° 17'N 101° 46'E
F34	Gombak FR	03° 17'N 101° 46'E
F35	Gombak FR	03° 17'N 101° 46'E
F36	Gombak FR	03° 17'N 101° 46'E
F37	Gombak FR	03° 17'N 101° 46'E
F38	Sungai Menyala FR	02° 48'N 101° 91'E
F39	Tranum FR	03° 88'N 101° 83'E
F45	Sungai Menyala FR	02° 48'N 101° 91'E
F47	Bukit Rengit FR	03° 45'N 102° 19'E

FR = Forest Reserve

Results and Discussion

Survival

Survival assessment was carried out at the age of six years. All of the half sib families survived well, with the percentage ranging from 43.8% to 84.4%, of which there were more than 90% of the overall half sib families that had a survival rate of more than 50% (Table 2). The mean survival rate was 64.5%. Death of trees was caused by disturbance of wildlife such as elephants, wild boar and 'tapir' and also due to crushing by tree branches (*A. mangium*) which had decomposed. There were no record of any diseases or pests that had caused the death of the trees.

This survival rate was higher compared to a report by Barnard (1954), that the survival percentage was only 45% for age 2 years old after planted on clear felled and burnt site. According to Symington (1974) and Appanah and Weinland (1993), saplings of *S. leprosula* demanded high light intensities in comparison to their seedlings which has better growth under 50% shaded light. Barnard (1954) also suggested that, these species can successfully has high survival rate if established under secondary forest.

Growth performances

The ANOVA table showed that there were highly significant differences in their growth performances (DBH and HT) among the 40 half sib families (Table 3). There was also highly significant difference among the eight blocks observed, probably due to variations in the study site. This analysis also revealed the effects of blocks and families to the growth performances. However, the emphasis of the study was on the variations from the family factor. The mean square for DBH and HT was recorded 7.74 ± 3.85 cm and 7.33 ± 3.02 m respectively (Table 4) which estimated the growth rate of 1.3 cm / year (DBH) and 1.2 m / year (HT).

Furthermore, the mean, standard deviation, coefficient of variation and the number of samples for each of the 40 half sib families for all traits tested were prepared according to their ranking, from highest to lowest (Table 5 and 6). Observation from the analyzed data of DBH and HT (Table 5 and 6), showed that the differences among families were narrow. However, there was significant difference between the averages of the 10 fastest and the 10 slowest families. F14 (DBH = 10.3 ± 10.4 cm; HT = 9.61 ± 2.92 m) was the family with the best characteristics of DBH and HT while the F23 (DBH = 4.71 ± 3.11 cm; HT = 4.87 ± 2.37 m) was the lowest.

Moreover the results also showed that the value of standard deviation was large when compared with the magnitude of the mean, which means the variation within the families is high. On the other hand, the coefficient of variation for both characteristics was between 28.4 to 66.0% (Table 5) and 16.3 to 55.6% (Table 6). This means that there were large variations between progenies in each half sib families.

It was mentioned earlier that *S. leprosula* was considered as a fast growing meranti. Appanah and Weinland (1993) documented that it can reach up to 30–40 cm stem diameter in 20 years. On the other hand, based on the experience of PT. Sari Bumi Kusuma, it can be concluded that the growth of meranti was best when the top canopy received sufficient sunlight at a perpendicular angle. In contrast, when young trees received sunlight from a horizontal direction, there is a tendency for the young trees to produce more branches (the height growth of the main stem would be low).

According to Loveless (1992), the high level of genetic diversity in *S. leprosula* can be due to several reasons, such as regional geographical range, high fecundates, out crossing, long life span, and occurrence in late-succession phase. However, since the genetic diversity obtained was relatively higher than other tropical species with similar life history traits, the species' evolutionary history in

which the ancestors of *S. leprosula* had acquired very high genetic diversity during speciation has also been considered as another reason causing the higher value of genetic diversity in this species. Out crossing and insect pollinated and widely distributed species of *S. leprosula* are considered as to be the other reasons in maintaining the high level of genetic diversity of the species (Rimbawanto and Isoda, 2001).

Table 2: Survival rate of the 40 half sibs families.

