ISBN: 978-967-344-188-4

TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 18

ENHANCING PLANT PRODUCTIVITY AND ECOSYSTEM SERVICES IN A CHALLENGING ENVIRONMENT

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20th Malaysian Society of Plant Physiology Conference (MSPPC 2009)

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

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held at Avillion Admiral Cove, Port Dickson, Negeri Sembilan, Malaysia
24-26 July 2009

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Malaysian Society of Plant Physiology (MSPP)

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ISBN: 978-967-344-188-4
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CHAPTER 1

PLANT GROWTH AND DEVELOPMENT

ISBN: 978-967-344-188-4
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Effects of Seeding Density and Watering Duration on Growth Characteristics and Sprouting Atmosphere of Black Gram (*Vigna mungo* L.) Sprouts Grown in a Chamber

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Introduction

Bean sprouts or 'tauge' are 3-5 day-old germinated seedlings of green gram (*Vigna radiata* L. Wilczek) or black gram (*Vigna mungo* L. Hepper) that have been grown in soilless culture and in darkness. Sprouts can be cooked in many different ways or eaten raw and are rich in vitamins, minerals and contain important phytochemicals for disease prevention and health promoting benefits. The bean sprout is a popular vegetable in Asian cuisine and the consumption is increasing in western countries due to their freshness, crunchiness and health benefits (Fernandez-Orozco et al., 2008). Sprouting activates enzyme processes that sustain the later growth stages (Fernandez-Orozco et al., 2006). These chemical changes increase the nutritive value and health qualities of seeds (Plaza et al., 2003) by mobilizing storage nutrients such as concentrated starch and protein into simpler carbohydrates and free amino acids (Ghazali and Cheng, 1991) that can be readily used by human (Sattar et al., 1988).

Seeding density is the amount of seeds per liter of pot and is important in sprout production and in determining the quality of sprouts (Chang and Yeh, 1984). Low seed density causes poor sprout growth producing thin and long sprouts. High density causes overcrowding, resulting in short, curved sprouts and ungerminated seeds in the bottom layer of the containers (Lee et al., 2007). Water acts as a cooling and cleansing agent during sprouting (Lee and Lee, 1992). Too much water produces sprouts with long, thin hypocotyls and long and extensive root-hairs. Studies showed that C_2H_4 is produced during germination. It acts as a growth inhibitor with the triple response of dark-grown seedlings as in *Pisum sativum* and *Arabidopsis* (Guo and Ecker, 2004). These responses consist of thickened hypocotyls, inhibition of both hypocotyls and root elongation and exaggerated apical hook formation.

The concentration of endogenous C_2H_4 produced plays an important role in determining the sprouts quality. Ahmad (1985) reported that a modified-atmosphere containing low level of O_2 and high level of CO_2 in the sprouting environment could be used to regulate growth of sprouts. There is little information available on the effect of seeding density and watering duration on sprout production. There is also limited information on the modified-atmosphere produced during the sprouting in an enclosed chamber. The objective of this study was to determine the effects of seeding density and watering duration on the growth characteristics of sprouts and modified atmosphere during sprouting in a chamber.

Materials and Methods

Selected seeds were washed, sterilized with 10% sodium hypochlorite (NaClO) (Clorox®) and pre-soaked in 150 mg/L Ca for 12 hours. Seeds were put into a pot and the pot was placed into the chamber equipped with an automatic watering system for 96 h of sprouting in darkness. The experiment was conducted in a completely randomized block with a split-plot design. The treatments included 3 watering durations (WD) of 10, 15 and 20 minutes with 3 hours interval x 4 seeding densities (SD) of 50, 75, 100 and 125g seeds/L, in 3 replicates. Watering duration was used as the main plot and seeding density as sub-plot. Hypocotyl length (HL), root length (RL) and hypocotyl diameter (HD) of sprouts were determined at harvest. The modified-atmosphere in the chamber was

measured every 12 h throughout the sprouting period. The data were analyzed by using ANOVA and mean comparisons were done by using DMRT. Regression analysis was done to determine the relationships between dependent and independent variables.

Results and Discussion

Results from this study showed that there were significant interactions between SD x WD on HL, RL and HD (Table 1). There was a significant quadratic relationship between HL and WD (Figure 1). For sprouts grown at 50 g seeds/L, there was a gradual decrease of HL from 10 – 15 minutes WD followed by an increase from 15 – 20 minutes WD. For sprouts produced at 75, 100 and 125 g seeds/L, there were increases in HL from 10 – 15 minutes WD followed by decreases in HL from 15 – 20 minutes WD. A similar growth trend was shown in root length (Figure 2). There was a significant positive correlation (r^2=0.79) between HL and RL indicating that a decrease in HL was followed by a decrease in RL. The decrease in HL and RL could be attributed to the effects of C_2H_4 produced during sprouting. With higher SD, HL of sprouts was shorter. When SD was increased, the sprouts grew closer to one another resulting in compaction within the container, thus inducing production of stress C_2H_4 (Ahmad, 1985). The heat and the endogenous C_2H_4 produced during sprouting inhibited sprout growth. Ethylene inhibited longitudinal growth of sprouts by slowing down cell division and stimulating cells to swell radially (Nicolás et al., 2001). The higher the SD used, the higher would be the production of stress C_2H_4. Studies showed that restricting the elongation of soybean seedlings with a strain gauge resulted in a 3-7 fold increase in the C_2H_4 production rate from hypocotyl, causing retarded elongation and increased hypocotyl expansion (Abeles et al., 1992).

Table 1. Main and interaction effects of seeding densities (SD) and watering duration (WD) on hypocotyl length (HL), root length (RL) and hypocotyl diameter (HD) of sprouts produced.

Seeding density (SD) (g seeds/L)	HL (cm)	RL (cm)	HD (cm)
50	6.65[a z]	5.92[a]	2.22[b]
75	5.44[b]	4.54[b]	2.18[c]
100	4.31[c]	3.58[c]	2.29[a]
125	3.24[d]	2.44[d]	2.19[c]
Watering duration (WD) (min/3 hrs)			
10	5.43[a]	4.24[b]	2.09[b]
15	5.11[b]	4.35[a]	2.28[a]
20	4.20[c]	3.77[c]	2.28[a]
SD x WD	**	**	**

Z For each treatment, means within a column followed by the same letter are not significantly different by DMRT at P≤0.05
*** significant at P≤0.01*

There was a significant quadratic relationship between HD and WD (Figure 3). For sprouts grown at 50 and 100 g seeds/L, there were linear increases in HD as WD increased. However, for sprouts that were produced at 75 and 125 g seeds/L, maximum HD was produced at 15 minutes WD and decreased at 20 minutes WD. The increase in HD in sprouts produced at 50 g seeds/L was due to the development of sprouts into seedlings, as longer WD enabled them to grow long and etiolated. HD of sprouts produced at 100 g seeds/L was affected by the interaction of SD x WD with C_2H_4 produced during sprouting, stimulating cell radial swelling. In sprouts produced at 75 and 125 g seeds/L, HD was increased from 10 -15 minutes WD but decreased thereafter as too much watering affected sprouts growth, causing HD to decrease.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

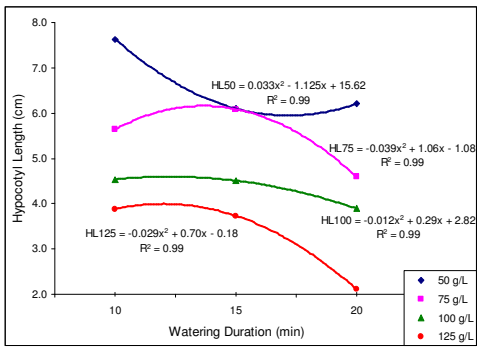

Figure 1. Relationship between SD x WD and hypocotyl length

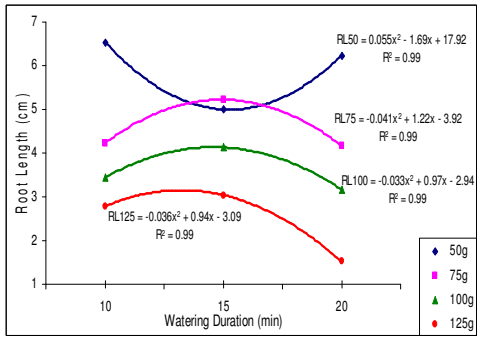

Figure 2. Relationship between SD x WD and root length

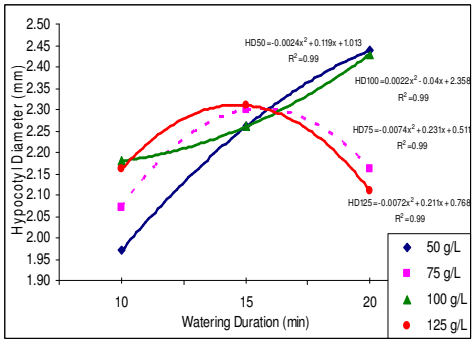

Figure 3. Relationship between SD x WD and hypocotyl diameter

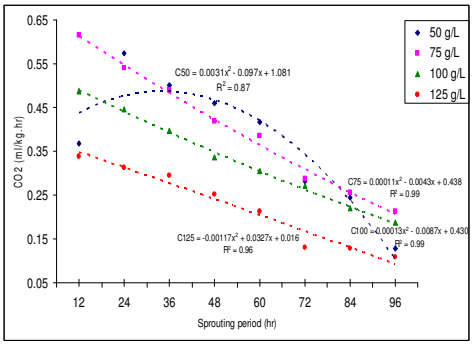

Figure 4. Respiration rate of sprouts during 96 sprouting hours

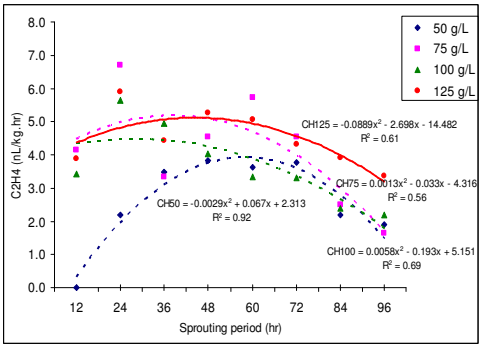

Figure 5. C_2H_4 production of sprouts during 96 sprouting hours

There was a significant quadratic and negative relationship between respiration rate and sprouting period (Figure 4). Sprouts produced at 50 g seeds/L had increasing CO_2 production from 12 to 36 hours after sprouting, followed by a decrease until 96 hours after sprouting. For sprouts that were produced at 75, 100 and 125 g seeds/L, respiration rate showed linear decreases as sprouting progressed. Results showed that sprouts respiration rate decreased gradually throughout the sprouting period in all the SD used. Respiration rate could be slowed down by the limited O_2 and the increasing CO_2 concentration in the chamber. The reduction of respiration rate too was due to exhaustion of soluble carbohydrates in the cotyledon. Sprouts grown in continual darkness were not able to photosynthesize to make their own food. As a result, cotyledonary food reserves became limiting as sprouting progressed (Ahmad, 1985).

Sprouts produced at 50 g seeds/L had a rapid increase in C_2H_4 production rate from 12 to 60 hours after sprouting followed by a decrease until day of harvest (Figure 5). For sprouts produced at 75, 100 and 125 g seeds/L, there were gradual increases of C_2H_4 production rate from 12 to 48 hours after sprouting. Higher production of C_2H_4 during early sprouting period was due to the production of C_2H_4

from imbibition until radicle protrusion that could be related to the rapid reactivation of the metabolism in seeds. Results from this study showed similar findings as reported on C_2H_4 production rate in germinating pea (Petruzzelli et al., 1994). During sprouting, radicle started to break the seed coat followed by root elongation and cell division. The production of C_2H_4 was decreased as sprouting progressed. A study on hypocotyl growth in etiolated lupin seedlings showed that production of C_2H_4 decreased as the germination progressed (Nicolás et al., 2001). This was due to the decrease in ACC oxidase activity and endogenous ACC concentration as the age of seedlings increased.

Conclusions

Results showed that during sprouting, the production of CO_2 and C_2H_4 in the sprout-chamber caused by different combinations of SD and WD created a modified atmosphere that regulated sprout growth. Thus, by regulating SD and WD during sprouting, healthy sprouts with acceptable appearance could be produced without the use of chemicals.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effects of Empty Fruit Bunch (EFB) Compost and Indigenous Microbes on Growth Performance of Cabbage (*Brassica oleracea* var. Capitata)

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Introduction

Consumer awareness on health and environmental concerns has led to an increased demand for organically produced foods. Hence, organic cultivation became one of the top priorities in our current national agriculture policy. In organic food production, particularly under protective structure the use of agriculture wastes as source of nutrients for vegetable production is important to control soil fertility, nutrient cycling, and salinity. Due to continuous application of chemical fertilizers, there have been reported that salt accumulation in soils under plastic houses is a common occurrence (Chang and Liao, 1995; Yoon, 1985) since there is no direct rainfall to wash off or leach away the applied fertilizer.

Vegetable crop growth, yield and product quality in relation to application of agrowaste compost has been widely reported (Togun et al., 2004). Compost as organic fertilizers enhanced the soil structure, organic compounds and nutrient uptake for the plants (Parkinson et al., 1999). Studies on composting of empty fruit bunch (EFB) indicated that its have positive effects on soil chemical and biological properties (Caliman et al., 2001), increased nutrient uptake and yield (Zaharah and Lim, 2000; Ismail et al., 2004). EFB compost considered as a slow nutrients releasing fertilizer, during high nutrients demand at peak time, the nutrients releasing process need to be accelerated by increasing microbial assisted biodegradation. Application of microbial consortium such as indigenous microbes could increase nutrient release from the compost. Indigenous microbes is a group of microorganisms which was extracted from natural resources and applied to soil in combination with organic matters like EFB compost. These microorganisms are important in increasing soil fertility by mineralizing the compost to the available form of nutrients in the soil, hence producing growth factors such as growth hormones and vitamins.

The use of organic fertilizer in cabbage production is recommended because of its nutritive aspects and contribution to human nutrition and other health benefits (Salunkhe and Kadam, 1998). A diet rich in brassica vegetables has been associated with inhibitions of chemical induced carcinogenesis in animals and humans (Kushad et al., 1999). Cabbage for examples is used to treat skin ulcers, premenstrual symptom and yeast infection (Ong, 2008). Hence, a study was conducted to evaluate the influence of EFB compost and indigenous microbes on the growth and yield of cabbage under protective structure.

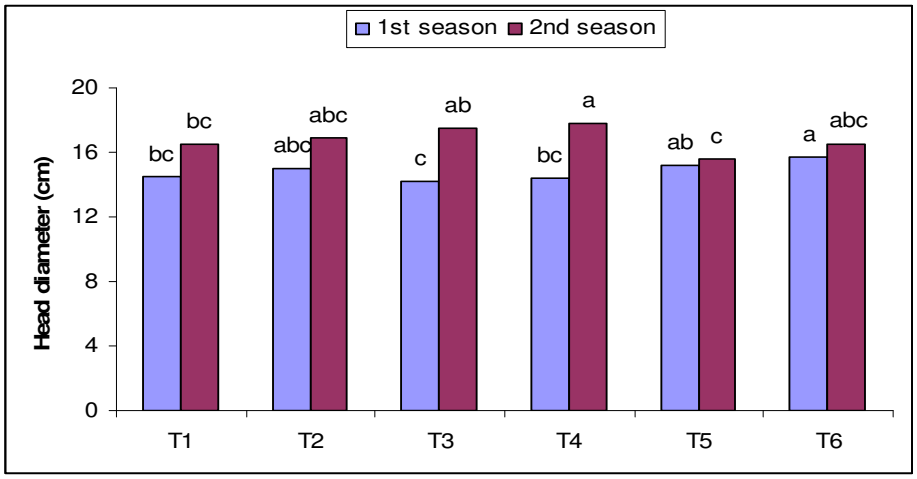
Materials and Methods

The study was carried out under protective structures and the cabbage variety KK Cross was used. Trial was undertaken in two growing seasons. The experiment was laid out in a randomized complete block design with three replications. The treatments were 50%-50% combination of EFB compost and inorganic fertilizer + indigenous microbes (T1), 50%-50% combination of EFB compost and inorganic fertilizer (T2), 100% of EFB compost + indigenous microbes (T3), 100% of EFB compost (T4), 100% of inorganic fertilizer with chicken manure + indigenous microbes (T5) and 100% of inorganic fertilizer with chicken manure as a control (T6). The amount of fertilizer applied was calculated based on 150 kgh^{-1} of N. Compost was incorporated into the planting beds a week before transplanting. Plants were drip irrigated as needed. The plant growth performance was observed and evaluated. Parameter taken at harvesting time were shoot dry weight, shoot canopy, head weight, head diameter and head maturity (days after transplanting, DAT). Data were subjected to an analysis of

varians and means where a significant F value ($p<0.05$) was obtained, Duncan's multiple range test (DMRT) was used for differences among means of the parameter.

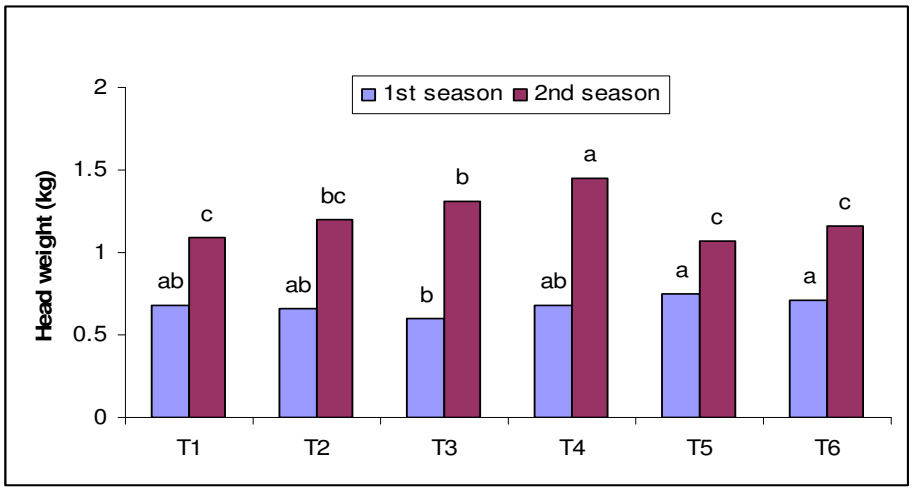
Results and Discussion

There were no significance effects among the treatments of organic and inorganic fertilizer on shoot growth in first growing season (data not shown). It showed that EFB compost alone and in combination with inorganic fertilizer could support shoot growth of cabbage. However yield parameter (head diameter and weight) was better in inorganic fertilizer treatments (Figure 1 and 2). This could be due to the organic matter in the compost formed highly stable N compounds that behaved as a slow release N source, which was not available to the plant on the short term (Nommik, 1965). In the 2nd growing season, it seems that nutrients slowly released to plants during their life cycle as the compost decomposes in the soil. Hence the shoot canopy of 100% EFB compost + indigenous microbes plants was slightly greater as compared to control although they are insignificantly different (Table 1). Similar effect was also found on shoot mass. But with 100% EFB compost, the shoot mass was significantly higher than control.



Means with the same letter(s) are not significantly different by DMRT at P≤0.05

Figure 1. Effect of EFB compost and indigenous microbes on head diameter in 1st and 2nd growing season



Means with the same letter(s) are not significantly different by DMRT at P≤0.05

Figure 2. Effect of EFB compost and indigenous microbes on head weight in 1st and 2nd growing season

Table 1. Effect of EFB compost and indigenous microbes on shoot canopy, shoot dry weight and head maturity in 2nd growing seasons of cabbage.

Trt. No.	Treatments	Shoot Canopy (cm)	Shoot dry weight (g)	Head maturity (DAT)
1	50% EFB Compost + 50% inorganic fertilizer + indigenous microbes	42.40[b]	33.91[b]	87.71[b]
2	50% EFB Compost + 50% inorganic fertilizer	47.66[a]	33.80[ab]	86.80[bc]
3	100% EFB Compost + indigenous microbes	49.07[a]	40.37[ab]	87.20[bc]
4	100% EFB Compost	46.87[a]	42.17[a]	84.00[c]
5	100% inorganic fertilizer + Chicken manure + indigenous microbes	48.04[a]	34.93[b]	92.14[a]
6	100% inorganic fertilizer + Chicken manure (Control)	44.79[ab]	33.91[b]	87.87[b]

Values with the same letter(s) within column are not significantly different by DMRT at $P \leq 0.05$

Head maturity was not affected by treatments in first growing season (data not shown). However, in the 2nd growing season, cabbages treated with EFB matured earlier as compared to those treated with 100% inorganic fertilizer + chicken manure + indigenous microbes (Table 1). The head diameter (Figure 1) and weight (Figure 2) were also affected by the treatments. Similar trends were observed on both parameters. Both 100% EFB compost treatment (T3 and T4) yielded greater head diameter compared to treatment without EFB (T5). The highest head weight however was recorded on cabbage treated with 100% EFB only. There were several studies where crop yields have been increased by the application of agrowaste compost (Maynard and Hill, 2000; Stofella and Graetz, 2000; Togun et al., 2004). However, application of indigenous microbes on EFB compost showed no significance effect on the growth and yield of cabbage. In general, plant growth and yield of cabbage were better in second growing season. This could be due to the high temperature for a period of time at first growing season, thus cause rapid loss of water from the plants.

Conclusions

Generally applying EFB compost would give optimum growth of cabbage. The use of organic fertilizer as the sole nutrient source provided sufficient nutrients for growth and yield of cabbage. EFB compost has great potential to be used in organic cabbage production replacing inorganic fertilizer. Further studies are required to investigate the nutritive quality of yield and the effect of EFB compost in a longer term production with extends to other vegetable crops.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

The Effect of Pre-Acidic Treatment on the Growth of Selected Slope Plants

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Introduction

Plants that are tolerant to high aluminium (Al) concentrations are classified as Al-accumulators. Watanabe (2008) classified Al-accumulators to be the plants that absorb Al in quantities more than 0.1% of the dry matter in leaves. These plants can be divided into three groups (Watanabe, 2002): (1) Al excluders which block the entry of Al at the root level; (2) plants in which roots accumulate Al to prevent the element from reaching the shoot; and (3) plants with accumulation of Al in the shoot. The last group includes plants defined as Al-accumulators (Yang, 2005).