Half sib family	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	No. of progeny	Survival rate (%)
F1	1	3	4	1	1	3	2	4	19	59.4
F2	4	4	4	2	0	3	1	2	20	62.5
F3	4	4	2	4	2	3	1	2	22	68.8
F4	4	3	3	3	3	2	3	2	23	71.9
F5	4	4	1	4	2	2	1	1	19	59.4
F6	0	3	3	4	2	1	2	2	17	53.1
F7	1	3	2	2	3	3	1	2	17	53.1
F8	2	3	3	4	3	2	3	4	24	75.0
F9	2	4	2	4	1	2	2	0	17	53.1
F10	2	2	4	3	3	3	3	1	21	65.6
F11	4	4	4	3	3	4	4	1	27	84.4
F12	2	4	4	1	2	4	1	1	19	59.4
F13	1	4	3	2	2	3	2	2	19	59.4
F14	3	3	2	3	2	4	4	4	25	78.1
F15	4	4	2	2	3	4	3	3	25	78.1
F16	4	3	3	1	2	3	1	3	20	62.5
F17	3	2	3	2	3	3	3	4	23	71.9
F18	3	3	4	1	4	4	2	1	22	68.8
F19	3	4	3	4	2	4	3	2	25	78.1
F20	3	3	4	4	4	3	3	1	25	78.1
F21	3	2	3	3	3	3	3	4	24	75.0
F22	3	1	4	3	2	2	0	3	18	56.3
F23	4	3	0	1	2	3	3	3	19	59.4
F24	2	3	3	1	3	4	3	2	21	65.6
F25	2	0	1	4	3	1	1	2	14	43.8
F26	2	1	1	2	2	2	2	2	14	43.8
F27	2	3	3	3	3	3	2	1	20	62.5
F28	2	2	4	4	0	4	1	1	18	56.3
F29	2	3	2	2	3	4	0	1	17	53.1
F30	2	2	3	3	2	1	2	3	18	56.3
F31	1	3	1	4	2	2	4	4	21	65.6
F32	2	4	4	3	4	4	2	3	26	81.3
F34	1	2	2	1	4	2	2	1	15	46.9
F35	3	1	4	3	3	3	4	1	22	68.8
F36	4	3	2	4	4	4	1	0	22	68.8
F37	2	4	0	2	3	2	3	3	19	59.4
F38	3	3	3	1	2	4	2	1	19	59.4
F39	2	2	4	3	3	4	2	2	22	68.8
F45	2	3	4	3	4	2	2	4	24	75.0
F47	3	2	3	3	4	3	4	1	23	71.9
Total	101	114	111	107	103	117	88	84	825	
%	63.1	71.3	69.4	66.9	64.4	73.1	55.0	52.5	Mean	64.5

Table 3: Analysis of variance (ANOVA).

Source	Df	DBH	HT
Block	7	37.35**	10.8*
Family	39	33.35**	19.14**
Block*Family	262	24.38**	17.0**
Error	516	7.37	3.89
Total	824		

*** Significant and highly significant at $P = 0.05$ and $P = 0.01$, respectively.

Table 4: The number of samples (N), mean value \pm standard deviation and coefficient of variation for the diameter at breast height (DBH) and total height (HT).

Characteristic	N	Mean (square) \pm standard deviation	Coefficient of variation (%)
DBH (cm)	825	7.74 \pm 3.85	49.7
HT (m)	825	7.33 \pm 3.02	41.3

In principle, forest tree species which has very large genetic distribution, open and cross pollination system, very wide seed separation and can be propagated generatively or vegetatively will have very large species or population genetic diversity compared to the species which have very narrow genetic diversity. Furthermore, species with very narrow genetic base usually produces a bad genetic planting stock material due to inbreeding depression (Mitton 1989, Hamrick 1989, Hamrick *et al.* 1992).

In order to prevent the species from becoming endangered, conservation strategy is needed. For widespread species such as *S. leprosula* however, in which species existence is not threatened, it is the long-term evolutionary fitness of the species that need to be safeguarded. The progeny test plantation done in this present study can also be considered as a genetic conservation strategy especially if the families observed could be taken from all available populations in natural distribution. This activity is very important in order to maintain the high genetic diversity of *S. leprosula*.

Table 5: The number of samples (N), mean value, standard deviation and coefficient of variation for the diameter at breast height (DBH).