In general, young seedlings are more susceptible to Al than older plants (Li, 2000). From physiological aspects, Al has been shown to interfere with the cell division in seedling roots. Moreover, it will decrease the root respiration in which subsequently interfering with certain enzymes governing the deposition of polysaccharides in root cell wall (Yang, 2005).

Three potential slope crops were chosen for this project; namely, *Acacia mangium, Leucaena leucocephala* and *Melastoma malabathricum,* respectively. *Acacia mangium* has the ability to grow well on infertile soils, especially where low phosphorus is available, make it a favorite plant for rehabilitation of mine spoils and eroded sites (Jansen, 2003). On the other hand, *L. leucocephala* grows on a wide variety of soil types including moderate acid but requires good levels of phosphorus and calcium for the best growth (Normaniza et al., 2006). *Melastoma malabathricum* is a plant that is very efficient in absorbing aluminium ions and classified as a good Al accumulator (Watanabe, 2008).

Therefore, an experiment was conducted to determine the growth performance of pre-acidic seedling in acidic condition and to determine the Al-accumulator characteristic of the plant studied. Some physiological aspects of the plant growth were also examined in this trial.

Materials and Methods

(a) Pre-acidic treatment

Seeds of *A. mangium*, *L. leucocephala* and *M. malabathricum* were germinated for 7 days on moistened filter paper at 25 °C. Seedlings were precultured for 2 weeks on treatment solutions (pH 4.3) of the following composition; 200 µM K_2SO_4, 200 µM $CaCl_2$, 100 µM $MgSO_4$, 200 µM $Ca(NO_3)_3$, 300 µM NH_4NO_3, 5 µM NaH_2PO_4, 10 µM Fe-EDTA, 5 µM $MnSO_4$, 0.38 µM $ZnSO_4$, 0.16 µM $CuSO_4$, 8 µM H_3BO_3, 0.06 µM $(NH_4)_6Mo_7O_{24}$. Controls (pH 7.0) received the basic nutrient solution while for the Al treatments; the solution was supplemented with a nominal concentration of 50 µM Al (non pre-acidic seed). This solution was renewed two times a week to prevent fungal activities. The seeds were grown in a growth chamber under the light intensity of 330 µE m^{-2} s^{-1}, photoperiod of 16 h light/8 h darkness, day/night relative humidity 50%/80%, and day/night temperature 24 °C/18 °C (Tolrá, 2004).

Glasshouse experiment

(i) Soil treatment

After laboratory experiment, both non pre-acidic and pre-acidic treated seedlings, with uniform height of 10 cm were transferred to polythene bag filled with sandy-loam and acidic soil, with soil pH 6.5 to

7.5 and 3.8 to 4.5, respectively. Each species were grown in five replicates. The plants were watered twice a day to maintain the water turgidity.

(ii) Measurements

Plant height and root length

Plant height was measured for 70 days (at 7-day interval). At the end of the experiment, the plant was pulled out from the soil carefully. Both the plant height and root length parameters were measured using measuring tape.

A 7-day interval, Leaf area index (LAI) of the species was measured using leaf area instrument (AccuPAR-LP80, UK).

Total biomass

Biomass of stem, leaf, and root were obtained at the end of the experiment. All parts were oven-dried (80 °C) to constant weight.

Statistical analysis

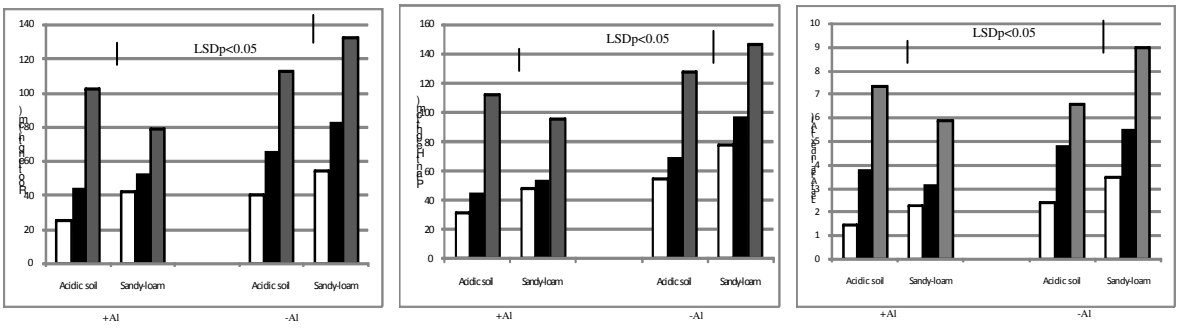
Statistical analysis was performed using Sigma Plot 10.0. The one way ANOVA was applied to evaluate the significant difference of the parameters studied in the three different species of plant in two different treatments. LSD (p=0.05) was calculated using the error mean square of the analysis of variance. The correlation test between the parameters studied was analysed using Microsoft Excel.

Results and Discussion

Plant growth performance

Pre-treated *M. malabathricum* in acidic soil exhibits the longest root length, 23.0% higher than pre-treated *M. malabathricum* in sandy-loam (Figure 1a). Watanabe (2008) in his experiment found that root of *M. malabathricum* exuded large amount of mucilage, generally known to immobilize metal cations such as Al in the rhizosphere. This result indicates that the root growth of *M. malabathricum* is much more tolerant to Al than that of *L. leucocephala* and *A. mangium* during the seedling growth stage. The height of the controlled plant in both sandy-loam and acidic soil is higher than the pre-treated species (Figure 1b). Interestingly, plant height for the pre-treated *M. malabathricum* in acidic soil is higher by 17.0% than those in sandy-loam. These results show that the growth of *M. malabathricum* in acidic soil is greater as compared to those in sandy-loam. In contrast, the pre-treated *A. mangium* shows greater height in sandy-loam by 54.8% than those in acidic soil. The differences in height between pre- treated and controlled *M. malabathricum* in sandy-loam is about 47.4% while in acidic soil is only 5.5%. The LAI of the pre-treated *M. malabathricum* in acidic soil is higher by 11.5% than those in sandy-loam (Figure 1c). This result indicates that *M. malabathricum* has survived extremely well in acidic soil. Watanabe (2008) found that water-soluble Ca, Mg, and oxalate concentrations in both shoots and roots of *M. malabathricum* grown hydroponically in the absence of Al were significantly lower than that in the presence of Al. The increment of Ca, Mg and oxalate in the presence of Al encourages the growth of the upper part of the species; thus, increases the LAI of the plant as well. As shown in Figure 2, the pre-treated *M. malabathricum* exhibits highest total biomass of 12.47 ± 0.21 g and 18.53 ± 0.53 g in sandy-loam and acidic soil, respectively. According to Godsey et al. (2007), aluminium adversely affects several physiological activities, producing a severe physiological stress which increases peroxide activity. Increased peroxide activity might be corresponded to the decreased growth rate, as found in *L. leucocephala* (Normaniza et al., 2006) and *A. mangium*.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18



(a) (b) (c)

Figure 1. (a) Root length, (b) Plant height and (c) Leaf Area Index of Al pre-treated and control seedling in two types of soil; acidic soil and sandy-loam. (□ *A. mangium*, ■ *L. leucocephala* and ▩ *M. malabathricum*). Vertical lines represent significant difference among the treatments at LSD p<0.05.

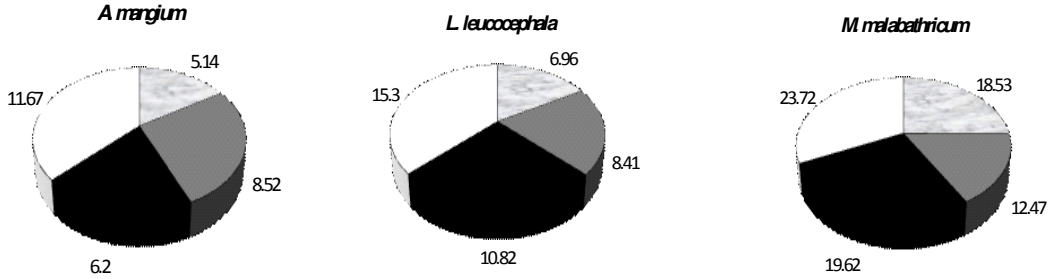


Figure 2. Total biomass of Al pre-treated and control seedling in two different types of soil; acidic soil and sandy-loam.
[□ Acidic soil (+Al), ▩ Sandy-loam (+Al), ■ Acidic soil (-Al), ▨ Sandy-loam (-Al)]

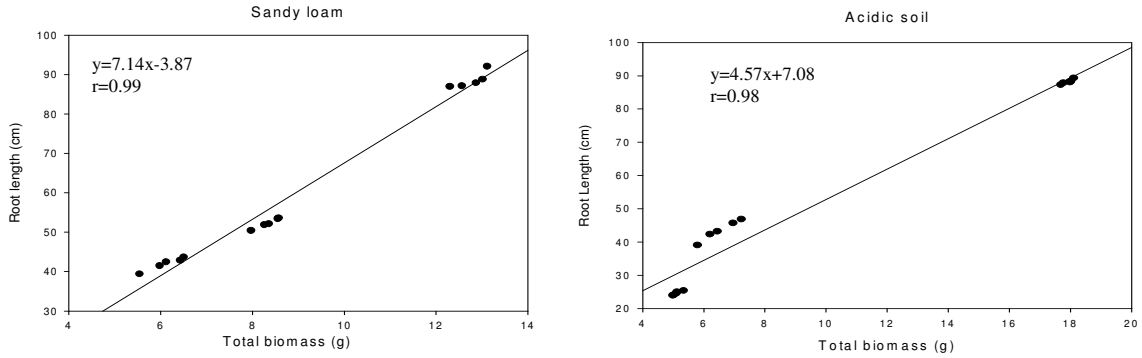


Figure 3. Relationship between root length and total biomass for pre-treated seedling in sandy-loam and acidic soil. Each point represents the mean of 5 determinations.

In this trial, the total biomass is linearly related to the root length both in sandy-loam and acidic soil (Figure 3). The results may be attributed to the adaptive feature of the higher root length of the species studied, presumably to maximize the water and nutrient uptake in order to survive in acidic condition. In addition, due to the contribution of water and nutrient uptake to the upper part of the plant, the photosynthesis rate of the species may increase, thus, the plant growth will increase as well (Sung-Ju and Matsumoto, 2006). The results indicate a positive relationship between LAI and plant height in both types of soil (Figure 4). It implied that high LAI will result in high photosynthetic rate, therefore, possibly high increase of plant height.

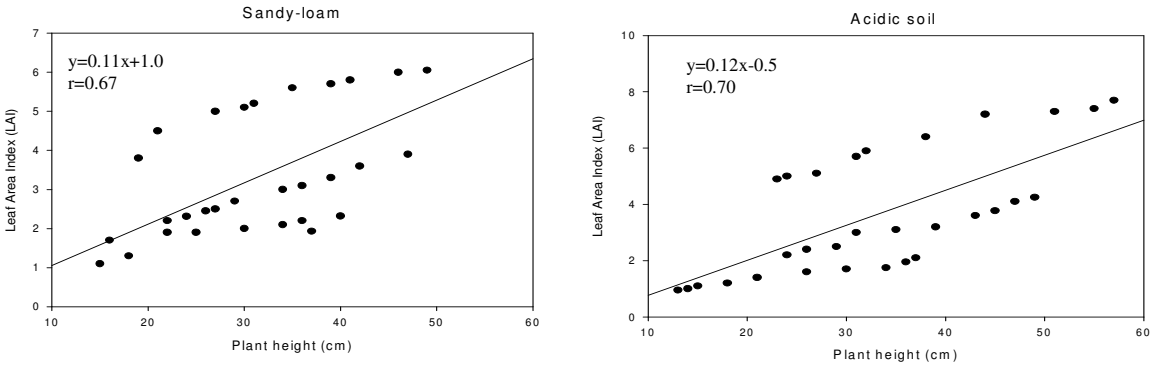


Figure 4. Relationship between Leaf Area Index and plant height for aluminium treated seedling in sandy-loam and acidic soil. Each point represents the mean of 5 determinations.

Conclusions

The pre-treated *M. malabathricum* in acidic soil shows the best growth among the species studied. From the experiment, we can conclude that *M. malabathricum* is highly tolerance to excessive aluminium condition whilst *L .leucocephala* and *A. mangium* are moderate.

Acknowledgements

This project is supported by University of Malaya (PS336/2009A).

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effects of Gibberellic Acid on the Vegetative Growth and Flowering of *Stevia rebaudiana*

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Introduction

Stevia rebaudiana Bertoni (Stevia) is a herbaceous perennial plant of the Asteraceae family. It is native to Paraguay, where it grows wild in sandy soils (Katayama et al., 1976). Stevia has been found to be a commercial interest in many countries due to the presence of diterpene glycoside, mainly steviosides and rebaudiosides in the leaves. It is a hypocaloric sweetener of high quality up to 200-300 times sweeter than sucrose and has recently gained importance as a natural sweetener (Tateo et al., 2004).

It was found that vegetative growth and stevioside levels of Stevia are reduced when day length is less than 12 hours (Yermakov, 1996). Flowering is photoperiod dependent and is enhanced by short day length (Brandle, 1998). When Stevia was first introduced into Malaysia in the mid-1970s, it failed to turn into commercial crop due to several numbers of problems especially the availability of suitable variety. All accessions of Stevia introduced are photo-sensitive and short day length in Malaysia (which is less than 12 hours) causing Stevia to flower, resulting in low leaf yield per harvest and lower sweetener content.

The objectives of this study were to determine the effects of Gibberellic Acid (GA_3) on the vegetative growth and plant phenology (flowering) of Stevia. The target was to obtain optimum concentration of GA_3 to lengthen the vegetative growth and delay flowering.

Materials and Methods

The study was carried out in an open field conditions at MARDI Station Serdang, Selangor from September 2008 to June 2009. Two node cuttings from Stevia mother plants (variety 012) were raised in trays filled with mixtures of coco peat and paddy burnt husk at 2:1 for a month. The rooted plantlets were transplanted to bigger pots with mixtures of top soil, coco peat and sand at 5:3:2. Seven days after transplanting, all shoots of the plantlets were cut 15 cm above the ground level to obtain uniform plants. The experimental field was irrigated with sprinkler daily in the morning and evening while fertilizers, NPK Green, at EC 4 dS/m were applied daily.

Fourteen days after transplanting, plants were treated with gibberellic acid (GA_3) with five different concentrations. The spraying applications were done until the whole plant became completely wet (about five to seven times spraying) at weekly intervals. A randomized complete block design (RCBD) with 16 plants per treatment was arranged with 3 replications (Table 1). The plants were harvested after flower bud emerged. Data taken were plant height, leaf area, leaf weight, leaf number, internode length, stem weight and days to flower bud emergence.

Data gathered was subjected to statistical analysis to confirm any significant effects of GA_3 to the vegetative growth and flowering of Stevia. Analyses of variance and means were done using SAS statistical package employing 0.05 probability level.

Table 1. Experimental treatments

Treatment	Concentration of GA_3 (ppm)
T1	20
T2	40
T3	60
T4	80
T5	100
Control	water

Results and Discussion

Vegetative growth

The results (Table 2) showed that 100 ppm of GA_3 induced the highest plant height (35.77 cm) and treatments with all concentrations of GA_3 produced plants which were significantly higher than control (29.77 cm). The increase in concentrations of GA_3 also showed increasing trend of plant height. The internode length also showed increasing trend with increasing GA_3 concentrations with 100 ppm showing the longest internode length. Although 60 ppm induced the highest stem dry weight (757.76 mg), the stem dry weight still showed the increasing trend with the increases of GA_3 concentration. These results showed that GA_3 lengthened the vegetative growth of Stevia although between 60 to 100 ppm, most parameters showed no significant difference. The increase in plant height, internode length and stem weight in all GA_3 applications suggested that GA_3 induced the accumulation of biomass in stem.

The application of 60 ppm of GA_3 induced the largest leaf area (179.01 cm^2). However, the leaf areas of all concentrations of GA3 and control plants were not significantly different. For the leaf number, all concentrations of GA_3 were not significantly different with control except for 40 and 60 ppm. Forty ppm of GA_3 induced the highest leaf dry weight (1056.61 mg). However, the leaf dry weight of all concentrations of GA_3 and control were not significantly different. These results showed that GA_3 did not significantly induce the biomass production in leaves.

Flowering responses

The application of GA_3 caused earlier flower bud emergence compared to control although there was no significant difference in any treatment and control (Table 2).

Conclusions

The application of GA_3 lengthened the vegetative growth of Stevia. The increase in plant height, internode length and stem weight in all GA_3 applications suggested that GA_3 induced the accumulation of biomass in stem. However, GA_3 did not significantly increase the leaf area, number and dry weight. Since sweeteners are extracted from Stevia leaves, it can be concluded that GA_3 did not significantly contribute to the yield performance during harvesting. However, 40 ppm of GA_3 has the biggest potential to obtain higher yield of Stevia as it induced the highest leaf dry weight. GA_3 also induced plant to flower earlier and thus failed to delay the flowering of Stevia.

Acknowledgements

The authors would like to thank MARDI for permission granted to present these findings. Thanks are also due to all supporting staff in the Physiology Laboratory for their help and support. The research is a sub-project No. 8 from project Development of Production Technology for Stevia (RI 2098KH10).

Table 2. Vegetative growth and flowering parameters of Stevia after harvesting as affected by several concentrations of GA_3

	control	T1	T2	T3	T4	T5
Plant height (cm)	29.77 ± 0.96 a	32.77 ± 1.03 b	33.94 ± 0.77 bc	35.40 ± 0.94 bc	34.19 ± 1.09 bc	35.77 ± 1.00 c
Leaf area (cm^2)	146.07 ± 13.07 a	148.60 ± 14.16 a	167.97 ± 12.09 a	179.01 ± 14.30 a	153.67 ± 11.57 a	159.08 ± 13.57 a
Leaf weight (mg)	923.38 ± 87.68 a	875.45 ± 86.15 a	1056.61 ± 76.01 a	1040.47 ± 91.20 a	882.05 ± 78.43 a	918.36 ± 85.76 a
Leaf number	54.15 ± 4.01 a	52.77 ± 3.11 a	65.54 ± 3.93 b	66.23 ± 3.94 b	60.26 ± 4.31 ab	61.60 ± 3.76 ab
Internode length (cm)	3.46 ± 0.16 a	3.60 ± 0.13 ab	3.72 ± 0.12 ad	4.11 ± 0.18 cd	3.88 ± 0.12 bcd	4.21 ± 0.15 c
Stem weight (mg)	484.96 ± 58.09 a	513.20 ± 51.33 a	666.66 ± 54.84 bc	757.76 ± 77.61 c	672.47 ± 74.81 bc	687.12 ± 60.66 bc
Days to flower bud emergence	26.00 ± 0.60 a	25.56 ± 0.64 a	24.67 ± 0.71 a	24.77 ± 0.52 a	25.91 ± 0.68 a	25.45 ± 0.75 a

** ± represents standard error ** Means with the same letter are not significantly different at P=0.05*

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Influence of Lengthening Day on Stevia Vegetative Growth

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Introduction

Stevia (*Stevia rebaudiana*) is an herbaceous perennial shrub from Compositae family and it is native to Paraguay. The natives have been using Stevia as a sweetener in medicinal teas for treating conditions such as obesity, high blood pressure, heartburn and diabetes. Recently, Stevia has received greater intention with the rise in demand for low-carbohydrate, low-sugar food and beverages additives. Leaves of this plant produce a zero-calorie, non-nutritive and high potency sweetener that is 300 times sweeter than sucrose (Soejarto et al., 1983). It is considered safe for consumption with no major contradictions, warning and side effects reported.

Stevia is an obligate short day plant (Lester, 1999) with a critical day length of about 13 hours. It can initiate flowering after a minimum of four true leaves have been produced (Carneiro, 1990). Day length in Malaysia which is less than 13 hours causes Stevia to flower readily, resulting in low leaf yield per harvest and a lower percentage of sweetener content. Metivier and Viana (1979) reported that Stevia vegetative growth was influenced by day length variation. The vegetative growth is reduced when the day length is less than 13 hours. Thus, increasing day length to 14-16 hours may increase vegetative growth and sugar contents in Stevia (Metivier and Viana, 1979; Ramesh et al., 2006). The objective of this study was to examine the effects of extending day length using lights to Stevia's vegetative growth, namely plant height, internode length and leaf number.

Materials and Methods

The study was conducted under a rain shelter in MARDI Serdang. A total of 180 plants aged one month were planted in pots (3 plants in a pot of 20 cm in diameter x 28 cm in height) containing subsoil, sand and coco peat at the ratio of 7:3:2. All plants were pruned and left with only 2 pairs of leaves in each plant. Each pot was watered with 500 mL daily and supplemented with fertilizer 1.4%N: 0.3%P: 2.4%K once weekly.

There were 6 treatments imposed to the plants; lengthening day with 5 minutes light (T1), lengthening day with 15 minutes light (T2), lengthening day with 30 minutes light (T3), lengthening day with 60 minutes light (T4), lengthening day with 120 minutes light (T5) and 12 hour-light for control plants (T6). Each treatment contained 10 pots arranged in a line with 3 energy saving bulbs (18 watts with irradiance of 430 $umolm^{-2}s^{-1}$) placed 1.3 meter above the plants. For T1 to T5, lights were switched on at 7 p.m. to lengthen the day. Plant height of new shoots, internode length and leaf number were recorded 2 weeks later. All data were subjected to ANOVA, using SAS version 9.1.

Results and Discussion

In Table 1, lengthening day with 15 minutes light allowed highest plant height (9.5 cm) but second lowest leaf number (10 leaves) as compared to other treatments. Control plants (no extended light) showed the lowest in plant height (7.2 cm) and leaf number (9 leaves). There was no significant difference in internode length among all treatments. The results above were similar to that of Metivier and Viana (1979). They reported that extending day length would increase plant height and leaf number. However, results from Metivier and Viana (1979) showed that plants maintained under long day conditions also had increased internode length.