Half sib family	N	Mean \pm standard deviation *	Coefficient of variation (%)
F14	25	10.3 \pm 4.10a	38.6
F5	19	10.2 \pm 3.81ab	37.4
F39	22	10.1 \pm 2.85ab	28.4
F27	20	9.66 \pm 3.16abc	32.7
F11	28	9.59 \pm 3.60abc	37.5
F37	19	9.45 \pm 4.57abc	48.4
F2	20	9.29 \pm 4.03abc	43.4
F6	17	9.28 \pm 4.51abc	48.6
F25	14	8.81 \pm 3.93abcd	44.6
F47	23	8.62 \pm 4.09 abcd	47.5
F24	21	8.56 \pm 4.10 abcd	47.9
F36	22	8.31 \pm 4.23 abcd	50.9
F22	18	8.28 \pm 5.36 abcd	64.7
F17	23	8.05 \pm 4.27 abcd	53.0
F20	25	8.03 \pm 3.23 abcd	40.2
F34	15	7.99 \pm 5.26 abcd	65.8
F15	24	7.78 \pm 3.22 abcd	41.4
F12	19	7.72 \pm 2.74 abcd	35.5
F31	21	7.70 \pm 3.71 abcd	48.2
F28	18	7.66 \pm 3.82 abcd	49.9
F8	24	7.60 \pm 2.89 abcd	38.0
F21	24	7.57 \pm 3.98 abcd	52.6
F19	25	7.46 \pm 3.59 abcd	48.1
F13	19	7.38 \pm 2.56 abcd	34.7
F10	21	7.38 \pm 3.27 abcd	44.3
F32	26	7.38 \pm 4.00 abcd	54.2
F3	22	7.20 \pm 3.74 abcd	51.9
F4	23	6.98 \pm 2.99 abcd	42.8
F18	22	6.95 \pm 2.58 abcd	37.1
F7	17	6.92 \pm 4.35 abcd	62.9
F16	20	6.71 \pm 4.32 abcd	64.4
F9	17	6.69 \pm 2.97 abcd	44.4
F1	19	6.48 \pm 3.67 abcd	56.6
F35	22	6.34 \pm 2.40 abcd	37.9
F29	17	6.21 \pm 3.63 abcd	58.5
F45	24	5.78 \pm 2.62bcd	45.3
F30	18	5.77 \pm 3.69bcd	64.0
F38	19	5.38 \pm 2.75cd	51.1
F26	14	5.30 \pm 2.47cd	46.6
F23	19	4.71 \pm 3.11d	66.0

* Mean with same alphabet showed no significant differences at level 0.05

Table 6: The number of samples (N), mean value, standard deviation and coefficient of variation for the total height (HT).

Half sib family	N	Mean \pm standard deviation *	Coefficient of variation (%)
F14	25	9.61 \pm 2.92 a	30.4
F5	19	9.48 \pm 2.81 ab	29.6
F39	22	9.27 \pm 1.51 abc	16.3
F6	17	9.02 \pm 3.34 abc	37.0
F24	21	8.62 \pm 3.39 abcd	39.3
F2	20	8.60 \pm 2.98 abcd	34.7
F20	25	8.24 \pm 3.10 abcde	37.6
F27	20	8.19 \pm 2.39 abcde	29.2
F37	19	8.12 \pm 3.17 abcde	39.0
F11	28	8.06 \pm 2.49 abcde	30.9
F25	14	7.71 \pm 2.67 abcde	34.6
F17	23	7.67 \pm 3.80 abcde	49.5
F22	18	7.57 \pm 3.86 abcde	51.0
F12	19	7.53 \pm 2.47 abcde	32.8
F4	23	7.48 \pm 2.53 abcde	33.8
F15	24	7.45 \pm 2.85 abcde	38.3
F28	18	7.44 \pm 3.18 abcde	42.7
F36	22	7.41 \pm 3.16 abcde	42.7
F34	15	7.39 \pm 3.93 abcde	53.2
F31	21	7.34 \pm 3.34 abcde	45.5
F9	17	7.24 \pm 2.36 abcde	32.6
F21	24	7.20 \pm 2.95 abcde	41.0
F7	17	7.19 \pm 3.86 abcde	53.7
F47	23	7.16 \pm 2.58 abcde	36.0
F3	22	7.15 \pm 2.87 abcde	40.1
F19	25	7.09 \pm 2.83 abcde	39.9
F13	19	7.07 \pm 1.90 abcde	26.9
F8	24	6.95 \pm 2.89 abcde	41.6
F32	26	6.78 \pm 2.90 abcde	42.8
F10	21	6.73 \pm 2.54 abcde	37.7
F30	18	6.62 \pm 3.63 abcde	54.8
F16	20	6.54 \pm 3.58 abcde	54.7
F1	19	6.45 \pm 3.09 abcde	47.9
F18	22	6.38 \pm 1.74 abcde	27.3
F35	22	6.27 \pm 2.44 abcde	38.9
F29	17	5.95 \pm 3.31 bcde	55.6
F45	24	5.73 \pm 2.18cde	38.1
F26	14	5.45 \pm 2.60de	47.7
F38	19	5.04 \pm 1.87e	37.1
F23	19	4.87 \pm 2.37e	48.7