Table 1. Effects of extending light treatment to plant height, internode length and leaf number

Extended light imposed/minutes	Plant height/cm	Internode length/cm	Leaf numbers
0 (control)	7.2c	1.9	9c
5	8.3abc	1.9	12a
15	9.5a	2.1	10bc
30	7.6c	1.9	11ab
60	8.2bc	2.0	12a
120	8.9ab	1.9	10bc

Means followed by the same letter(s) in column are not significantly different at P<0.05

Conclusions

Stevia planted under light treatment gives positive effect on vegetative growth. Plants treated with extended day length increase the plant height and leaf number as compared to plants with no extended light (day length of 12 hours). Further experiment will be carried out to gain more reliable data on vegetative growth, time to flower, biomass production and bioactive compound in Stevia.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Preliminary Study of Floral Induction of *Hibiscus rosa-sinensis* by the Application of Uniconazole

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Introduction

Hibiscus rosa-sinensis or locally known as Bunga raya is an evergreen woody shrub and is commonly planted in Malaysia. Declared as the National Flower of Malaysia in 1960, this species has distinctive red flower that symbolizes bravery and strength (Lian, 2005). The flowers, however, only last for a day; they bloom in the morning and fade in the evening. Flower development is dependent on many intrinsic and extrinsic factors. The aim of this study was to evaluate the effectiveness of a plant growth retardant, uniconazole, in inducing flowering of *H. rosa-sinensis*. It was also aimed to increase flowering with this species within the shortest possible time.

Materials and Methods

Hibiscus rosa-sinensis plants in polybags sized 20 cm x 25 cm were purchased from a local nursery. The plants were propagated by using stem cuttings and were grown under 100% sunlight. The potting medium used was a combination of soil and cocopeat at equal amount. Organic fertilizer was applied for promoting the plant growth prior to sale. The plants were five months old when used for experimentation. Application of uniconazole at rates of 0 g/L, 0.09 g/L, 0.18 g/L, 0.27 g/L and 0.36 g/L was carried out on the plants. Each treatment was replicated seven times in a RCBD. The application volume for each plant was 1 L. Watering of the plants was done when necessary. Weekly, NPK 12:12:17 was applied at a rate of 2 g/plant. Weeding was carried out manually.

Vegetative growth (number of wrinkled leaves, leaf length and number of branches) was measured weekly. The number of flower buds was recorded daily. Data were subjected to ANOVA and means where a significant F value ($p<0.05$) was obtained, Duncan's multiple range test (DMRT) was used for differences among means of the parameter.

Results and Discussion

The leaves of the treated plants were severely affected by the treatment of uniconazole at rates ranging from 0.09 to 0.36 g/L. The wrinkled new leaves were found on the treated plants at seven day-after treatment with the plant growth regulator. Increasing concentrations of uniconazole increased the number of wrinkled leaves (Table 1). The number of wrinkled leaves was also increasing throughout the 28 days of observation with the treated plants. The highest dosage of uniconazole under study resulted in significantly more wrinkled leaves as compared to the untreated plants and those treated at lower dosages. However, the plants were still acceptable for landscaping purposes.

Table 1. Number of wrinkle leaves of *H. rosa-sinensis* after treatment with uniconazole

Uniconazole treatment (g/L)	Period (days)				
	0	7	14	21	28
0	0	0^{c}	0^{c}	0^{c}	0^{c}
0.09	0	11.14^{b}	7.57^{b}	14.29^{b}	15.29^{b}
0.18	0	11.29^{b}	10.43^{b}	13.86^{b}	17.29^{ab}
0.27	0	10.86^{b}	9.57^{b}	18.14^{ab}	23.00^{a}
0.36	0	15.29^{a}	16.00^{a}	19.57^{a}	21.71^{a}

Means followed by the same letter(s) within the same column do not differ (p<0.05) by DMRT

The leaves on the treated plants were relatively smaller as compared to the untreated plants. The average leaf length of the plants treated with 0.36 g/L uniconazole was 9.25 cm before application and 8.29 cm at 28 day-after application of this plant growth regulator, showing a reduction of 10.38% (Table 2). Meanwhile, the plants treated with the lowest dosage of uniconazole, i.e. at 0.09 g/L, showed a reduction of leaf length of only 4.24% for the same length of period. Plants treated with the highest concentration of uniconazole, i.e. 0.36 g/L, had significantly smaller leaves than the untreated plants at 28 day-after treatment. The number of branches was, however, not affected by the application of uniconazole within this experimentation period of only 1 month (Table 3). Ahmad Nazarudin et al. (In press) also reported that uniconazole reduced the height and leaf size of ornamental species, *Syzygium campanulatum*.

Table 2. Leaf length of *H. rosa-sinensis* after treatment with uniconazole

Uniconazole treatment (g/L)	Period (days)				
	0	7	14	21	28
0	9.29	9.03	9.20	9.47	9.60[a]
0.09	10.10	9.67	9.65	9.63	9.26[ab]
0.18	9.19	9.15	9.24	8.98	8.59[ab]
0.27	9.32	8.77	8.99	8.69	8.61[ab]
0.36	9.25	9.12	8.88	8.58	8.29[b]

Means followed by the same letter(s) within the same column do not differ (p<0.05) by DMRT

Table 3. Number of branches of *H. rosa-sinensis* after treatment with uniconazole

Uniconazole treatment (g/L)	Period (days)				
	0	7	14	21	28
0	4.14	4.43	4.86	4.86	4.86
0.09	3.57	4.00	4.29	5.00	5.14
0.18	4.14	4.71	5.14	5.57	5.29
0.27	3.86	4.57	5.00	5.29	5.43
0.36	4.43	4.57	5.29	5.57	6.29

ANOVA showed no significant difference at P<0.05

On the contrary, plants treated with the highest concentration of uniconazole had increased number of flower buds at 28-day after application (Table 4). Flower buds were observed in every plant treated with the highest concentration of uniconazole. Some of the untreated plants as well as those treated with lower dosages did not show as many flower buds as those treated with the highest concentration of this plant growth regulator as studied.

Table 4. Number of flower buds at 28 days after treatment with uniconazole

Uniconazole treatment (g/L)	Average no. of flower buds per plant
0	4.14
0.09	4.42
0.18	2.71
0.27	6.14
0.36	6.14

ANOVA showed no significant difference at P<0.05

Conclusions

Uniconazole resulted in wrinkled and smaller leaves probably due to inhibition of growth of cells. The inhibition effect of this compound can be used as a plant growth maintenance tool in the landscape areas. Floral induction by the application of uniconazole is yet to be concluded. Extended studies are necessary.

Acknowledgements

The authors wish to thank Universiti Teknologi MARA Shah Alam, Selangor for financial support in this study.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Cytokinin Application Effects on Growth Performance and Flowering of *Cattleya* Blc. Haadyai Delight Orchid

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Introduction

Cytokinins and auxins are two important hormones in plant growth and development. Cytokinins promote cell division and are required for plant growth and development (Zhang et al., 2005; Howell et al., 2003), floral development (Taylor et al., 2005) and senescence of plant organs (Sergiev et al., 2007). The application of cytokinin in plants has been shown to induce new shoot and promote flowering in several orchid species. For example, the application of exogenous 6-benzylaminopurine (BAP) has been reported to promote growth of new shoots and flowering of *Deritaenopsis* and *Phalaenopsis* orchids (Blanchard and Runkle, 2008). This plant growth regulator also promoted new vegetative growth of *Miltoniopsis* orchid hybrids as compared to untreated control plants. Increased in vegetative shoot production was alleviated by the adding of gibberellic acid in combination with BA (Matsumoto, 2006). Combining application of BAP and phosphorus enrichment, however, restricted nitrogen supply in *Cymbidium niveo-marginatum* (Kostenyuk et al., 1999).

The application of cytokinin has been reported to induce flowering in a number of *Dendrobium* hybrids, such as *Dendrobuim* Louise, *Dendrobium* Lady Hochoy, *Dendrobium* Buddy Shepler and *Dendrobium* Peggy Shaw (Goh and Yang, 1978; Goh, 1979). Flower induction in plants is also influenced by environmental factors such as temperature, light, photoperiod and water availability (Bernier and Perilleux, 2005). These environmental factors can promote the synthesis of floral stimulus that is transported through tissue to shoot meristem and subsequently induces flowering. Rotor (1959) listed 15 orchid species and hybrids including *Cymbidiums*, *Phalaenopsis*, *Dendrobiums* and *Cattleyas* which responded to low temperature and short day treatment for floral initiation. *Cattleya warscewiczii* Rchb. and *Cattleya mossiae* Hkr. flowered under continuous 9-hours light at 13 °C, whereas few plants flowered under 16-hours light at 13 °C (Rotor, 1952; 1959). *Cattleya liabiata* Lindley and *Cattleya schilleriana* Rchb. flowered under short days regardless of the growing temperature and flowered under long days when the temperature was maintained below 16 °C.

The objective of this study was to determine the effects of cytokinin application on *Cattleya* Blc. Haadyai Delight orchids by injection method. The effects of cytokinin and the application concentration on vegetative growth and flowering of this *Cattleya* species were recorded to determine the potential of such treatment for improvement of quantity, quality and physical attributes of this orchid.

Materials and Methods

A total of 98-potted orchids were bought from a local nursery at Sungai Buloh, Selangor. Plants were 2 year-old after being transferred from tissue culture laboratory. The orchids were planted in plastic pots. Charcoal was used as planting media. All the plants were maintained under the rain shelter and hung on racks to ensure good air circulation around the plants. Black shade netting with 50% relative light intensity was used to avoid direct sunlight reaching the plants.

To maintain the orchids in good growing condition, the coconut husk was applied to the orchids to keep the charcoal moist for longer period. A total of 12.5 g goat dung and 7.5 g dry shrimp shell were applied to each plant monthly. Watering was done when necessary to prevent the orchids from drying. After one month of acclimation under the rain shelter, NPK fertilizers 21:21:21:TE and 18:33:18:TE were alternately applied at a concentration of 2.2 gL^{-1} weekly to enhance the growth of orchids.

Cytokinins of BAP and kinetin were applied to the plants when they were 3 year-old. The plants were treated with three concentrations of BAP and kinetin respectively, i.e. 0.0001 M, 0.001 M and 0.01 M. The treatments were carried out by means of pseudobulb injection. A total of 1 mL of cytokinin solution was injected (with plunge removed) directly into the pseudobulb of each plant. The syringe remained attached to the pseudobulb and the solution was allowed to flow into the tissue freely. The application of cytokinin was repeated monthly on other pseudobulbs of the same plant for a period of four months. Control plants were treated similarly with distilled water (Goh, 1979).

The observation recorded in this study included the emergence of new shoots, length of mature pesudobulbs, length and width of mature leaves and flower development. The length of mature pesudobulbs and length and width of mature leaves were measured by using the measuring tape. The data were recorded weekly. The experiment was based on a Randomized Complete Block Design (RCBD). Each treatment was replicated seven times. There were two potted orchids for each replicate. Analysis of Variance was carried out and the treatment means were compared using Tukey's Simultaneous Test.

Results and Discussion

The effects of cytokinin application on *Cattleya* Blc. Haadyai Delight orchids are summarized in Tables 1 and 2. Such treatment did not significantly affect the number of mature pseudobulbs, length of pseudobulbs, length of leaves and width of leaves respectively within the same growth period throughout the study period of approximately 25 weeks (Table 1). Application of BAP at 0.0001 M, however, significantly induced the potted orchids to produce more total new shoots throughout the study period (Table 2). Application of higher concentrations of BAP at 0.001 M and 0.01 M resulted in necrosis at the points of injection. Some of the treated pseudobulbs died; some were convalescing but the aesthetic value of orchid was reduced and growth of orchids may also probably be affected.

Flowering

As the pseudobulbs and leaves attained full vegetative growth, flowering can be induced from the flower sheath by manipulating the nutrient and plant growth regulator status in plants coupled with optimum environmental requirement for flowering (Lopez and Runkle, 2005). When conditions are not favorable for flowering, the mature pseudobulb will go into dormant stage. The application of cytokinins did not give conclusive results in this study in terms of flowering induction although it was reported to induce flowering in other orchid species such as *Dendrobium* (Sakai et al., 2000), *Aranda* (Goh, 1977) and *Phalaenopsis* (Blanchard and Runkle, 2008). It was probably due to the light availability factor. Further study to manipulate the light availability in combination with the application of cytokinin may be attempted to induce flowering with this *Cattleya* species.

Conclusions

The application of 0.0001 M BAP significantly induced growth of more new shoots in *Cattleya* Blc. Haadyai Delight orchids. The mature leaf size and length of mature pseudobulbs, however, were not affected by the application of cytokinins. Flowering was also not assured probably due to other environmental factors, e.g. availability of light. Further studies on manipulation of availability of light coupled with application of cytokinin should be carried out to induce both new shoot development and flowering with this orchid species.

Acknowledgements

The authors wish to thank Universiti Teknologi MARA Shah Alam, Selangor for financial support in this study.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Table 1. P value of Analysis of Variance of vegetative growth of *Cattleya* Blc. Haadyai Delight orchids after application of cytokinin

Measurement	Period (Day after application)																		
	0	14	22	41	48	55	62	76	84	90	104	111	118	132	139	146	160	167	174
Total number of mature pesudobulbs	0.079	0.134	0.148	0.066	0.132	0.236	0.338	0.353	0.353	0.353	0.242	0.156	0.205	0.141	0.185	0.216	0.419	0.744	0.726
Length of pseudobulbs (cm)	0.599	0.605	0.605	0.649	0.808	0.798	0.798	0.802	0.802	0.802	0.739	0.751	0.755	0.722	0.751	0.748	0.684	0.659	0.649
Length of leaves (cm)	0.791	0.783	0.765	0.706	0.867	0.857	0.869	0.933	0.933	0.933	0.877	0.902	0.902	0.913	0.917	0.921	0.917	0.744	0.726
Width of leaves (cm)	0.471	0.445	0.460	0.374	0.258	0.214	0.355	0.352	0.364	0.367	0.247	0.208	0.204	0.226	0.203	0.192	0.244	0.404	0.389
Total number of new shoots																			0.020

Table 2. Mean comparison of total emergence of new shoots of *Cattleya* Blc. Haadyai Delight orchids at 174 day-after application of cytokinin

Concentration of cytokinin (M)	Total number of new shoots
Control	3.5^{ab}
0.0001M BAP	5.5^{a}
0.001M BAP	4.5^{ab}
0.01M M BAP	4.5^{ab}
0.0001M Kinetin	3.71^{ab}
0.001M Kinetin	3.36^{b}
0.01M Kinetin	3.57^{ab}

Means with the same letter(s) are not significantly different at 5% level of significance

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effect of Culture Medium on Vegetative Growth and Yield of Rockmelon (*Cucumis melo* L. var. Waka Natsu 1)

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Introduction

Growing media is made up of inorganic or organic materials other than soil in which plants are grown. It should be porous and well drained, yet retentive of sufficient moisture to meet the water requirements of plants growing on it between irrigations. It also should contain relatively low soluble salts, but has adequate exchange capacity to retain and supply the elements necessary for plant growth (Calile, 1997).

Coconut coir dust (CD) is a suitable growing media for many crops (Prasad, 1997) and proved to have ion exchange and gas absorptive properties that can be utilized to absorb N in its NH^+ and NO_3^- forms, protecting it from loss into the environment (Evans et al., 1996). When used as growing media, it may be used alone or in combination with other materials. Utilization of 100% CD generally causes initial wettability problem. Therefore, addition of other substrates into CD is needed to stabilize water content in growing medium and possibly enhance its quality as growing media.

Addition of additives such as compost into CD can enhance plant growth and increase fruit quality. It generally improves the chemical properties of container substrates by increasing pH, cation exchange capacity (CEC), and concentrations of plant-available nutrients (Kraus et al., 2000). The use of Agro by-products such as rice straw, empty fruit bunches and rice burn husk as a growing media is becoming popular nowadays. Rice straw has the ability to stimulate phosphorus availability in the soil which can result in higher yield of plants growing in it (Verma and Bhagat, 1992). The production of rice straw compost reduces burning activity in a way while eliminating rice straw waste from paddy field after harvest. In Malaysia, about 8 million tons of empty fruit bunches (EFB) are produced after the oil is extracted and only commonly utilized as mulch for field crop production.

Peat moss is a growing media-based substrate that has high salt buffering capacity and good aeration quality. However, it is suitable for growing plants that require more acidic condition and generally not suitable for growing crops without mixing with other media (Molitor and Bruckner, 1997). Peat is a basic component of potting media in horticulture (Molitor and Bruckner, 1997). Rice husk charcoal is another by-product used to improve properties of growing media.

A study was carried out to determine the best organic material to be used as an additive in coconut coir dust culture for rockmelon production in fertigation system.

Materials and Methods

The study was carried out at Agrotech Unit, Taman Pertanian Universiti, Universiti Putra Malaysia, Serdang, Selangor. Experiments were conducted under a simple rain shelter sized 27.5 m x 3.3 m which can accommodate 180 polybags. The distant between polybags was 45 cm x 45 cm. Rockmelon seeds variety Waka Natsu 1 were germinated in peat moss. After 10 days, the seedlings were transferred to polybags sized 25 cm x 30 cm containing different media treatments (Table 1). The treatments were arranged in Completely Randomized Design (CRD) with five treatments and six replications. By using a fertigation system, fertilizer solution was dripped directly to the root systems. Fertilizer used was based on Cooper Formulation solution and was applied to the plants four times a day (500 mL each time).

Table 1. Five different media treatments tested

Treatment	Composition	Ratio % (v/v basis)
Medium 1	Coconut Coir Dust : Rice Straw Compost	70:30
Medium 2	Coconut Coir Dust : EFB Compost	70:30
Medium 3	Coconut Coir Dust : Peat Moss	70:30
Medium 4	Coconut Coir Dust : Rice Husk Burn	70:30
Medium 5	Coconut Coir Dust (Control)	100

Parameters observed were total leaf area, dry weights (leaf, stem, and root), fruit diameter and total soluble solid (TSS). Total leaf area was determined using Automatic Leaf Area Meter (MODEL LI-3100, LI-COR). The diameter of fruit was measured by using vernier calipers and total soluble solid was obtained using Refractometer.

Results and Discussion

Seedlings of rockmelon var Waka Natsu 1 started to form true leaves at four to five days after sowing. In the third week, strings were tied up to plant stem to support the growth of plant. During this time, the height of plant reached 20 cm. The plants started to form flowers at the fourth week after sowing and assisted pollination was done. Fruit setting was observed at one week after pollination (fifth week). To ensure fruit quality, only two fruits were allowed to develop on each plant. At the early stage of fruit setting, the outer surface of fruits was smooth but after the second week, rough skin resembling netting was formed on the outer surface of the fruits. The fruit 'net' became harder and obvious over time. At the beginning, the net colour was white but it turned to creamy when turned mature. Mature fruits were harvested on the twelve week after sowing.

Vegetative growth

Figure 1a shows the effect of different medium on total leaf area after 12 weeks of cultivation. The highest total leaf area was obtained from plants grown in medium 2 (9185.8 cm^2) and it was significantly different from the control medium 5 (5285.4 cm^2), medium 1 (7136.8 cm^2), medium 3 (6407.4 cm^2) and medium 4 (5411.2 cm^2). The effects of medium on leaf, stem and root dry weight after 12 weeks of cultivation are shown in Figure 1b. Analysis of variance showed that plants grown in medium 2 gave the highest leaf dry weight (56.61 g) and it was significantly different as compared to the control (medium 5, 31.55 g), medium 1 (47.96 g), medium 3 (41.93 g) and medium 4 (35.11 g). Stem dry weight of plants grown in medium 2 was not significantly different from plants grown in medium 1 (27.70 g) and medium 3 (24.68 g). However, stem dry weight of plants grown in medium 2 and medium 1 showed a significant different with the control medium 5 (18.27 g). Root dry weight of plants (Figure 1b) grown in medium 2 (2.20 g), medium 1 (1.89 g) and medium 3 (1.79 g) were not significantly different among one another but significantly different from the control (1.18 g). Root dry weight of plant grown in medium 4 (1.78 g) did not show any significant difference from those obtained from plants grown in medium 1, medium 3 and the control (medium 5).

Root to shoot ratio (Figure 1c) of plants grown in medium 4 was 0.031 and did not significantly differ from plants grown in medium 1 (0.027), medium 3 (0.029) and medium 5 (control). The lowest root to shoot ratio was observed in plants grown in medium 2 (0.025). Incorporation of EFB compost (medium 2) into coconut coir dust culture increased total leaf area, leaf dry weight, stem dry weight and root dry weight of plants. It could be due to the improvement of aeration in the medium which allowed the plant to grow healthier. Hartley (1980) reported that EFB compost could improve soil condition through an increase in soil organic content, soil structure, aeration capacity and microfauna activities. Ismail et al. (2004) suggested that EFB compost can be used to substitute peat in the production of vegetables in combination with coconut dust in soilless culture system.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

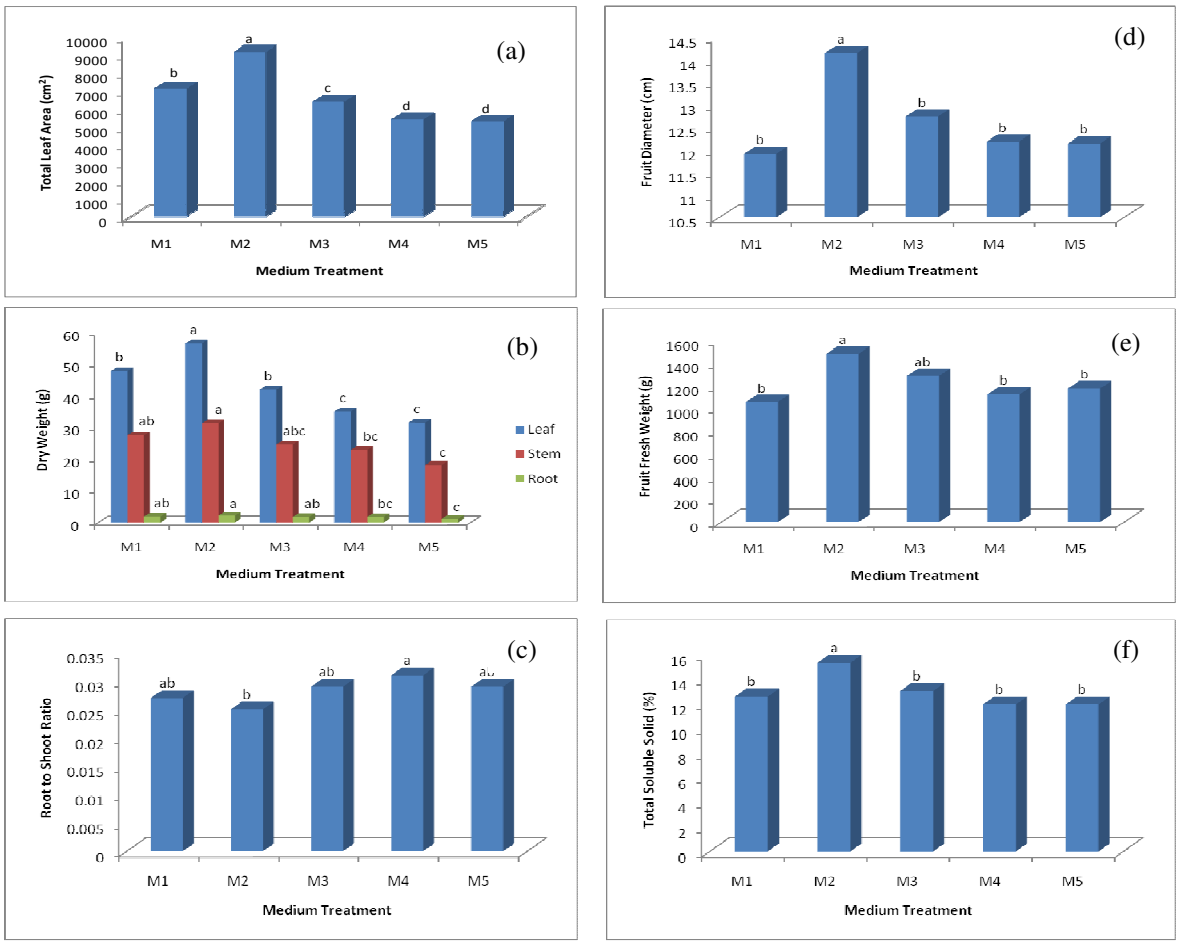

Figure 1. Effect of medium treatment on (a) total leaf area; (b) leaf, stem and root dry weight; (c) root to shoot ratio; (d) fruit diameter; (e) fruit fresh weight and (f) Total Soluble Solid of Rockmelon var. Waka Natsu 1 after 12 weeks of cultivation. Means with the same letter were not significantly different based on LSD (P=0.05).

Yield

As shown in Figure 1d, the highest fruit diameter was found in plants grown in medium 2 (14.15 cm) and it was significantly different as compared to the control (12.14 cm), medium 1 (11.91 cm), medium 3 (12.74 cm) and medium 4 (12.18 cm). Effect of medium treatment on fresh fruit weight was shown in Figure 1e. The control plants were found to have significantly the lowest fruit weight. As shown in Figure 1f, TSS for fruits derived from plants grown in medium 2 was the highest (15.33%) and significantly different from that subjected to the other treatments. The TSS of fruits treated with medium 1, medium 3, medium 4 and control treatment was not significantly different from one another. The recommended minimum eating quality of rockmelon is 10% TSS. The result showed that plants with higher total leaf area produced bigger fruits and higher TSS content. Higher leaf dry weight was associated with higher leaf area and it may suggest a higher whole plant assimilation rate. This, hence, increased the TSS content (Awang and Ismail, 1997).

Conclusions

The growth of rockmelon var. Waka Natsu 1 was enhanced when grown in media containing 70% coconut coir dust incorporated with 30% EFB compost (medium 2). Results obtained in this study were similar as results obtained by Ismail et al. (2004) which reported that cauliflower and Pak Choy grown in coconut coir dust supplemented with EFB compost resulted in better growth and higher

yield as compared to plant grown in either coconut dust or coconut dust mixed with peat. Fruits formed from plants grown in medium 2 also showed better quality. The size was bigger and the TSS was higher as compared to fruits formed from plants subjected to other medium treatments.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Correlating Morphological and Physiological Characters of *Hopea odorata* Planted in Urban Areas

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Introduction

Hopea odorata is suitable to be planted outside their natural habitat and is one of the few Malaysian dipterocarps that can grow in the open. It is a light demanding species where seedling planted in the open areas showed better growth than those planted in partially shaded and closed areas. It is a fast growing species and is found to be favoured for planting in the open environment. Since its introduction for urban planting, it has turned to adapt well to the harsh urban environment and can grow reasonably fast in such conditions. The growth performance of tree depends largely on the maintenance of higher physiological status. Tree species which excel in growth for the first 10 years had higher photosynthetic rate, light interception and water use efficiency. The rate of net photosynthesis was lower in dipterocarps, than in the fast growing species such as *Acacia mangium* and *Tectona grandis.* However, in contrast, Maruyama et al. (1997) reported that net photosynthesis for *H. odorata* was similar to that of fast growing species. This observation has been carried out in a quest for a better understanding on the growth of *H. odorata* planted in urban areas and to examine the relationship between morphological and physiological parameters.

Materials and Methods

The study was conducted on 36 *H. odorata* trees at the New Klang Valley Expressway (NKVE). The seven years old trees were planted by PLUS Berhad, the highway concessionaire. Total height, stem diameter at breast height (dbh), crown diameter was measured at the onset of the study and after 12 months. Differences between initial measurement and measurements after 12 months were obtained to get increments. Relative leaf chlorophyll content was measured by a non-destructive measurement using a portable chlorophyll meter, SPAD-502. Net photosynthesis (Pn) was measured using a portable infrared gas analyzer, Li-6200 photosynthesis system. Measurement of leaf area index (LAI) was performed using a plant canopy analyzer, LAI 2000. Specific leaf area (SLA) was determined using the formulae; L_A/L_W (Hunt, 1982) where L_A = leaf area and L_W = leaf weight. Light response curve was recorded using portable photosynthesis system, Li-6400. At light saturation point of the light response curve, the maximum photosynthesis rate (A_{max}) and transpiration rate (E) was recorded. Water use efficiency (WUE) was then determined using formulae; A_{max}/E (Li et al., 2003). Apparent quantum yield (Qn) was calculated using linear regression across three or more points where net CO_2 assimilation was linearly related to photosynthetic photon flux density (Singsaas et al., 2001). Light radiation beneath and above tree canopy was recorded using a light meter, Solarimeter (EMS, UK). Light use efficiency was determined using formulae; Pn/I_O (Vandermeer, 1989) where Pn = net photosynthesis and I_O = light above the canopy. Foliar nitrogen (N) was analyzed using the classical Kjeldahl method. All statistical analyses were carried out using Statistical Analysis System (SAS).

Results and Discussion

Correlation analysis was carried out to determine the relationship among all the parameters examined in this study. The results showed that there were no significant correlations among the morphological characters of *H. odorata* (Table 1). This indicates that within the study period of only 12 months, no relationship can be observed among height, stem dbh and crown diameter increments. Such relationship may be observed in a longer period of time as the result in this study also indicated no significant improvement in terms of stem dbh for *H. odorata* at the study site. Among the physiological characters, LAI was found to have positive significant correlations with relative

chlorophyll content (n=35, r=0.632, p=0.05) as well as with foliar N content (n=35, r=0.593, p=0.05) of *H. odorata.* A positive significant correlation was also found between SLA and foliar N content (n=32, r=0.384, p=0.05), implying that LAI increased with N supply and was associated with increase in mean leaf size and number. N supply was significantly related to foliar N content and SLA (Chang, 2003). Fertilization was found to increase LAI and subsequently growth of *Eucalyptus nitrus* plantation at ex-forest sites (Smethurst et al., 2003). A positive correlation was also found between LAI and transpiration rate (E) of *H. odorata* (n=33, r=0.408, p=0.05). This is in agreement with Pierce and Running (1988) whom reported a positive correlation between both variables. An increase in LAI would increase transpiration rate (Linder and Rook, 1984).

Foliar N was also found to have a strong positive correlation with relative chlorophyll content (n=36, r=0.852, p=0.05). Havstad and Aamlid (2006) found a significant relationship between chlorophyll readings and nitrogen concentration of the last fully developed leaf of *Phleum pretense* and suggested that it can be an indicator for plant N status. Pn and LUE showed a strong positive correlation (n=34, r=0.931, p=0.05) indicating a linear relationship between both variables. In measuring association between morphological and physiological characters some positive relationships were observed. A positive significant correlation were found between LAI and height increment (n=33, r=0.468, p=0.05) and between LAI and crown diameter increment (n=35, r=0.556, p=0.05). Smethurst et al. (2003) found a strong relationship between LAI and growth of *Eucalyptus nitens* and suggested that LAI may be a better indicator of growth. Height increment was found to have positive correlations with foliar N, (n=34, r=0.575, p=0.05), relative chlorophyll content (n=34, r=0.582, p=0.05) and SLA (n=30, r=0.426, p=0.05). Relative chlorophyll content was also found to have positive correlations with crown diameter increment (n=36, r=0.392, p=0.05). Relative leaf chlorophyll content was found to be positively correlated with leaf nitrogen content (Wood et al., 1992). High nitrogen supply affect leaf nitrogen status and thus have an effect on leaf chlorophyll content and plant growth. Plant responds to N supply by stimulating shoot growth and increasing top growth (Harris, 1990).

Result of the correlation analysis also showed relationship between parameters determined from the light response curve and their associations with the rest of the growth parameters. A_{max} was found to give a strong positive correlation with E (n=34, r=0.723, p=0.05) and WUE (n=34, r=0.706, p=0.05) and Qn (n=34, r=0.875, p=0.05) and net Pn (n=34, r=0.543, p=0.05). Moroco et al. (2002) reported that Qn and A_{max} for drought-stressed plants were decreased relative to well-watered plants, indicating a relationship between both parameters. Qn was also observed to have positive correlations with E (n=34, r=0.845, p=0.05) and WUE (n=34, r=0.464, p=0.05), net Pn (n=34, r=0.567, p=0.05) and LUE (n=34, r=0.534, p=0.05) whilst WUE showed a positive correlation with net Pn (n=34, r=0.448, p=0.05).

Conclusions

Preliminary results indicated that no relationship can be observed among the morphological parameters of *H. odorata* namely height, stem dbh and crown diameter increments after 12 months. Among the physiological parameters, (LAI) and SLA were found to have significant correlations with relative chlorophyll as well as foliar N content. In measuring associations between morphological and physiological parameters, significant relationships were observed, notably between height increment and LAI, SLA, relative chlorophyll content as well as foliar N content. Relationships found between LAI and foliar N, relative chlorophyll content as well as morphological characters suggested that LAI may be a better indicator of growth.

Acknowledgements

The authors are grateful to FRIM and UPM for the institutional support and to PLUS Expressways Berhad for the provision of the study site. The study was funded by the Malaysian Government (IRPA 01-04-01-0016).

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ISBN: 978-967-344-188-4
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Table 1. Coefficient of correlation between growth parameters of *H. odorata*

	Height increment	Stem dbh increment	Crown diameter increment	LAI	SLA	Chlorophyll content	Foliar N	Net Pn	LUE	Amax	E	WUE	Qn
Height increment	-	-0.0212 ns	0.1353 ns	0.4685 *	0.4268 *	0.5825 *	0.5754 *	0.1738 ns	0.1070 ns	0.2636 ns	0.2984 ns	0.1442 ns	0.3712 ns
Stem dbh increment		-	0.1610 ns	0.1808 ns	0.1718 ns	0.0960 ns	0.0000 ns	0.0282 ns	0.0272 ns	0.3957 *	0.3485 ns	0.1741 ns	0.3780 *
Crown diameter increment			-	0.5568 *	0.0209 ns	0.3921 *	0.2637 ns	0.3415 ns	0.4945 *	0.2771 ns	0.2310 ns	0.1912 ns	0.2557 ns
LAI				-	0.2594 ns	0.6322 *	0.5932 *	0.2633 ns	0.3463 ns	0.2193 ns	0.4083 *	-0.0618 ns	0.4171 *
SLA					-	0.3665 *	0.3842 *	0.3057 ns	0.2241 ns	0.1626 ns	0.1720 ns	0.1168 ns	0.2156 ns
Chlorophyll content						-	0.8527 *	0.2681 ns	0.2483 ns	0.2232 ns	0.3424 ns	0.0203 ns	0.4183 *
Foliar N							-	0.2774 ns	0.2371 ns	0.1831 ns	0.3465 ns	-0.0142 ns	0.3758 *
Net Pn								-	0.9313 *	0.5437 *	0.3954 ns	0.4487 *	0.5671
LUE									-	0.5467 *	0.4267 *	0.4145 *	0.5340*
Amax										-	0.7233 *	0.7064 *	0.8760 *
E											-	0.0670 ns	0.8455 *
WUE												-	0.4463 *
Qn													-

Notes: **: significant different at p<0.05*
ns: not significant

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effect of Organic and Inorganic Fertilizer on the Chlorophyll Content of *Polianthes tuberose* L.

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Introduction

Polianthes tuberose L. is one of the most popular odorous flowering ornamentals and is an excellent summer blooming flowering bulb well suited to the summer. It is a perennial plant of Agavaceae from Mexico and commercially grown for its attractive and luring cut flowers and also for production of new bulbs. Also it produces a showy, conspicuous, fragrant yield of cut flowers of a high marketable value due to the lack of other flowering bulbs in summer and autumn (El-Naggar, 1998). *Polianthes tuberose* is a night-blooming plant that consist about 12 species and its extract were used as a middle note in perfumery. Its scent describes as a complex, exotic and sweet. The flowers are always used in wedding ceremonies, garlands, decoration and various traditional rituals especially for Indian culture. This is due to the long lasting of scent and structure of the flowers after being harvested. A lot of studies have been done to increase the production of this unique and important flower plant. As we know, one of the factors that influence the plant production is the type of fertilizer supplement to the plant. Fertilizer contain compounds such as potassium, calcium, magnesium, sodium, and nitrogen that play an important role in complex plant biochemical process that will contribute to the plant development (Hättenschwiler et al., 2000). Mineral fertilizers are important and quickest way of nutrient supply to soil and play an important role in activating various enzymes (Tisdale et al., 1990). According to the Minimum Liebig Rule, certain nutrient shortage will inhibit the plant growth although the other nutrients are sufficient. Fertilizer can be categorized as organic fertilizers (composed of decayed plant/animal matter) or inorganic fertilizers (composed of simple chemicals and minerals). Organic fertilizers are naturally occurring compounds, such as peat, manufactured through natural processes, or naturally occurring mineral deposits whereas inorganic fertilizers are manufactured through chemical processes. Organic matter is of great importance for the maintenance of soil structure, soil bioactivity, soil exchange capacity and water holding capacity. Comparison between inorganic and organic fertilizer-nitrogen (N) sources are hard to perform since there is usually a dramatic difference in N availability from these two sources of N. Comparisons on the basis of similar amounts of total N applied are therefore of limited relevance to agricultural practice, whereas comparisons on the basis of similar N availability are hindered by the lack of reliable nitrogen release estimates for organic fertilizer sources derived from animal manures (Van and Reeves, 2002). Nitrogen is the most important nutrient in plant that is responsible in the chlorophyll production in leaves. In this study we attempt to identify the effect of organic and inorganic fertilizer on the chlorophyll content of *P. tuberose* L. Chlorophyll is vital in photosynthesis that allows plants to obtain energy from light. Thus, chlorophyll content is one of the factors that can contribute in plant growth and development. The objective of this study was to identify the most suitable types of fertilizer application for enhancing the chlorophyll content of *P. tuberose* L.

Materials and Methods

Polianthes tuberose L. was planted at the Pusat Bioteknologi Tumbuhan, Universiti Kebangsaan Malaysia at Selangor. For this experiment, a total of 20 seedlings were planted in 20 different pots filled with 3:2:1 composition of soil, sand and compost materials. These 20 potted plants were placed with distance of 30 cm from each other. Besides that, all the seedlings were planted 8 cm depth from the soil level in the pot. Direct sunlight (assumed as 100% light intensity) was exposed to the plants and twice watering per day was done for each experimental plants. Ten from the planted tuberose

were treated with organic fertilizer (Thank-Q), while the other 10 plants were treated with inorganic fertilizer (BBSCKAB) that composed of 12% nitrogen, 12% P_2O_5, 17% K_2O, 2% MgO and pH value neutral. Fertilization treatments were done after 30 days from planting and continued once a month with the same fertilization technique. Observations were made on the chlorophyll content of the plant. The chlorophyll contents were measured from matured leaves using Spectrum Chlorophyll Meter Model SPAD-502.

Results and Discussion

The chlorophyll contents were observed every month for 3 times. Different fertilizer application showed differences in the chlorophyll content (Figure 1) in which plant that used inorganic fertilizer showed higher chlorophyll content compared to plant treated with organic fertilizer. This probably caused by difference in the nitrogen content found in both fertilizers. Nitrogen is important for the chlorophyll production of leaves. Most nitrogen absorbed by plant is in nitrate form where sufficient nitrogen supply enabled plant to grow faster and exhibit its green color. Lack of nitrogen caused plant to become chlorosis which normally starts under the leaves. Plus, it can also caused remobilization on the seed growth, fasten the aging process of the leaves and reduced the chlorophyll content (Shukla et al., 2004). Previous study also indicated that inorganic fertilizer applied on *Lactuca sativa* L. showed an increased in the concentration of nitrate in the leaves. Therefore, inorganic fertilizer used may contain more nitrogen source than organic fertilizer because it can increase the chlorophyll content of the plant more efficient than the organic fertilizer. Chlorophyll is important in many plant metabolic functions such as growth and respiration. The increase of chlorophyll content will contribute the growth of plants. Chlorophyll a and b contained in leaves of higher plants are the main pigments of photosynthesis in the chloroplasts, and have important functions in the absorption and exploitation of the light energy, thereby influence photosynthetic efficiency (Pan and Dong, 2002).

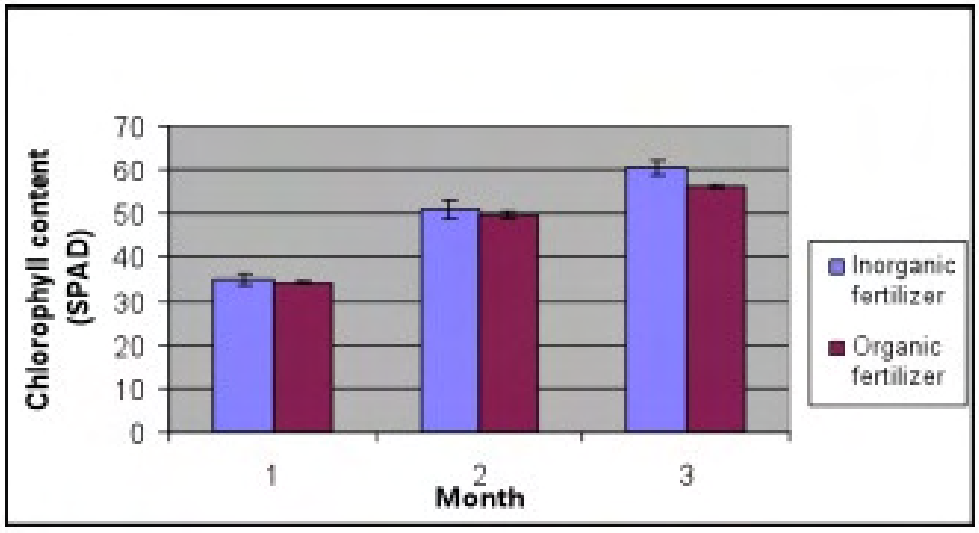

Figure 1. Effects of inorganic and organic fertilizers on the chlorophyll content of *P. tuberose* L.

Some studies have demonstrated that chlorophyll content is positively correlated with photosynthetic rate (Araus et al., 1997; Thomas et al., 2005). In this experiment, *P. tuberose* L. plants that have more chlorophyll content shows more leaf production and larger leaf size than the plants which have lower chlorophyll content (Figure 2). This indicates there is relationship between chlorophyll content and plant development. In orchids, increasing level of nitrogen increased the flower sized and also affects both vegetative (length and leaf production) and reproductive (inflorescence length) growth (Hew and Yong, 1997). By using inorganic fertilizer the chlorophyll content can be increased effectively. Therefore, this may become an alternative to enhance the growth and promote flowering of *P. tuberose* L. plant for commercialization.

(a) (b)

Figure 2. *Polianthes tuberose* L. plant treated with (a) inorganic fertilizer and (b) with organic fertilizer.

Conclusions

Different fertilizer used can affect the chlorophyll content of *P. tuberose* L. From this experiment it is concluded that inorganic fertilizer give better results in increasing the chlorophyll content of *P. tuberose* L. as compared to the organic fertilizer. However, there is an evidence to indicate that combinations of organic and inorganic fertilizer are recommended as it generally gives better growth and flowering. Thus, the correlation between all the other environmental factors such as light intensity and irrigation that can contribute to the growth of *P. tuberose* L. should be studied further.

Acknowledgements

This project was funded by School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

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ISBN: 978-967-344-188-4
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Flowering Behaviour, Fruit Set and Development of *Jatropha curcas* L.

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Introduction

Jatropha curcas L., is a multipurpose plant that belongs to the family Euphorbiaceae and is valued not only for its medicinal properties and resistance to various stresses but also for its use as an oilseed crop (Heller, 1996; Openshaw, 2000). In recent years it has drawn the world's attention as it has the potential to replace fossil fuel as biodiesel. However, the biggest setback in Jatropha cultivation in Malaysia is the low percentage of fruit set and the high ratio of male to female flowers. Bhattacharya et al. (2005) reported about only 50% of female flowers set to fruit in Lucknow, India. In order to further understand the flowering and fruit characteristics of Jatropha, floral and fruit development timeline is needed in order to develop solutions for the problems in low fruit set. One approach is to study the floral biology, floral ontogenesis, pollination ecology and pollen-style interaction. The objectives of the present study were to determine the timeline of floral and fruit development and to describe the floral biology and flowering pattern of *J. curcas.*

Materials and Methods

Field observations were carried out from November 2008 to June 2009 on twenty, five to six years old *J. curcas* plants at Field 2, U.P.M, Serdang from a population of 121 *J. curcas* plants. Floral phenology, male to female ratio and flower to fruit ratio was studied. Male to female flower ratio and flower to fruit ratio were recorded based on 10 inflorescences. Each inflorescence was derived from different trees in the same planted area. For floral development, observations were based on macro-morphological changes and vegetative shoots were tagged and observed for their developmental changes up to fruiting stage.