* Mean with same alphabet showed no significant differences at level 0.05

Conclusion

From the results of the progeny test in Ulu Sedili FR, it can be concluded that:

1. In the progeny test plantation, there was a significant genetic variation in growth performances among families observed. Thus, breeding by selection would be very important in supplying good quality seeds for commercial scale.
2. This important early information would support many tree improvement programmes to include genetic conservation strategy of the species in the near future.

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Preparation of Lotus Planting Stock at Tasik Chini for Sustainable Availability

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Introduction

Lotus (*Nelumbo nucifera*) is a unique aquatic plant which represent as a major tourists attraction to the lakes beside contribution in socio-economic factors and lake conservation at Tasik Chini, Pahang. The lotus that naturally live in the lake has the potential to filter the contaminants or nutrients from water (phytofiltration) and submerged soil (phytoremediation). Those particular substances are absorbed by these aquatic plants for growth via photosynthesis processes (Paresh and Bill, 2006). In terms of socio-economic, lotus helps local people to generate income through ecotourism activities. For preservation of the ecosystem of the lake, lotus help in oxygen cycle in the lake for aquatic life habitat. This in turn will increase the biodiversity of aquatic life in the lake.

However, lotus is a sensitive aquatic plant. Natural disaster such as fluctuations in water level of the lake due to drought and flood incident have been major threat to the lotus population in the lake. Hence, the lotus population had been threatened and diminished. Therefore, the conservation efforts on the lake is very important to restore the sustainability of lotus community and ecosystem stability. Replanting of lotus at the edge of the lake is an effective effort for the conservation of the lotus population. Thus, preparation of lotus planting stock at Tasik Chini is inevitable. This study showed that at least six months were needed to stabilize the lotus community planted in the lake. This aquatic plant is grown well in the muddy soil with water depth less than one meter (Mohd Ghazali et al., 2014).

Materials and Methods

The seedling preparation method is using a ball-rooted plant in pot. Planting stock was collected from living plant in the lake. At least three nodes of stolon need to be taken into pot containing media mixture. The root ball is preserve and grow in the mixture of soil, organic materials and muds (1:1:1) for prolific rooting. The rooting pots need to be submerged in the shallow water for a month at the edge of the lake for refreshing the newly transferred planting materials. After that, it must be transferred into larger pots and put into the nursery pond for three months in order to stabilize the roots and enable new shoots to grow. The new buds may grow profusely when given enough nutrients supply. Then, this young lotus shoots can be planted firmly into the lake muds which has water depth less than 1 m.

A boat is needed for planting lotus in the lake. Planting distance can be made at 3 m intervals at the suitable places, normally parallel to the water fringe. Illustration of the planting procedure is shown in Figure 1.



Figure 1: Planting procedure of *Nelumbo nucifera*.

Results and Discussion

Observation showed that the rate of lotus reproduction in Chini Lake is fast by using appropriately prepared planting stock. A month after lotus replanting into the lake, more than thirty pieces of new leaves were emerged as compared to only eight pieces leaves in the early plantings. Lotus growing area also covered up to 4 m in diameter. The area of plant growth then reached up to 36 m² after two months of planting (Figure 2).



Figure 2: Tasik Chini, (a) before lotus conservation and (b) four months after lotus conservation.

Planting lotus root ball method can be practiced due to its high percentage of success. For this purpose, a nursery for aquatic plants is needed to store prepared the planting stock. The lotus stock in the pot should have at least five leaves above the water surface, before it can be transferred to the lake. Slow release fertilizer at a rate of 200 g should be supplied in the pot to provide ample nutrients supply to the lotus seedlings.

Conclusion

Preparation of lotus mass planting stock is a manageable activity. However, appropriate technique is needed in order to succeed. This study showed a success story of a sound experiment for lotus mass planting stock preparation.

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