Results

Floral and fruit development

Floral bud becomes visible after 24 days from the first day of observation in vegetative stage (Figures 1(a) and (b)). It takes about 26 days from the day of visible floral bud to floral anthesis (Figure 1(c)). Once it begins flowering, the flowers open daily. The flowering last about 8 days (Figure 1(d)) and followed by 33 days for the fruit to be matured (Figure 1(e)). Fruit senescence occurred 7 days after the fruit maturity (Figure 1(f)). It takes about 100 day to complete a cycle of fruit set development (Figure 2).

Floral behaviour and phenology

Jatropha curcas in this field is monoecious and produces individual flowers in dichasial cyme pattern (Figure 3(a)) and grouped together in the cymose corymb inflorescence (Figure 3 (b)). The flowers are unisexual, both staminate and pistillate on the same inflorescence (Figure 3). Normally, the female flowers are produced at the central of the inflorescence surrounded by the male flowers (Figure 3). In some cases, the expected female flower positions are replaced with male flowers. The ratio of male to female flowers is 22:1 and flower to fruit ratio is 6:5. Numerically, 3-10 female flowers and 78-215 male flowers are produced in the same inflorescence (Table 1).

ISBN: 978-967-344-188-4
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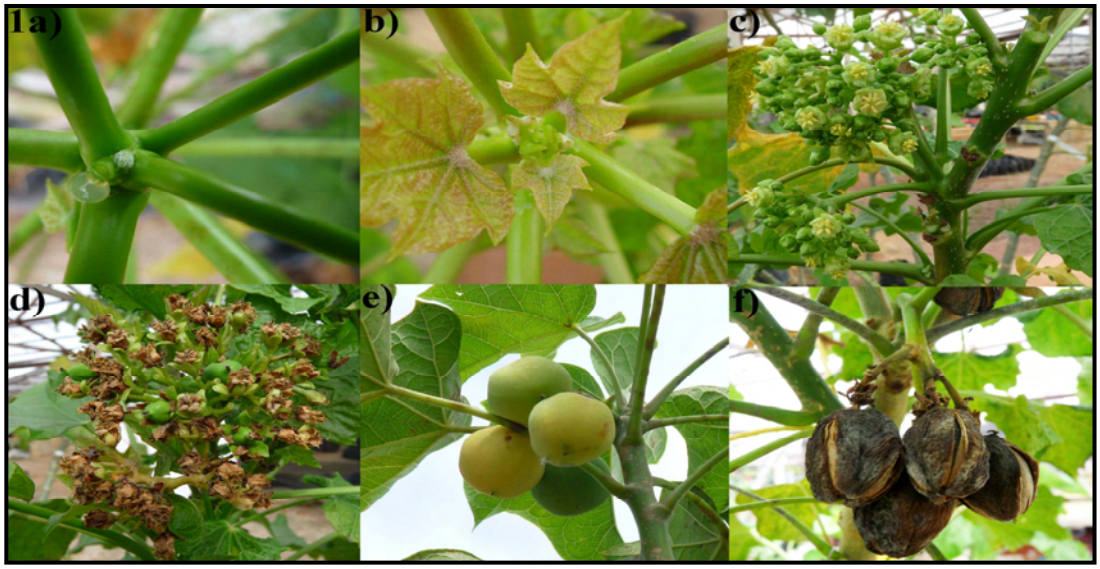

Figure 1. Macrophotographs of flower and fruit development showing (a) Vegetative stage at Day 0, (b) First visible flower bud at Day 24, (c) Anthesis at Day 50, (d) Flower senescence and fruiting at Day 58, (e) Mature fruit at Day 93 and (f) Fruit senescence at 100 days.

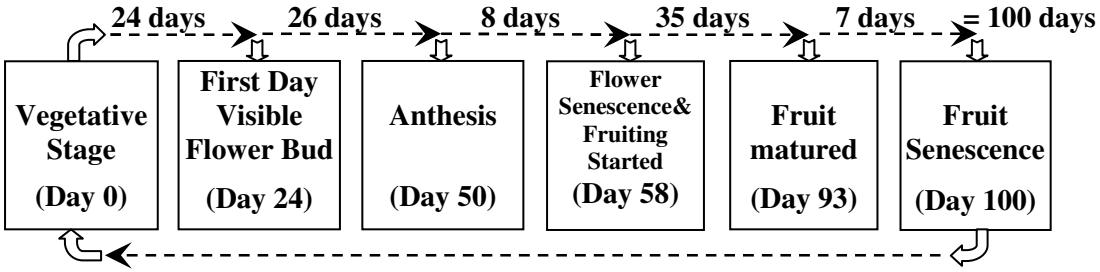

Figure 2. Timeline of flowering and fruit set showing *J. curcas* takes about 3 months to complete a fruiting cycle.

In this study, the terminal stems of Jatropha profusely bears fruits in January and May in year 2009 (Figure 4). Fruiting in both months was during the dry period and when the shrub is leafless. In the month of June after fruiting, flowering took place.

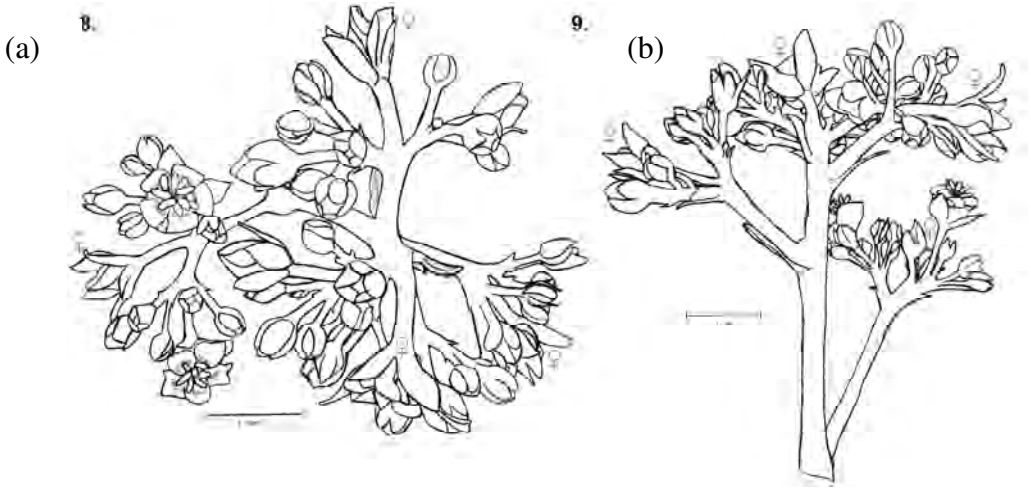

Figure 3. *Jatropha curcas* inflorescence in dichasial cyme pattern and grouped together in the cymose corymb inflorescence (a) Upper view and (b) Side view. Abbreviations: ♀, female flower; cm, centimeter.

ISBN: 978-967-344-188-4
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Table 1. Mean and number of staminate and pistillate flowers in an inflorescence and number of fruits produced from the pistillate flowers.

Inflorescence no.	No. of staminate	No. of pistillate	No. of fruits
1	78	3	3
2	98	4	4
3	183	5	4
4	105	8	7
5	112	6	6
6	95	8	8
7	109	3	3
8	215	10	10
9	155	3	2
10	143	6	6
Mean	129	6	5

Figure 4. Terminal stems of Jatropha profusely bears fruits.

Discussion

Jatropha shows the characteristic year round free bearing habit but with multiple cyclical fruiting peaks in Malaysia. This understanding can be related to the fruit set development that takes about 3 months from vegetative stage until fruit maturity. In this study, Jatropha showed two fruiting peaks in January and May in year 2009. The natural peaks can be altered by the changing of weather conditions or by cultural manipulations (Milan, 2008). Floral and fruit development takes about 3 months or 90 days interval and similar to the finding by Heller (1996). This understanding confirmed the results that we have for floral and fruit development that takes about 3 months from the initiated floral bud stage until the fruit maturity. Flowering usually triggers after a dry and dormant period and induced by prolonged periods of soil water availability (Jongschaap et al., 2007). Flower formation maybe influenced by the weather condition at the time of bud differentiation. Dry weather induces flower bud formation and heavy rainfall promotes the formation of vegetative buds.

Flowering is one of the most important stages for *J. curcas* oil production as the number of pistillate flowers and their fertilization determines how many fruits eventually will develop. The initial fruit set of Jatropha is quite high as much as 83% of the pistillate flowers set fruit. This shows that the plant does not suffer from under-pollination. The production of pistillate flowers in small number, and surrounded by a large number of staminate flowers with male to female ratio 22:1 promotes pollination to the maximum point. This result was similar to Bhattacharya et al. (2005) which recorded 29:1 male to female ratio. The arrangement of individual flowers grouped together into

inflorescences also promotes attraction and foraging rate by the foragers (Solomon Raju and Ezradanam, 2002). Large number of flowers tends to increase the attraction on pollinators because the emission of perfumes is more intense and flowers are more visible (Tcherkez, 2004).

Conclusions

In conclusion, the problem of low fruit set is mainly caused by the small number of pistillate flowers in an inflorescence. The correct time of fertilizer application and the correct type and rate of fertilizer can also be studied in order to increase the number of pistillate flower.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Sour to Sweet Magical Properties of the Miracle Fruit - *Synsepalum dulcificum* Daniell (Sapotaceae)

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Introduction

Miracle fruit (*Synsepalum dulcificum* Daniell) from the family Sapotaceae is a tropical evergreen shrub or small tree growing to 2 – 5 m tall (Keay, 1992; Duke, 1993). The plant is indigenous to tropical West Africa (Opeke, 1984), distributed from Ghana to the Congo region and commonly found growing in the wild in virgin forest, costal areas and in mountainous forest. The plant can be usually found growing in partial shade area under the canopy of the rain-forest in its native habitat and is able to tolerate full sunshine and drought (Duke, 1993).

Methodology

Samples of inflorescent, fruits and seeds were randomly collected from five matured miracle fruit plants in Ladang 2, UPM. Samples were measured and observed under a light microscope. Ten 3 years old potted *S. dulcificum* were randomly selected for observation of tree form and plant growth characteristic. Fifty seeds were sowed in peat and placed under a rain shelter in Ladang 2, UPM to observe the germination performances.

Results and Discussion

The tree produces small ellipsoid berries around 2 – 3 cm long (Figure 1c and Figure 1d). The fruits turn bright red when ripe with a thin layer of edible pulp surrounding a single seed. The most unusual thing about the fruit is the extraordinary effect of the fleshy pulp on the taste buds of the tongue. When the fleshy pulp is allowed to coat the taste buds of the tongue, it causes sour or acidic food eaten or drunk to taste sweet. The taste modifying effect will last for 30 minutes to 1 hour or more. The sweetening property is due to the presence of a glycoprotein in the pulp of the berry called miraculin which is only activated in the mouth by acid substances (Temussi, 2006). This taste-modifying protein is not sweet by it self, but it able to alter the taste of other foods from sour into a sweet taste (Paladino et al., 2008). The taste modification in the miracle fruits occurs when the miraculin are firmly binds to sweet receptor cells in tongue when sour substance present. The protein then transmits a false message to the brain and causing the perception of strong sweet taste (Kant, 2005; Yamamoto et al., 2006). The fruit could possibly help diabetic people taste sweet food without taking in sugar. Miraculin has been investigated as a food additive and is a possible source of a natural food sweetener. Now miraculin is accepted as a dietary supplement and a harmless additive in Japan (Jaafar, 2008). A fresh harvested miracle berry will only last for a few days. Frozen miracle berries and miracle fruit powder with longer shelf life are available in the market. These products have the same effect as the fresh miracle berry. Recently a new product from Japan has come to the market. It is called miracle berry tablet which is actually compressed freeze-dried *S. dulcificum* in tablet form with a shelf life that will last for 12 months (Jaafar, 2008).

Miracle fruit produces small creamy white complete flowers measuring 0.5 cm in cauliflorous flushes throughout the year. The flowers only partially open during anthesis (Figure 1b) and produce coco butter like fragrance. Flowers emerge from the axillary buds, nearly sessile, in clusters (Figure 1a), and the calyx is ribbed and covered with long hairs. The corolla contains five petals and forms a narrow corolla-tube by their basal coalescence. There are five petaloid appendages forming an inner circle and coalescing at the base with the corolla. The petaloid appendages have the same colour with the corolla but differ from the petals in size and shape where the petaloid appendages are much

narrower and tapering on the tip. The flower contains five epipetalous stamens where each stamen located opposite a petal. The style of the flower is simple and erect with a very inconspicuous stigma. The ovary is superior and the outer wall of ovary is heavily covered with hairs (Ayensu, 1972) (Figure 2).

Miracle fruit has been cultivated not only for its fruit but also as an ornamental shrub or potted plant (Figure 3). The slow growing and free branching characteristic of miracle fruit makes it an ideal potted plant. Pruning is not necessary for this plant since it is bushy and forms a beautiful tree form naturally. The red to orange colour of its young shoots adds to the esthetic value of the plant (Figure 4).

Most of the miracle fruit plants are propagated through seeds. The seed is large, elongate-ovoid in shape. The seed coat is hard and smooth with dark brown to black colour (Figure 1e). Matured and fresh seeds are able to germinate within 10 days after sowing with 89.5% of germination rate under ambient temperature of 36 – 40 ºC. Large scale productions of miracle fruit face some restrictions as the seeds are recalcitrant and propagation by cuttings is difficult to root (Okhapkina, 2006). Seed grown plants will only begin to bear fruits 3 to 4 years after planting (Duke, 1993). Propagation through stem cuttings has been investigated to produce early fruiting planting materials.

Conclusions

Since the past 20 years, extensive researches on miraculin have been carried out but aspects on plant propagation, establishment and reproductive biology have not given much attention. Propagation using cuttings should be investigated for early fruiting planting materials. Studies on reproductive biology, pollination ecology, fruit set and development are important to understand the flowering and fruiting behavior of miracle fruit.

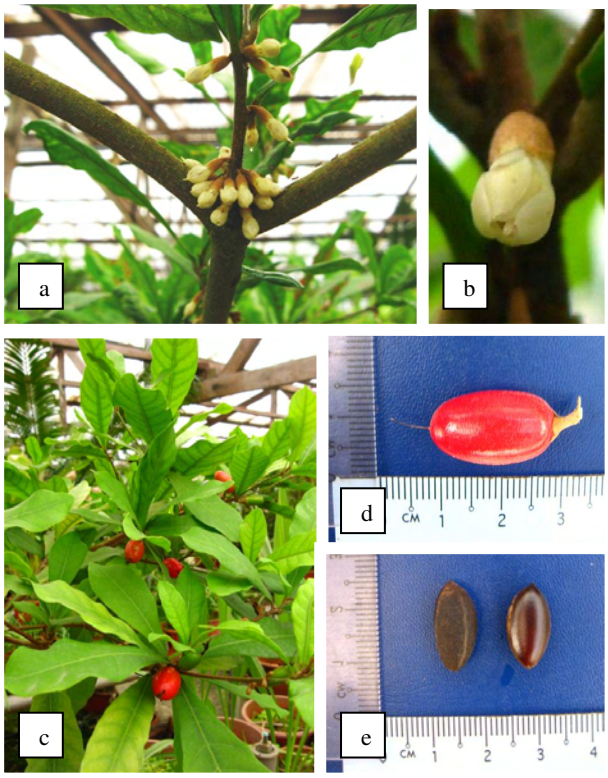

Figure 1. (a) flowers emerged from the axillary buds along the stem; (b) the flower of miracle fruit only partially opened during full bloom; (c) tree with ripen miracle berries; (d) miracle fruit; (e) seeds of miracle fruit

ISBN: 978-967-344-188-4
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Figure 2. Complete flower of *Synsepalum dulcificum* with hairy calyx (c), 5 petals (p), 5 petaloid appendages (pa), 5 stamens with anthers (a) and filaments (f), a simple style (s), an inconspicuous stigma (st) and a superior ovary (o) which is heavily covered with hairs

Figure 3. Three years old potted plant of *Synsepalum dulcificum* Daniell

Figure 4. Flushing of new shoots on *Synsepalum dulcificum* Daniell

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 2

POSTHARVEST TECHNOLOGY AND QUALITY CONTROL

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Mineral Contents of Red Dragon Fruit (*Hylocereus polyrhizus)* Following Pre-Harvest Calcium Fruit Spray

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Introduction

Calcium is a vital macronutrient in plant cycle especially in fruit development. Lack of Ca might cause an abnormal growth in fruit (Jones, 1998). Ca content in fruit generally decreases as the fruit grow (Bernadac et al., 1996; Saure, 2005) and this could result in Ca deficiency. Fruits with low Ca may depict poor quality retention (Serrano et al., 2002) and becoming more sensitive to physiological disorders and disease infection (Fallahi et al., 1997). Many of studies showed a positive relationship between Ca, shelf life and fruit quality (Luna-Guzman and Barret, 2000, Alcaraz et al., 2003).

Since the mobility of Ca in plants is low (Jones, 1998) Ca root uptake from soil applied fertilizer is less effective in increasing Ca content in fruit. Application of liquid source of Ca on leaves and fruit may offer an alternative solution. In this study, we were aiming to increase Ca content in red dragon fruit (*Hylocereus polyrhizus*) via direct application of a liquid source of Ca given at different concentration. As the absorption of Ca into fruit may interact with other nutritional elements, the fruit contents of N, P, K and Mg were also considered in the study.

Materials and Methods

Pre-harvest Ca application

The study was conducted at a commercial farm at Pajam in Negeri Sembilan, Malaysia. Prior to the treatment, freshly opened flowers (regarded as one day after anthesis) were tagged with four fruits per replication and four replications for every treatment. The fruits that developed from the flowers were then sprayed till dripping, approximately 20 seconds, for every fruit (Figure 1) with five different concentrations of Ca (using $CaCl_2$) : 0 (distilled water), 1000, 2000, 3000 and 4000 mgL^{-1} Ca with addition of 5% Tween 20 as wetting agent. The sprays were performed at day 7, 14, 21 and 28 after anthesis. The fruits were wrapped in clear plastic bags (Figure 2) after every spray and harvested at fully ripened stage, approximately 32 to 35 day after anthesis.

Determination of N, P, K, Ca and Mg uptake

After harvest, the fruits were divided into flesh and peel portions, cut into small pieces and dried at 60 °C in a air-circulating oven and finely grounded once dried. Fruit sample of 0.25 g was digested in 5 mL of sulfuric acid (H_2SO_4) on hot plate at 450 °C in a fume chamber for 7 min. Ten mL of hydrogen peroxide (H_2O_2) was added into the mixtures and the heating was continued for another 4 mins. The solution mixtures were made-up to 100 mL with distilled water. N and P contents in the samples were determined using an auto-analyzer (LACHART Instruments, Model Quikchem IC + FIA 8000 Series) while K, Ca and Mg were measured using an atomic absorption spectrophotometer (Perkin Elmer, Model AAS 3110).

The experiment was conducted in a completely randomized design (CRD) with four replications. Data were subjected to an analysis of variance (ANOVA) and comparison of means using Tukey HSD with SPSS (version 13).

Results and Discussion

Results of the study are shown in Table 1. Fruit Ca content increased markedly with the increasing concentration of Ca applied. However, the effects of Ca treatment on Ca content in fruit tissues were more apparent in peel compared to the flesh. Although not significant, Ca content in the flesh was also enhanced at higher Ca concentration. The peel of the fruits treated with Ca treatment at 4000 mgL^{-1} showed the highest concentration of Ca (835.6 µg/100g).

Generally, increasing Ca concentration in the applied solution did not produce significant affect on fruit N, P, K and Mg contents; such result was in agreement with previous finding reported by Alcaraz et al. (2003) on plum. Such results would give a good indication on the positive effects of increasing Ca in the fruit tissue as this did not affect the balance of nutrient composition in fruit. Lack of interaction between the content Ca and other elements measured could be attributed by differences in the regulation of uptake and absorption process for Ca compared to the other element since Ca ion move passively and highly dependent to transpiration flux whilst other nutrients are not (Alcaraz et al., 2003). Transpiration rate was considerably influenced and regulated the uptake of Ca ion into fruit but have little influenced on the other element such as K and Mg (Jones, 1998).

During fruit development, Ca was highly demanded and cause a dilution of Ca in such tissues (Saure, 2005), fruit spray may create a concentration gradient of calcium on exogenous and endogenous of fruit which resulted in the uptake of Ca by fruit passive and extensively. Other fact is, as N, P, K and Mg are very mobile (Jones, 1998), the N, P, K and Mg can easily move to fruit via movement from root or other parts of plant without influenced by fruit calcium treatment. Besides, examination of the results in Table 1 indicated that the contents of N and P were higher in flesh than in peel, while the content of K, Ca and Mg were higher in peel.

Increase in Ca in fruits following treatment indicated that Ca applied as liquid spray could be effectively utilized in elevating fruit Ca during its development suggesting that Ca from such source could easily penetrate the fruit epidermis and retained in fruit tissues. Results recorded here were inline with studies involving other fruit species e.g. peach (Elmer et al., 2003), kiwi (Gerasopoulos et al., 1996), strawberry (Hernandez-munoz et al., 2006) and nectarine (Vasilakakis et al., 2006). Increase in Ca in fruit peel may have an implication impact on the shelf life and storability of fruits as Ca could play important role in fruit softening.

Table 1. Effects of calcium fruit spray on mineral concentrations in flesh and peel of *H. Polyrhizus*

Inoculum (1x106 spore mL-1)	Calcium treatment (mgL-1)	Nutrient concentration (µg/100g)				
		N	P	K	Ca	Mg
Flesh	0(control)	2089.0ab	195.3a	1486.8bc	66.8f	201.9b
	1000	2344.0a	217.6a	1447.4c	81.2ef	211.9b
	2000	2384.0a	214.9a	1474.7c	88.5ef	206.8b
	3000	1735.0bc	173.8a	1734.3b	92.7def	185.9c
	4000	2089.0ab	188.0a	1573.3bc	105.3def	205.7b
Peel	0(control)	1269.0cd	102.8b	2433.0a	227.7cd	251.0a
	1000	1008.0d	108.1b	2511.7a	206.2cde	253.6a
	2000	971.0d	107.6b	2508.3a	286.2c	253.6a
	3000	955.0d	123.7b	2499.7a	667.9b	250.9a
	4000	1007.0d	109.9b	2439.1a	835.6a	261.6a
Tukey HSD $_{0.05}$		**554.19**	**45.31**	**253.17**	**138.70**	**12.26**

Figure 1. Foliar spraying was conducted manually

Figure 2. Fruits were wrapped in plastic bag after every calcium application.

Acknowledgements

This study was funded by the Ministry of Science, Technology and Innovations (MOSTI) Malaysia through Sciencefund Scheme (05-01-04-SF0174).

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Maturity Index and Respiratory Pattern Indicate Optimal Harvesting Time and Post-harvest Handling of *Jatropha curcas* Linn Fruits

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Introduction

The main harvesting and post-harvest problem of jatropha is that any single branch the fruits ripens at different times. It leads to laborious and time consuming harvesting as farmers have to select the ripe fruits only or fruits have to be harvested manually at regular intervals (Heller, 1996; Biswas et al., 2006). Therefore, this problem has been highlighted in many publications that may jeopardise the economic viability of production of this crop (GEXSI, 2008; Hambali, 2008). Jatropha fruits are still harvested by hand in small and plantation scale farms. Mechanical harvesting of jatropha is considered to be impossible due to the non uniformity of fruit ripening.

In many crops, maturity or ripening index is developed as measurement that can be used to determine or estimate when the particular commodity is mature and ready to be harvested. High CJCO content and fruit color changes after elapsed days from full bloom have been recommended as indication of harvesting time in Jatropha by many researchers; Heller (1996) and Hambali et al. (2007) recommended 90 days after anthesis or black dry fruits; Santoso et al. (2008) recommended 55 days after anthesis or fully yellow fruits, Wanita and Hartono (2008) recommended 45 days after anthesis of yellow fruits and Annarao et al. (2008) recommended 37 days after anthesis or just before drying. Varying and contradict recommendations indicate a need for development of maturity index in this crop.

In general, fruits can be classified as either climacteric or non-climacteric based on their respiration pattern during ripening. Climacteric fruits display a characteristic peak in respiratory activity during ripening. It is interesting to note that climacteric fruits, such as banana, tend to ripen rapidly. It has led to the regulation of respiration as a possible target of biochemical manipulation of shelf life. In contrast, non-climacteric fruits, such as pineapple, simply exhibit a gradual decline in their respiration during ripening. They have to be harvested at optimal stage of maturity on the tree. However, no information is available on the Jatropha's respiration pattern during ripening. Therefore, the main objective of this study was to determine the maturity index and its physico-chemical characteristics and to better understand the fruit respiration pattern during storage.

Materials and Methods

Sample for maturity index study

Jatropha fruits of local variety were obtained from Sabah Land Development Board, Malaysia Jatropha Demonstration Plot at Jatropha Estet Binakaan, Sook, Keningau, Sabah, Malaysia. Five fruits of each of seven maturity indexes were randomly harvested from different trees in the farm. The visual colour characteristics are described in Table 1. Measurement of physical characteristics of fruits, fruit coats, seeds, shells and kernels was made on the next day after harvest.

Samples for respiration study

This study was carried out at Bogor Agricultural University, Indonesia. Two types of sample were used. Samples for no pre-handling treatment were harvested form Bogor University Farm near the laboratory. Samples with pre-handling treatment were brought from Jatropha Plantation at Serang,

Banten, Indonesia. Open air transportation to bring the samples from farm to laboratory took eight hours. Pre-handling interruption before respiration test of the pre-handling fruits was 28 hours. Fruits were kept in the respirometer bottle from the open topside and were kept closed with the lid while inserting neoprene gasket in between. The three storage temperatures used were 27±3 ^{o}C, 15±3 ^{o}C and 7±3 ^{o}C.

Physical characteristics measurement

Fruit coat colour (L, c and h value) of each sample was measured at a single point on the equatorial region of the fruit, seed and kernel using a Konica Minolta Colour Reader (CR-100, Minolta Corp., Japan). Fruit firmness was determined by using penetrometer (WAGNER U.S.A) fitted with 6 mm plunger. Weight of each bunch, fruit, fruit coat, shell and kernel was determined by using a balance.

Carbon dioxide measurement

The gas inside the airtight respirometer bottle of 3300 ml was measured using Infrared Continuous Gas Analyzer Model IRA-107 (Shimadzu, Japan). Gas composition was analyzed at varying intervals depending on the storage temperatures. Preliminary experiment was carried out to determine a suitable interval for measurement of CO_2 and minimum weight of fruit required per respirometer bottle. Gas measurement was stopped when the fruits were fully senesced or black in color.

Calculation of respiratory rates

The respiration rates in terms of CO_2 at given temperature were calculated using the following equation as given by Kays (1991). The amount of gas in milliliters was converted to milligrams to remove the effect of temperature on the volume of gas according to Kays (1991).

$$\text{ml kg}^{-1}\text{hr}^{-1} = \frac{(\Delta\ \%\ \text{x}\ 10)(\text{free space volume of respirometer bottle in liters})}{(\text{product fwt in kg})(\text{time respirometer bottle is close in hours})}$$

Where $\Delta = \Delta CO_2$ or concentration time 2 – concentration time 1

Seed oil extraction and measurement

Seed oil was extracted by using modified portable hydraulic presser. The principal components of the hydraulic presser are screw, disk pressing, pressing chamber, bearing pressing, hydraulic jack, heater and thermostat. The percentage oil yield was later computed from the ratio of mass of oil to the mass of sample before oil extraction.

Results and Discussion

Respiration pattern of jatropha fruit

The respiration data corresponding to different storage temperatures and different samples indicated an upsurge in CO_2 concentration (Figure 1). The result of this experiment confirmed that jatropha fruit is in the class of climacteric fruit. The point of upsurge in the CO_2 concentration was different according to storage temperature. The peak was observed as early at 54 hours at storage temperature of 27+3 ^{o}C but it was only observed at 90 and 116 hours at storage temperature of 15+3 ^{o}C and 7+3 ^{o}C respectively. The present recommendation of harvesting only yellow and black fruit is a waste of time. According to the results, the mature green fruits can be harvested. It is important to note that a must in harvesting a mature fruit is pre-requested in some fruits (avocado and mango) due to the fact that respiratory upsurge was reported inhibited while the fruits are attached to the tree (Kays, 1991).

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

The result of this study offers future study on jatropha fruit postharvest ripening treatment and its effect to the quantity and quality of crude jatropha curcas oil (CJCO).

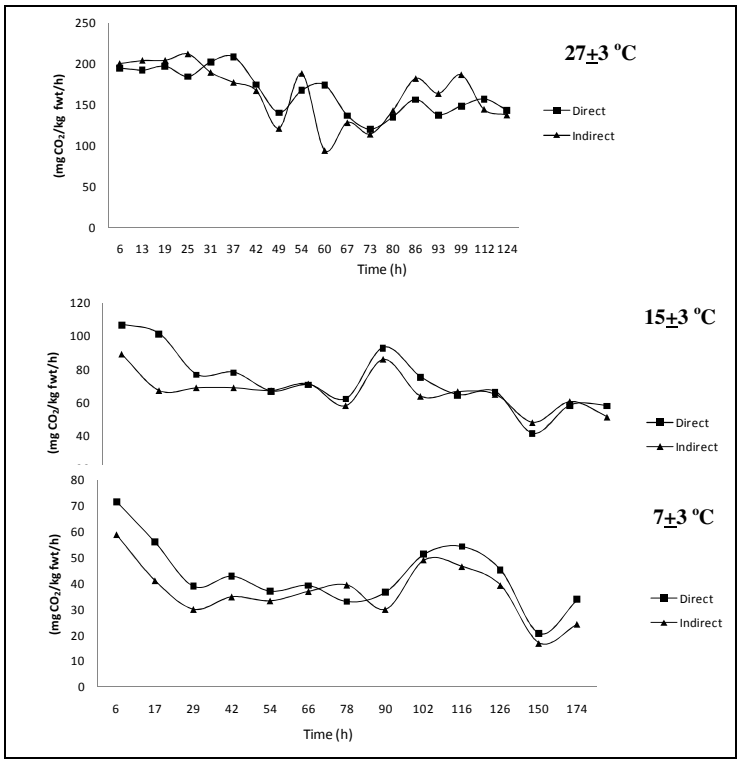

Figure 1. Respiratory rate of jatropha fruit at three different storage temperatures (27±3 °C, 15±3 °C and 7±3 °C) and two different postharvest handling of samples (freshly harvested and 28 hours after harvesting).

Maturity index and physico-chemical characteristics of jatropha fruit

The young and mature fruit showed significant differences in colors (Table 1). Matured fruit or index two showed darker and more saturated green fruit coat color as compared to young fruit which was brighter and had less saturated green color. The color differences between young and matured fruit could be used as physiological maturity indicator for this fruit. Fruit weight was significantly greater in fruits of maturity index 5; i.e. 15.32 g per fruit. Fruits at maturity index 6 and 7 were less than 10 g per fruit. Seeds and kernels of the black dry fruit group were significantly lighter as compared to that of fruits of other maturity indices. A change on firmness value was interesting in this study. Firmness of fruit coats increased when mature green fruits turned to fully yellow fruits but decreased when senescence started. The shell firmness increased with increasing maturity index. However, the kernels of young and matured fruits were much firmer as compared to that of other maturity indices.

CJCO quantity and quality

Result of this study showed that CJCO quantity and quality were different according to different maturity indices (Table 2). The CJCO quantity was different between dry and fresh seeds. High CJCO was found in the dried seeds at maturity index 4, i.e. about 27% as compared to only about 5% in the fresh seeds of similar maturity index. Result of this study indicated the importance of drying before extraction to maximize oil yield. Therefore, this report was not in agreement with most of the harvesting time recommendations, i.e. harvesting the black dried fruit. Instead, harvesting should be carried out at ripening index three, four and five. On the other hand, irrespective of using dried or fresh seeds at any maturity indices, the free fatty acids were less than 2%. It indicated that any of these materials were good for single transesterification processes. It is important to note that if free

fatty acids are found more than 5% in the feedstock by using catalyzed reaction, the material will only form soap and water (van Gerpen and Knothe, 2005).

Table 1. Physical characteristics (color – L, c and h value; weight (g), and firmness (N)) according to seven maturity indexes (1: young fruit, 2: Matured Green, 3: half green and half yellow, 4: fully yellow, 5: half yellow and half black, 6: black wet fruit, 7: black dry fruit) of *Jatropha curcas* L. fruits, coats, seeds, shells and kernels.

Physical Characteristics	Parts	Maturity Index							Sig.
		1	2	3	4	5	6	7	
Weight (g)	Fruits	12.83c[z]	13.41bc	14.28b	15.32a	12.71c	7.98d	02.97e	*
	Seeds	03.18a	03.44a	03.47a	03.72a	03.59a	03.08a	2.23b	*
	Kernels	01.74a	01.76a	01.87a	01.93a	02.05a	01.78a	01.20b	*
Color	Coats (L value)	47.41d	56.78c	60.26b	73.96a	52.36c	25.80e	27.00e	*
	Coats (c value)	30.74d	39.04c	45.43b	55.06a	29.47d	04.66e	06.68e	*
	Coats (h value)	117.10a	113.20a	99.44b	90.72c	67.66d	55.70e	51.00e	*
Firmness (N)	Coats	14.32ab	13.24b	17.36a	16.67ab	10.93bc	07.45c	13.80b	*
	Shells	23.83b	24.42b	34.48ab	35.99ab	42.72a	41.40a	33.90ab	*
	Kernels	06.67b	06.28b	10.79a	11.47a	11.04a	12.00a	11.00a	*

** Significant by ANOVA at 5% level of significance*
[z] Mean for each treatments followed by the same letter at a similar rows are not significantly different at p>0.05 with Duncan's multiple range test (DMRT).

Table 2. Percentage of crude jatropha curcas oil (CJCO) at different maturity indexes of dried and fresh jatropha seeds and free fatty acids (FFA) extracted with modified hydraulic presser.

Maturity Indexes	CJCO (%) of fresh seeds	CJCO (%) of dried seeds	FFA (%) of fresh seeds
Index 2	0.19	17.93	0.59
Index 3	0.97	26.80	0.60
Index 4	4.87	26.76	1.04
Index 5	3.16	24.97	1.08
Index 6	2.02	21.10	0.59

Conclusions

Result of respiration test showed that jatropha fruit is in the climacteric class according to its respiration pattern. The physio-chemical data indicate an optimal seeds oil extraction at ripening index 3 and 4. The percentage of extracted seed oil is higher with drying before extraction. Therefore, the results of this study indicate a possibility to harvest physiological matured fruits followed by ripening off the tree. It is hoped to manipulate the fruit ripening to reduce the cost of harvesting of individual ripened fruits on the tree which was reported laborious and time consuming. However, the result of this study offers future study to confirm the effect of ripening treatment to the harvested mature green fruits and its effect on CJCO quality and quantity.

Acknowledgments

The authors acknowledge Agri-Biotechnology Institute, Malaysia for funding the project. Thanks are also extended to Mr. Jabi from Seberang Tenom, Agricultural Research Centre, Department of Agriculture, Sabah, Malaysia and Mr. Makunsai from Sabah Land Development Board, Sabah, Malaysia for allowing us to use their research plots.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Activity of Dragon Fruit (*Hylocereus polyrhizus*) Cell Wall Degrading Enzymes after Postharvest Calcium Treatment

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Introduction

Dragon fruit (*Hylocereus polyrhizus*) is an exotic climbing cactus, nutritious and delicious. It is a non-climacteric fruit and its eating quality does not change much after harvest. The fruit is normally harvested at full maturity. Once harvested, the fruit will disintegrate rapidly unless it is stored under a proper storage condition. Among others, disintegration of fruit can be seen as softening of the tissue which is partially linked with the activity of cell wall degrading enzymes such as polygalacturonase (PG), pectin methylesterase (PME) and β-galactosidase (Chin et al., 1999). Activity of these enzymes is dependant on many factors such as temperature, pH and mineral composition (Chun and Huber, 1998; Almeida and Huber, 1999). Previous research has also shown that the activity of the enzymes was negatively correlated with the concentration of calcium in fruit tissues, thus calcium could play a role in reducing firmness loss and mechanical damage (Laminkara and Watson, 2004). This study was conducted with the aim of determining the activity of PG and PME in red flesh dragon fruits following post-treatment of calcium and upon fruit maturity.

Materials and Methods

Plant materials and calcium treatment

Dragon fruit of uniform size with maturity indices 3 and 5 were purchased from a commercial farm in Nilai, Negeri Sembilan, Malaysia. Whole fruits were dipped into four levels of calcium concentration ($CaCl_2$: 0 g/L, 2.5 g/L, 5.0 g/L and 7.5 g/L) for two hours, air-dried, cut into small pieces (~2 cm^3), snapped frozen in liquid nitrogen and kept at -80 ^oC until needed for analysis.

Enzyme extraction and assay

Extraction and assay of the enzymes were carried out following procedures as outlined by Ali et al. (2004). Ten grams of fruit tissue for each sample were homogenized using a domestic blender in 20 mL of 0.1 M sodium citrate, containing 1 M NaCl, 13 mM EDTA, 10 mM β-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (PVP-40) at pH 4.6. Extraction was carried out at 4 ºC. The extracts were left for 30 min with occasional stirring. The supernatant was recovered by centrifugation at 29000 x g for 30 min. The supernatant obtained was used for PG and PME assays.

PG enzyme was assayed in a mixture containing 0.75 mL of 1.5% (w/v) polygalacturonic acid, 0.1 mL 0.6 M sodium chloride and 1.0 mL supernatant at pH 5.2, adjusted using HCl. The mixture was incubated for 1 hour at 37 °C. Reducing sugars released were estimated by the cyanoecetamide method (Gross, 1982) with monogalacturonic acid as a standard. Enzyme activity was expressed as ŋkatal/g fresh weight (FW). PME was assayed using 0.5 mL crude extract added to 25 mL 1% (w/v) pectin, containing 0.3 M NaCl and titrated with 0.01 M NaOH at pH 7.3 for 10 min at room temperature (25 ^oC). Enzyme activity was expressed as ŋ equivalent carboxyl $group.g^{-1}.s^{-1}$ fresh weight (FW). The activities for PG and PME were determined at day one until seven.

Statistical analysis

The study was conducted using Complete Randomized Design (CRD) with three replications. Each plot consists of three fruits. Data collected was analyzed using Analysis of Variance (ANOVA). Mean

separation were performed using Least Significance Difference (LSD) at $p \leq 0.05$ level with SAS (version 9.0, Cary, NC, USA).

Results and Discussion

Ripening of dragon fruit is characterized by a relatively slow softening rate which was paralleled with an equally gradual increase in activities of the major cell wall degrading enzymes, PG and PME (Figure 1) and also perhaps with β-galactosidase and cellulase which would link to a slow rate of modification of the various pectic and hemicellulosic components of the cell wall (Manganaris et al., 2005). These enzymes may probably be significant to cell wall modifications at any stage of ripening.

Post harvest application of calcium chloride has been proven to reduce the enzyme levels and increase the neutral sugar in fruits. Exogenously applied calcium binds the negative charges of de-esterified uranic acid residues that are generated by PME during ripening and therefore enhancing the tissue's mechanical strength (Magee et al., 2003). Such hypothesis was clearly manifested in result shown in Figure 1. Regardless of fruit maturity, the activity of both enzymes increased with time of storage, suggesting that the rate of fruit disintegration would be faster at a later stage of storage.

The results also revealed that increasing $CaCl_2$ markedly reduced the activity of both PG and PME enzymes (Figure 1). This is somewhat expected as high Ca in the external environment would increase the rate of Ca absorption into fruits. High calcium content in fruit leads to the formation of salt-bridge cross-links which resulted from the integration of COO- groups of the fruit pectin with Ca^{2+} ions, making the cell wall becoming less accessible to the enzymes that cause softening (Saftner et al., 2003).

Beside a clear effect of Ca, it was also shown that PG activity in index 5 fruits (7.028 $\eta katg^{-1}$) was higher than that of index 3 fruits (6.753 $\eta katg^{-1}$). Contrasting result was obtained for PME activity whereby PME activity of index 3 fruits (77.149 $\eta eqg^{-1}s^{-1}$) was higher than that of index 5 fruits (59.438 $\eta eqg^{-1}s^{-1}$). The data suggested that PME may act first to demethylate polygalacturonate and may be followed by the action of other enzymes resulting in cell wall disassembly and fruit softening in dragon fruit. A diverse number of species have shown high PME activity at the beginning of ripening (Gaffe et al., 1994; Iannetta et al., 1999; Trinchero et al., 1999). Relatively high activity of PME in dragon fruit during the earlier phase of ripening suggested that demethylation of polygalacturonic chains occured as an antecedent to make it more easily hydrolyzed by PG.

Conclusions

The study clearly showed that PG and PME activities were markedly reduced by postharvest calcium dip treatment which might have an impact on fruit softening.

Acknowledgements

This study was supported by the Ministry of Higher Education, Malaysia through Fundamental Research Grant Scheme (01-01-07-096FR).

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

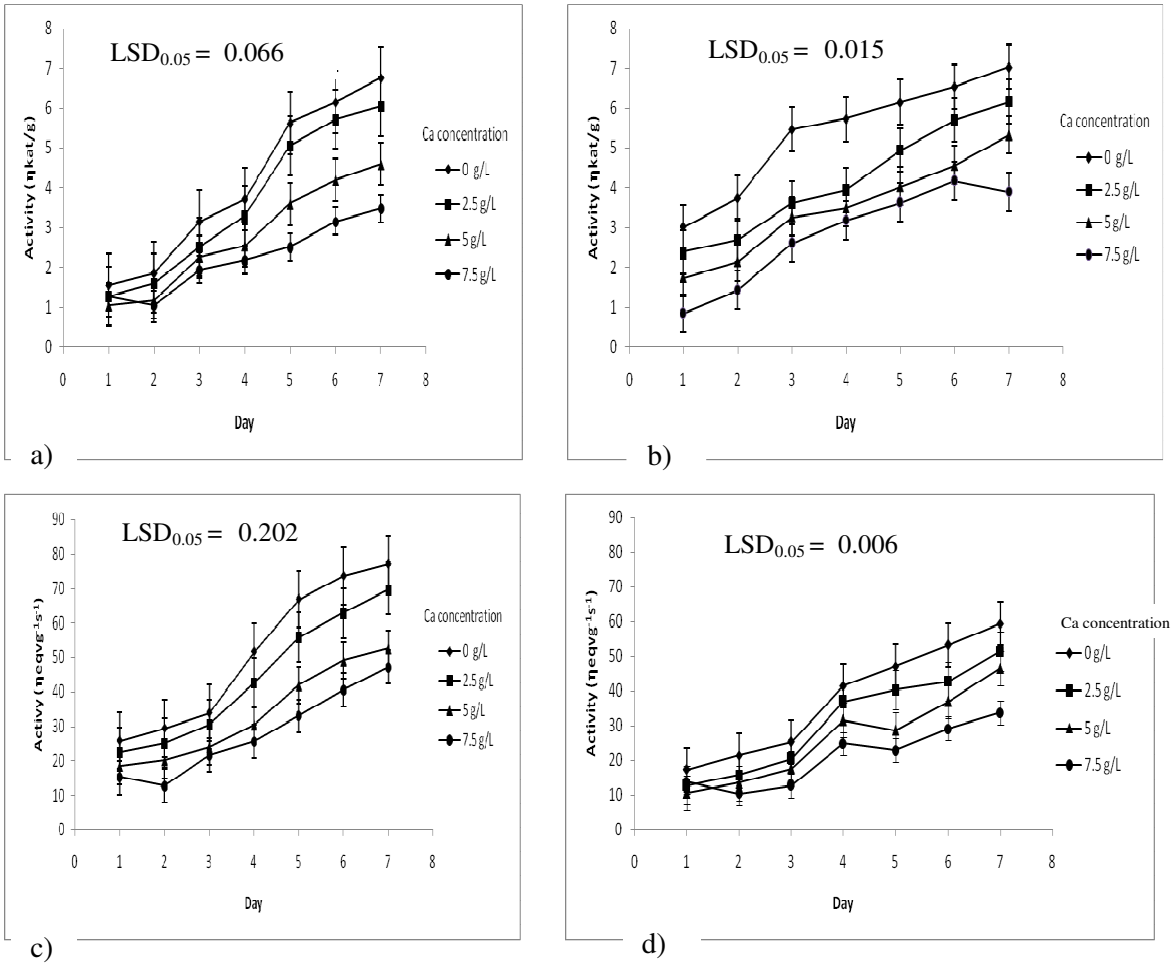

Figure 1. Changes in activity of PG and PME as affected by $CaCl_2$ concentration (a) PG activity for fruit at maturity index 3, (b) PG activity for fruit at maturity index 5, (c) PME activity for fruit at maturity index 3, (d) PME activity for fruit at maturity index 5.
* Values are means ± SE and n = 3

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effect of Different Fruit Positions on Postharvest Quality of Watermelon (*Citrullus lanatus*)

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Introduction

Watermelon, *Citrullus lanatus* (thunb.) Matsum and Nakai, is an annual plant of the Cucurbitaceous family. Watermelon is one of the most widely cultivated crops in the world and its global consumption is greater than that of any other cucurbit (Gichimu et al., 2008). It accounts for 6.8% of the world area devoted to vegetable production (Guner and Wehner, 2004; Goreta et al., 2005). Watermelons are important export fruits for Malaysia. In 2007, Malaysia exported 64,203 tonnes of watermelon which worth USD14,153,000 (Anon, 2008).

Anon (2006) stated that good taste is the most important reason for buying watermelon and some buyers require fruit must have some minimum soluble solids concentration (SSC) (Rushing et al., 2001). SSC above 10% indicate a high quality watermelon. According to Genard and Bruchou (1992) positions of fruit can affect several aspects of quality such as colour and flavor, and may contribute to the variation in fruit quality at harvest. Pyke et al. (1996) reported that the positions were expected to provide fruit which exhibited the greatest differences in SSC at harvest. Effects of fruit position on SSC were reasonably consistent between fruit at harvest and after storage.

Little is known regarding effect of fruit position on postharvest quality of watermelon even though it is a popular fruit. Therefore this study was carried out to determine the effect of different positions of watermelon fruit along the vine on fruit size and postharvest quality.

Materials and Methods

Plant materials

The watermelon seeds of variety F_1 hybrid watermelon Hi-U 16 were sown under shade and the seedlings were ready for transplanting into field after 12 days. The planting distance was 2.5 m between rows and 1 m between plants. Silvershine plastic mulch was applied to the raised beds. A vine was trained and maintained. The treatments were represented by three fruit positions and fruit was set on the same vine as follows: (a) first position, on the 8-11th nodes (average vine length was 75 – 100 cm from the main stem); (b) second position, on the 13-16th nodes (average vine length was 135 – 150 cm from the main stem); and (c) third position, on the 18-21th nodes (average vine length was 250 – 350 cm from the main stem). Fruit was tagged during anthesis and harvested at 35 days after anthesis. Watermelon was harvested by cutting the fruit from the vines using secateurs. After harvesting, the fruits were sent to laboratory.

Determination of postharvest quality

Fruit weight of watermelon was determined by weighing individual fruit using a balance (AND EK-600H, Japan) while diameter of fruit was determined by measuring the widest midpoint of each fruit with a measuring tape. Skin colour was determined using a chroma meter (model CR-300, Minolta Corp., Japan) and results were expressed as lightness (L*), chroma (C*) and hue (h^o). The flesh firmness of fruits was evaluated using a penetrometer (FT 327Bishop, Italy) with 11 mm cylindrical probe. The SSC of fruits were determined using a hand refractometer (Model N1, Atago). A drop of watermelon juice was placed on the prism glass of the refractometer to obtain the reading of %SSC. The titratable acidity (TA) of the fruit was determined by slicing out 10 g of the fruit. Then, 40 mL of

distilled water was added to the 10 g of fruit and then blended in a high speed blender (National model MX V2N) for 1 min. The macerate was filtered with a filter paper (Whatman no.1) into conical flask. After that, 5 mL of filtrate was titrated with 0.1 N NaOH and three drops of phenolphthalein indicator. The indicator added filtrate was titrated until it turns pink color. The remaining watermelon juice from the TA determination was used to measure the pH of the juice by using the glass electrode pH meter model Crison Micro pH 2000. The pH meter was calibrated with buffer at pH 4.0 and 7.0 before being used. Vitamin C determination was carried out using dye, 2,6-dichlorophenol-indophenol method (Ranggana, 1977).

Statistical analysis

The experimental design was a randomized complete block design with four replications consisted of four plants each. Data were analysed using the analysis of variance (SAS Institute, Cary, NC) and means were separated by Duncan's multiple range test.

Results and Discussion

There was no significant difference in L*, C* and h^o values of watermelon skin as affected by fruit position (Table 1). Similarly C* and h^o values of watermelon flesh was also not affected by fruit position, however, L* values of flesh showed significant increase as fruit position progressed from position 1 and 2 to 3 (Table 1).

Table 1. Effects of three different fruit positions on skin and flesh lightness (L*), chromaticity (C*) and hue (hº) of watermelon.

Fruit position	Skin			Flesh		
	L*	C*	hº	L*	C*	hº
1	57.70 a[z]	27.58 a	117.88 a	38.03 b	25.90 a	24.15 a
2	57.41 a	26.96 a	117.92 a	38.37 b	26.59 a	24.54 a
3	57.12 a	28.78 a	117.82 a	42.59 a	26.33 a	24.18 a

n = 48

[z] *Means followed by the same letter are not significantly different by DMRT at* $p \leq 0.05$

The weight of fruit increased significantly as fruit position progressed from 1 to 2, then followed by significant decreased as fruit position progressed from 2 to 3 (Table 2). The diameter of fruit showed similar trend as fruit weight where it increased then decreased significantly as fruit position progressed from 1 to 3 (Table 2). McFarlane (2007) reported that watermelon fruits vary in weight from less than 4 to over 18 kg depending on the variety.

The firmness of fruit decreased significantly as fruit position further from main stem (Table 2). The SSC of watermelon also showed similar result where the concentration decreased significantly as the fruit position progressed far from main stem (Table 2). This indicated the firmness and sweetness of fruit decreased as fruit position further from main stem.

The pH of watermelon fruit decreased significantly as fruit position progressed from 1 to 3 (Table 2). Contrary, the TA of fruit increased significantly as fruit position progressed from 1 to 3 (Table 2). The vitamin C content of watermelon showed a decrease trend as fruit position progressed however there was no significant difference among them (Table 2).

Table 2. Effects of three different fruit positions on fruit weight, diameter, soluble solids concentration (SSC), firmness, pH titratable acidity (TA) and ascorbic acid (AA) of watermelon.

Fruit position	Weight (kg)	Diameter (cm)	SSC (%SSC)	Firmness (N)	pH	TA (% malic acid)	AA (mg 100 g^{-1})
1	4.86 b[z]	63.57 a	9.35 a	23.52 a	5.76 a	0.27 b	6.96 a
2	5.20 a	65.08 a	9.07 b	21.62 b	5.69 a	0.27 b	6.42 a
3	4.14 c	60.91 b	7.64 c	20.75 b	5.26 b	0.30 a	5.97 a

n = 48

[z] *Means followed by the same letter are not significantly different by DMRT at $p \leq 0.05$*

Conclusions

The fruit position of watermelon in vine affect fruit quality and the best quality of fruit is nearest to main stem. With this finding, we could grade the fruit according to its position in a vine.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Influences of Pollen Load and Quality on Fruit Development of Red-Fleshed Dragon Fruit

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Introduction

Pitaya or dragon fruit belongs to the Cactaceae family which is a native from the tropical regions of Mexico and Central and South America (Nerd and Mizrahi, 1997). Fruit size and weight depend on the pollination technique used, as well as the variety involved. The average weight for a mature fruit ranged between 350–900 g. The optimal time to harvest dragon fruit is within 28 to 30 days after flower anthesis at full colour development (Nerd et al., 1999). In Malaysia, there are two species of red-fleshed dragon fruit; *Hylocereus polyrhizus* and *H. costaricensis* and one white-fleshed *H. undatus.* The fruits of the red-fleshed dragon fruits were of better quality when hand cross-pollinated compared to self pollinated (Weiss et al., 1994). Hand cross-pollination could be done by transferring the pollens (from another concurrent flower) using a brush to a receptive stigma. Under such circumstances, profitable yields depend entirely on hand cross-pollination where variations in weight and size of the dragon fruits were produced.

A developing seed has a very important role to play in the early development of fruit since the numbers of viable developing seeds influences the final size and weight of the dragon fruit (Weiss et al., 1994) and guava (Nagar and Rao, 1983). Developing seeds induced hormones that trigger maturation and control the final size and weight of the fruit (Varoquaux et al., 2000). The number of pollen grain (pollen load) on a receptive stigma can increase the seed number in a fruit, which is known as the pollen dose-response relationship (Cane and Schiffhauer, 2003). Like many fruit species, fruit size and fruit set have been reported to be positively correlated with seed numbers such as kiwi (Gonzalez et al., 1998) and dragon fruit (Weiss et al., 1994). The number of seeds per fruit increased with increasing pollen load indicating that seed number is limited by pollen load. This is in agreement with observations obtained in sweet pepper (Marcelis and Baan, 1997), zucchini (Stephenson et al., 1988), cherimoya (Gonzalez et al., 2006) and cranberry (Cane and Schiffhauer, 2003). For dragon fruit, the flesh developed mainly from the funiculli (that connect the ovules to the ovary wall) which originated from the developing seed (Nerd and Mizrahi, 1997). Therefore, the objective of this study was to elucidate the effects of different pollen load size on the red-fleshed dragon fruit in terms of fruit set, size and shape.

Materials and Methods

The experiments were carried out using a 5-year-old red-fleshed dragon fruit farm in Sepang. Three sampling units were used for each treatment combination. The hand cross pollination treatments (modified from Gonzalez et al., 2006): 0.01 x, 0.5 x, 1.0 x, 1.5 x and 2.0 x pollen load were applied using a small spoon. The usual pollen load applied by the grower was used as control (C). Pollination was done at night; a period between 9.00 - 11.00 pm. Three fruits were harvested randomly at 5 days interval after pollination (DAP) from day 5 until day 35.

Determination of postharvest qualities

Fruit length and fruit diameter were measured using 0.01 mm precision caliper. Fresh fruit weight were taken by weighing individual fruit from each pollen load treatment using electronic weighing balance (EK-600H A&D, Japan). For gloss value, three readings on the dragon fruit surface (top, middle and bottom) were determined using a small area glossmeter of 2 mm^2 (Rhopoint Novo-Curve^{TM}, UK). Peel and pulp color measurements were made by triplicate measurements in chromaticity values of lightness (L*), chroma (C*) and hue (h°) by using a Minolta CR-300 Chroma meter (Minolta Corp., Japan) with the Illuminate C (CIE, 1976).

The experiment was conducted using a complete randomized design with a factorial arrangement (2 application methods x 6 levels of pollen load treatments) and two replications. Data were analysed using ANOVA and means were separated using LSD.

Results and Discussion

In this study, the use of different pollen load during pollination had significantly affected the production of fruit weight, length, diameter, peel thickness and pulp diameter of red-fleshed dragon fruit (Table 1). In terms of colour production, there were no significant differences in L* and C* of peel and hue value of pulp. However, there were significant effects of pollen load on the hue value of peel and L* and C* of pulp of red-fleshed dragon fruit. In terms of fruit weight, 0.01 x pollen load produced the smallest fruit (187.5 g) followed by 0.5 x (303.02 g), 2.0 x (330.35 g), 1.5 x (419.25 g), control (435.8 g), and lastly 1.0 x pollen load produced the largest fruit weight (534.37g) at 35 DAP. Each pollen load treatment was significantly different from one another except for 1.5 x, 2.0 x and control since these three treatments produced fruits that fall within the range of one another (Table 1). From the results, it was found that the fruit weight began to increase when the amount of pollen was increased and maximum fruit weight was achieved at 1.0 x pollen load as fruit weight began to decrease at 1.5 x and 2.0 x pollen load.

By increasing the pollen load received by the stigma of red-fleshed dragon fruit, the fruit weight was able to increase until a maximum threshold was achieved. As the pollen load increased, there were significant increases in fruit weight, length and diameter. In this study, the maximum threshold for red-fleshed dragon fruit was 1.0 x pollen load. A significant decrease in fruit weight was noticeable when 1.5 x and 2.0 x pollen load treatment were applied. The decrease could be due to numerous growing pollen tubes that ended up congesting the style and preventing other pollen tubes from reaching the ovules (Dogeterom et al., 2000). Thus an excess of pollen load does not guarantee production of a bigger fruit as found in blueberry (Dogterom et al., 2000) and cranberry (Cane and Schiffhauer, 2003).

Conclusions

In conclusion, 1.0 x pollen load was the maximum threshold for red-fleshed dragon fruit. However, the application of high pollen load at 1.0 x proved to be daunting as more pollen grains were needed to be collected during pollination. By taking time and energy used into consideration, a better pollination method using other applicator that require less pollen grains collection would be economically feasible.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Table 1. Effects of different pollen load and day after pollination (DAP) on weight, length, diameter, peel thickness (peT), pulp diameter (puD), glossiness and peel (k) and pulp (i) colour of red-fleshed dragon fruit.

Factor	Weight (g)	Length (cm)	Diameter (cm)	peT (cm)	puD (cm)	Gloss (GU)	kL*	kC*	kh°	iL*	iC*	ih°
Pollen load treatment (T)												
Control	234.62 bc	9.07 a	7.20 a	0.57 abc	5.56 ab	1.60 ab	50.75 ab	28.72	91.27 a	63.38 a	15.68 d	161.53
0.01x	141.00 e	7.03 d	5.95 c	0.62 a	4.28 d	1.55 b	50.04 b	28.10	81.36 b	58.89 bc	17.91 c	146.14
0.5x	204.53 d	8.25 c	6.60 b	0.54 c	5.09 c	1.64 ab	54.55 ab	28.00	81.26 b	58.18 bc	21.26 ab	171.78
1.0x	279.83 a	8.99 a	7.27 a	0.61 ab	5.78 a	1.61 ab	50.41 ab	27.46	83.19 b	59.35 b	22.60 a	147.28
1.5x	261.36 ab	8.72 ab	7.30 a	0.56 bc	5.61 ab	1.59 ab	51.93 a	28.75	84.31 b	58.15 bc	20.12 b	147.22
2.0x	225.79 cd	8.52 bc	6.77 b	0.55 c	5.36 bc	1.70 a	50.66 ab	27.80	81.23 b	57.89 c	21.47 ab	145.88
F-significant	**	**	**	*	**	ns	ns	ns	*	**	**	ns
Day (D)												
5	54.19 f	6.30 d	4.19 e	0.97 a	1.94 f	1.39c	52.94 a	26.09 c	108.76 b	83.03 a	22.77 b	94.29 d
10	118.52 e	7.82 c	5.81 d	0.68 b	3.78 e	2.05 a	52.71 a	24.72 c	121.74 a	81.31 b	17.23 c	87.33 d
15	179.96 d	8.50 a	6.94 c	0.68 b	4.61 d	2.02 ab	53.90 a	26.43 c	123.93 a	80.80 b	17.29 c	86.19 d
20	211.93 c	9.11 a	6.88 c	0.72 b	4.95 c	1.87 b	53.03 a	25.05 c	122.85 a	67.77 c	4.45 d	102.04 d
25	271.32 b	8.93 a	7.42 b	0.60 c	6.14 b	2.01 ab	52.28 a	25.12 c	86.47 c	40.26 d	27.28 a	324.52 a
30	366.98 a	9.24 a	8.36 a	0.22 d	7.79 a	1.19 d	45.69 b	33.81 b	13.79 d	31.96 e	27.07 a	219.42 b
35	366.76 a	9.19 a	8.33 a	0.16 e	7.75 a	0.75 e	45.67 b	35.76 a	9.08 d	30.01 f	22.79 a	159.36 c
F-significant	**	**	**	**	**	**	**	**	**	**	**	**
Interaction												
TxD	**	*	*	**	*	**	ns	*	*	**	**	ns

n = 252

[z]Means followed by the same letter in each column and factor are not significantly different based on LSD at P < 0.05

ns,, ** non significant, significant or highly significant at P ≤ 0.05, respectively.*

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effects of Different Ripening Temperatures on Postharvest Quality of Berangan Banana

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Introduction

Banana (Musa sp.) belongs to the Musaceae family which is one of the popular tropical fruit grown in the world and Cavendish banana is the most widely consumed dessert banana. Bananas are harvested when they are mature green and then initiate to ripen using ripening gas. Berangan banana is popular among Malaysian and it ripens at 27 °C. The genome make up of Berangan banana is AAA which is similar as Cavendish banana. For Cavendish bananas, it failed to develop a fully yellow peel when ripening was conducted at warm temperatures above 24 °C (Li et al., 2006). Latest study also proved that Cavendish bananas could ripen successfully at 18 °C and fail to degreen when the bananas ripened at 27 °C (Ding et al., 2007). There was no report on ripening characteristics of Berangan banana at 18±2 °C. Therefore, a study was carried out to determine the ripening characteristics of Berangan banana at 18 and 27 °C.

Materials and Methods

Berangan banana fruits were obtained from Pasar Borong Selangor at mature green stage (ripening stage 1). Fruits were dehanded and washed to reduce the contamination on the peel surface. The fruits were divided into two batches, the first batch of fruits were ripened at 20°C while second batch of fruits were ripened at 25 °C using 100 mL/L of ethylene at 65% relative humidity. After 24 hours, the ripening gas was released and fruits were allowed to ripen in respective condition as ripening initiation. The fruits were analyzed for peel color, pulp firmness, soluble solids concentration (SSC), vitamin C, pH, and malic acid content on day 0, 1, 3 and 5.

The peel and pulp colors were measured using a chroma meter (model CR-300, Minolta Corp., Japan) with the measurements expressed as lightness (L*), chroma (C*) and hue (h°). Flesh firmness was evaluated using a penetrometer (model FT 327, Bishop, Italy) while SSC was determined using a refractometer (Model N1, Atago, Japan). The pH of the fruit juice was determined using a pH meter (model Micro pH 2000, Crison Instruments, Spain). Titratable acidity and vitamin C content of flesh were determined using method described by Ranganna (1977). Dry weights of the peel and pulp were measured using an electronic balance (BP 2100, Sartorius, Germany) and moisture content of the peel and pulp can be derived. Ethylene and CO_2 productions were determined using a gas chromatography (Clarus 500, Pekin Elmer, Shelton, USA). The experiment was conducted using a complete randomized design and the data were analyzed using t-test (SPSS version 12.0).

Results and Discussion

There was a significant differences in peel and pulp color (L*, C* and h°) of Berangan banana ripened at 20 and 25 °C. For peel and pulp colors, the L* and C* values of banana ripened at 25 °C increased as ripening progressed (Figure 1a) indicating the peel and pulp colors became lighter and more intense. For bananas ripened at 25 °C, peel h° values decreased while pulp h° values increased as fruit ripened (Figure 1). This reflected the color changed from green to yellow for the peel (Ding, 2008) while pulp color changed from creamy white to yellow. The changed of green color to yellow in the peel is due to the breakdown of grana-thylakoid membranes in chloroplast as ripening progressed (Ding et al., 2007). Bananas ripened at 20 °C remained green with no significant differences in peel colour.

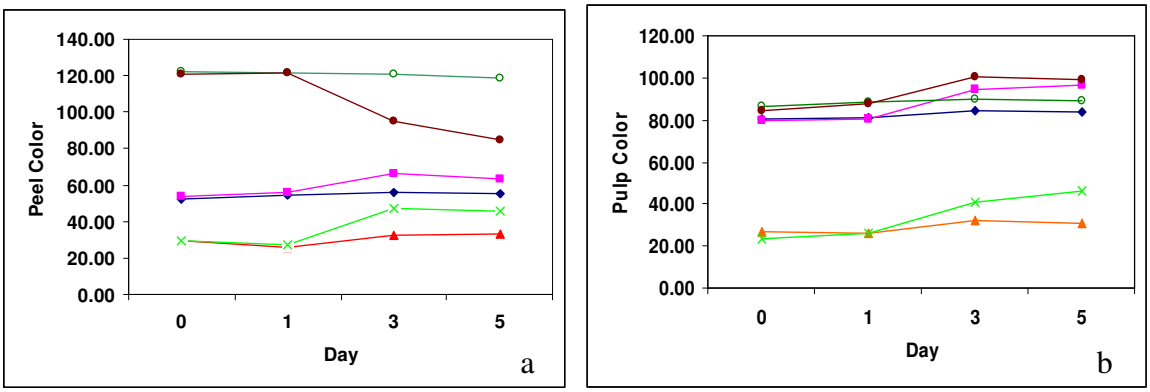

Figure 1. Mean differences showed in peel and pulp color (L*, C*, and h°) of Berangan bananas ripened at 20 and 25 °C measured at ◊ (h° values at 20 °C), ⌂ (h° values at 25 °C), ♦ (L* values at 20 °C), □ (L* values at 25 °C), Δ (C* values at 20 °C) and x (C* values at 25 °C).

The firmness of Berangan bananas ripened at 25 °C decreased significantly (P<0.05) with ripening days while bananas ripened at 20°C retain its firmness at an average of 9.0 N as ripening progressed (Figure 2a). In contrast, the SSC in bananas ripened at 25 °C increased markedly (P<0.05) on day 3 by 50.7% and increased by 53.5% on day 5 as fruit ripening progressed (Figure 2b). This is because the cell wall of banana degraded and the starch loss during ripening period and caused the texture of the pulp turned hard to soft (Seymour, 1993) and the starch also degraded to form soluble sugars which contribute to increase SSC. According to Asif and Nath (2005), the softening of banana during ripening is also a result from the action of at least four polygalacturonase genes. At 20 °C, the total sugars content in the banana was only 2.07% on day 1 and increased to 3.58% on day 3 and still present at a substantial level, about 3.87% on day 5.

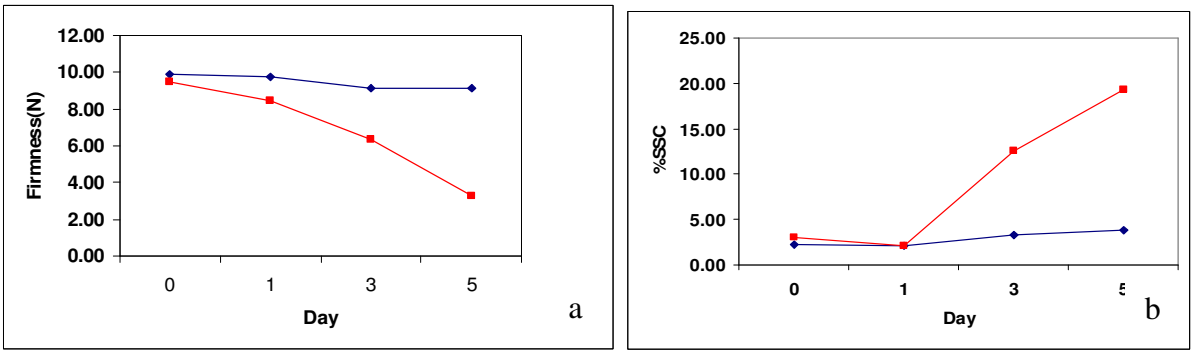

Figure 2. Mean differences showed in pulp firmness (a) and SSC (b) were measured for the Berangan bananas ripened at 20 and 25 °C whereby ♦ (bananas ripened at 20 °C), and ■ (bananas ripened at 25 °C).

As ripening progressed, malic acid content increased markedly (P<0.05) by 531.3% from day 1 to 5 compared to the bananas ripened at 20 °C which only increased by 100% during the same period of ripening (Figure 3). The increased in malic acid had explained for the significant decreased (P<0.05) of pH by 5.87 on day 0 to 4.38 on day 5 for bananas ripened at 25 °C while the pH maintained at 5.7 for bananas ripened at 20 °C as ripening progressed.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

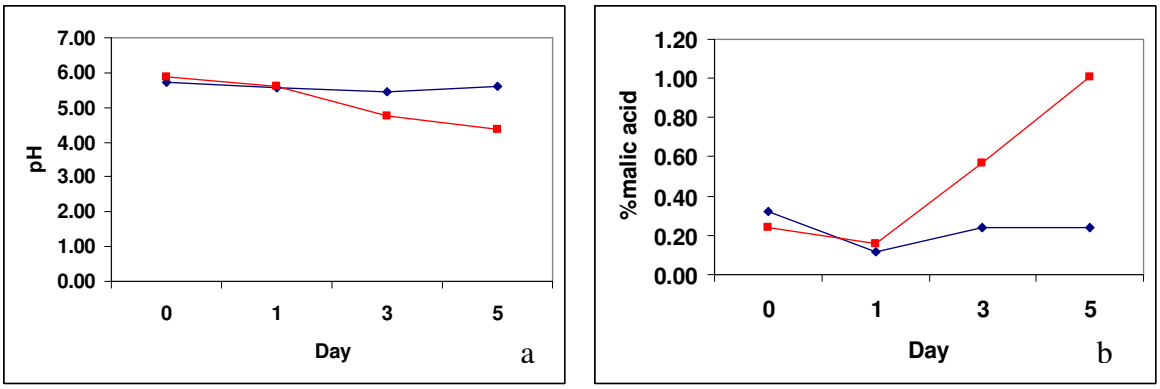

Figure 3. Mean differences showed in pH (a) and %malic acid (b) were measured for the Berangan bananas ripened at 20 and 25°C whereby ♦ (bananas ripened at 20 °C), and ■ (bananas ripened at 25 °C).

There was an increased in respiration rate of bananas ripened at 20 and 25 °C, respectively, on day 3 ($P<0.05$) (Figure 4). Rastali banana ripened at 20 and 25 °C exhibited climacteric patterns of respiration as there were sudden increased in CO_2 production at the onset of ripening. However, bananas ripened at 25 °C produced four times higher amount of CO_2 compared to bananas ripened at 20 °C as fruit ripened. The rise in respiratory activity on day 3 coincided with the rise in ethylene production. Ethylene production increased significantly and tremendously ($P<0.05$) by 37% on day 1 and decreased steadily on day 3 to day 5 by 10.8% (Figure 4) for bananas ripened at 25°C as fruit ripened. For bananas ripened at 20 °C, there was no ethylene gas found on day 1 and it produced only 0.02 µl C_2H_4/ kg-hr of ethylene on day 5 as ripening progressed.

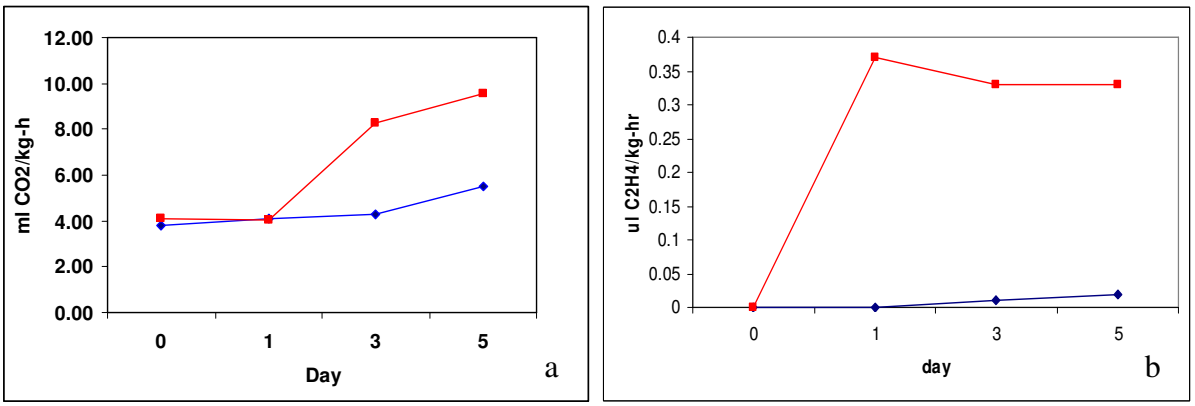

Figure 4. Mean differences showed in CO_2 and C_2H_4 productions for banana ripened at 20 and 25 °C whereby ♦ (bananas ripened at 20 °C), and ■ (bananas ripened at 25 °C).

Conclusions

Berangan bananas ripened at 25 °C has sweeter taste and softer pulp with green peel turned to yellow as ripening progressed while fruits ripened at 20 °C failed to degreen. With this study, Berangan bananas although having the same genome (AAA) as Cavendish banana, it failed to ripen at 20 °C.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effect of Calcium Chloride on Selected Quality Attributes of Fresh Cut Red Dragon Fruit (*Hylocereus polyrhizus*)

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Introduction

Hylocereus Polyrhizus is a member of cactus family, and non-climacteric fruit. It is contains highly valued for their antioxidant properties, less sugar content than most popular tropical fruits. This fruit can be formed into minimally processed or fresh cut fruit product that is very susceptible to softening during storage and product display. Prolonging its storage and shelflife would reduce its losses, increase product safety and extend saleability. Treatment with Ca, for example using $CaCl_2$ could be seen as one the cost effective and safe technique in extending the quality of fresh horticultural produce. The role of $CaCl_2$ as firming agent has been demonstrated in fresh-cut honeydew (Saftner et al., 2003), cantaloupe (Luna- Guzman et al., 1999) and whole apples (Chardonnet et al., 2002). There are two main ways of application of the calcium in fresh cut fruits have been reported: dipping and vacuum infiltration.

Materials and Methods

Sample preparation

Fully ripened and uniform fruits were purchased from a commercial Pitaya fruit farm at Nilai, Negeri Sembilan and stored for 24 hours at 13 ± 1 ^{o}C before used. The fruits were then washed, rinsed with 100 ppm sodium hypochlorite solution, peeled and each fruit was cut into eight pieces. The fruit portions were treated with 5 levels of Ca concentration 1000, 2000, 3000, 4000 mgL^{-1} Ca either by dipping into Ca solution for 10 minutes or vacuum infiltration. For the vacuum infiltration, fruit pieces were vacuum infiltrated by submerging in calcium chloride solutions at different concentration according to treatments for 30 seconds, then the pressure was reduced to 33 kPa and this partial vacuum was maintained for 10 minutes. After the vacuum was released, the fruits were maintained in the solutions for another 5 minutes. Then the fruit pieces were removed and allowed to dry at ambient temperature before packed in polystyrene trays and wrapped with polyethylene cling film. Each tray contained 200 g of fruits. The fruits were kept at 13 ± 1 ^{o}C for five days before analysis.

Measurement of quality and calcium concentration

Firmness, soluble solids content (SSC), titratable acidity (TA), pH, calcium, ascorbic acid, total phenolic contents and total antioxidants activity of cut fruit were analysed. Analysis of cut fruit quality was conducted as described by Ranggana (1977) and Singh et al. (2007).

The firmness was determined using a texture analyzer (Instron Universal Testing Machine, Model 5543, Instron Corp, USA) by measuring the maximum penetration force (N) required during tissue breakage using a 5 mm diameter flat probe. The measurement of firmness was done at three locations for each sample.

Soluble solids content (SSC) of the cut fruit were determined using a digital refractometer meter (Model PR-32, Atago, Japan) by squeezing the juice of cut fruit onto prism of the refractometer while titratable acidity (TA) was measured using diluted fruit juice (1 juice: 4 distilled water) prepared using the same cut fruit as for the SSC measurement. Ten ml of the diluted juice was titrated with 0.1 N NaOH to pH 8.1 (Model CRISON GLP 21). pH value was measured using a glass electrode pH meter

(CRISON GLP 21) and was calibrated with pH buffer 4.1 and pH 7 before being used. The TA was calculated and expressed as percentage of citric acid using the following equation:

$$\% \text{ citric acid} = \frac{\text{Titre x NaOH Normality (0.1) x Made-up volume x Citric acid equiv. x 100}}{\text{Sample vol x Sample wt. x 1000}}$$

For determination of fruit Ca content, the cut fruits were dried at 60 ^{o}C in a air-circulating oven. 0.25 g of the finely ground fruit samples were digested in 5 mL of sulfuric acid (H_2SO_4) on hot plate at 450 °C in a fume chamber for seven minutes. Ten mL of hydrogen peroxide (H_2O_2) was added into the mixtures and the heating was continued for another four minutes. The solution mixtures were made-up to 100 mL with distilled water. Ca content was measured using an atomic absorption spectrophotometer (Perkin Elmer, Model AAS 3110).

Ascorbic acid determination was carried out by using dye, 2,6-dichlorophenol-indophenol method. The reading was measured by using a spectrophotometer (Model PRIM Light 230V) at 518 nm. The ascorbic acid content was estimated using a standard curve.

Total phenolic content was carried out by using Folin-Ciocalteu solution. The reading was measured by using a spectrophotometer (Model PRIM Light 230V) at 760 nm. The measurement was compared to a standard curve of prepared gallic acid equivalents in milligrams. For determination of total antioxidant activity, the cut fruit extract was mixed with DPPH and the reading was also measured by using a spectrophotometer (Model PRIM Light 230V) at 517 nm.

Enzymes extraction and assay

Extraction of enzymes was as described in Zainon et al. (2004). Extraction was carried out at 4 ºC. About 10 g of tissue were homogenized in blender in 20 mL 0.1 M sodium citrate, pH 4.6, containing 1 M NaCl, 13 mM EDTA, 10 mM β-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (PVP-40). The extracts were left for 30 minutes with occasional stirring. The supernatant was recovered by centrifugation at 29000 x g for 30 minutes.

Enzymes assay was as described in Zainon et al. (2004). The assay mixture for PG consisted of 0.75 mL 1.5% (w/v) polygalacturonic acid (Sigma) pH 5.2, 0.1 mL 0.6 M sodium chloride and 1.0 ml supernatant. The mixture was incubated for 1 hour at 37 °C. Reducing sugar released was estimated by the cyanoecetamide method with monogalacturonic acid as a standard. Enzyme activity was expressed as nkatal/g fresh weight (FW).

PME was assayed using 0.5 ml crude extract added to 25 mL 1% (w/v) pectin (Sigma) containing 0.3 M NaCl and titrated with 0.01 M NaOH at pH 7.3 for 10 min at room temperature using digital pH meter. Enzyme activity was expressed as nequivalent carboxyl group formed $s^{-1}g^{-1}$ fresh weight (FW).

Statistical analysis

Experiments were arranged in a Complete Randomized Design (CRD) with three replications. Each plot consists of three fruits. Data collected were analyzed using Analysis of Variance (ANOVA). Mean separation were performed by using Least Significance Difference (LSD) at the $p \leq 0.05$ level. Effect of discreet continuous independent variables (treatment) on various parameters will be analysed using regression analysis with SAS (version 9.0) statistical data analysis.

Results and Discussion

From the result, there was interaction found between calcium concentration and application technique towards firmness and enzyme, but no interaction was found in soluble solids content (SSC), ascorbic acid (vitamin C), titratable acidity, pH, total phenolic content and total antioxidant activity.

In Figure 1, calcium content increased with calcium concentration in applied solution with both application techniques but a marked increase in the fruit firmness was more apparent when the fruit was subjected to Ca infiltration application. For the PG and PME (Figure 1: c and d), the activities were reduced as the concentration of Ca in applied solution increased, thus strengthening the fact that Ca could play a significant role in fruit firmness *via* depressing the activity of the enzymes as reported elsewhere (Aguayo et al., 2007; Laminkara and Watson, 2004; Siti Hajar et al., 2009). Beneficial effects of increasing Ca in dragon fruit tissue recorded here is parallel with the positive effects of Ca in reducing disease severity (Yahya et al., 2009).

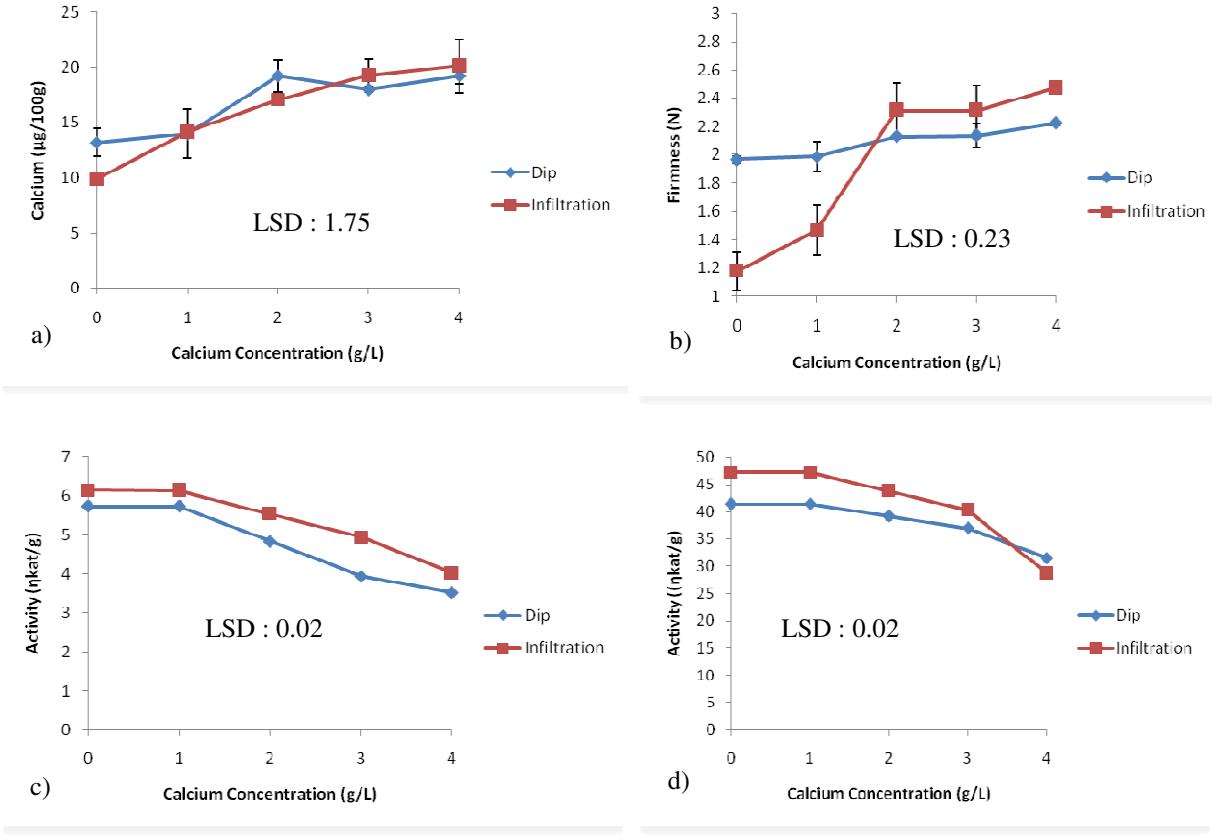

Figure 1. Effect of different calcium application techniques and calcium concentrations on a) Ca content, b) firmness, c) PG activity, and d) PME activity of fresh cut dragon fruit treated with five different concentrations of calcium.*Values are means of 3 readings.

This result was also consistent with those reported by other researches which indicated that cantaloupe cylinder was firmer when treated with 1%, 2.5% and 5% $CaCl_2$ solutions (Luna-Guzman et al., 1999). Hence, firmness and resistance to softening can be increased by addition of calcium due to stabilization of membrane systems and formation of Ca pectates that helped increasing the rigidity of middle lamella, cell walls and retarding polygalacturonase (PG) activity. Therefore, calcium treatments can extend postharvest life of fruits.

Conclusions

It is proven that increasing Ca^{2+} concentration reduced the activity of PG and PME enzymes in fresh cut red pitaya, suggesting that $CaCl_2$ could be effectively used in texture retention of fresh cut red pitaya in both application techniques. A marked increase in the fruit firmness was more apparent when the fresh cut fruit was subjected to Ca infiltration application.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effects of Storage Temperature, Packaging Materials and Storage Duration on Postharvest Quality Characteristics and Anthocyanin Pigmentation of Lemongrass (*Cymbopogon citratus*)

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Introduction

'Hijau' lemongrass (*Cymbopon citratus*) is a perennial turf grass and has a stalk formed by layers of leaf sheaths with broad aromatic leaves. There are three popular accessions of lemongrass, 'Galah', 'Paha ayam' and Hijau that have been planted commercially in Malaysia. Hijau lemongrass is highly demanded as additive ingredients in soft drinks and cosmetics. The lemongrass suffers from 'red pigmentation' on the pseudostem during postharvest, thus, reducing its market value.

The formation of anthocyanin is mainly influenced by cultivar, genetic, light, temperature and cultural practices (Lancaster et al., 1997). Anthocyanin accumulation is also influenced by different packaging methods (Ritenour and Khemira, 1997). The formation of anthocyanin can occur rapidly in presence of light. Pietrini et al. (2002) reported that the formation of anthocyanin in the fruit and vegetables will be reduced under storage at low temperature. Temperature of 35 ^{o}C speed destruction of anthocyanin resulting in production of chalkons which are responsible for brown color development on Berberis spp. (Laleh et al., 2006). According to Siomos et al., (2000) combination of low temperature and low light intensity reduced or slowed down the anthocyanin synthesis in stored white asparagus.

However, a specific recommendation for storage temperature and type of packaging for lemongrass is not available. Thus, the objective of this study was to determine the lemongrass quality characteristics and anthocyanin pigmentation as affected by storage temperature, type of packaging materials and storage duration.

Materials and Methods

Plant material

Mature-green (7 months old) Hijau lemongrass was harvested from a Farm in Kuala Selangor, Selangor. After trimming, well formed and free from any damages and diseases lemongrass stalks were selected for the experiment and were transported to the Postharvest Laboratory, Faculty of Agriculture, Universiti Putra Malaysia and stored in a cold room (10-12 ^{o}C) for 12 hr.

The next day, the lemongrass stalks were cut to 25 cm length, about three to five outer leaf layer of pseudo stem were discarded until white pseudo stem appeared. Then, the lemongrass were packed into two types of packaging materials (black and clear plastic bag) and stored at 10 and 15 ^{o}C for 0, 5, 10 and 15 days. Five stalks of lemongrass per replication were packed in each plastic bag.

Total monomeric anthocynin

Test solutions were adjusted to pH 1.0 and pH 4.5 buffers and absorbance was read at 520 nm and 700 nm using a visible spectrophotometer (Prim light 230V, Secomam, France).

Fruit quality evaluation

Firmness was measured with a penetrometer (Bishop FT 327, Italy). The firmness was measured on the equatorial diameter of five pseudo stems and results were expressed as newton (N).

SSC (%) was determined with a digital refractometer (Baush Lomb Abbe 3 L, Rochester, NY). Juice pH was determined by using pH meter (Crison Micro pH 2000, Crison Instruments, Spain). pH meter was calibrated using buffer solutions of pH 4 and 7. Titratable acidity was analyzed using the titration with 0.1% NaOH. The results were expressed as percentage of citric acid per 100 g fresh weight. AA was determined using the 2,6-dichlophenol-indophenol dye method. The AA content (Vitamin C) was expressed as mg^{-1} .100 g) of fresh stalk.

Results and Discussion

Effects on anthocyanin concentration

There were significant ($P \leq 0.05$) linear relationships between anthocyanin and storage durations (SD) of lemongrass stored at 10 and 15 ^{o}C (Figure 1A). There were significant interaction effects of storage temperature (ST) x packaging (P), ST x SD and ST x P x SD on anthocyanin (Table 1). Anthocyanin content of lemongrass packed with black plastic bag and stored at 15 ^{o}C was lower compared to other treatments (Figure 1B). According to Ritenour et al. (1997) packaging can prevent light from reaching the fruit. Thus, inhibiting chlorophyll production gave the apple an ivory color instead green. The formation can occur rapidly in presence of light. Saks et al. (1996) reported that anthocyanin accumulation in strawberries could occur under 2 hours of illumination with cool florescent light at 2 ^{o}C. Pietrini et al. (2002) reported that the formation of anthocyanin in the fruit and vegetables will be reduced under storage at low temperature. Temperature of 35 ^{o}C speed destruction of anthocyanin resulting in production of chalkons which are responsible for brown color development on *Berberis* spp. (Laleh et al., 2006). According to Siomos et al. (2000) combination of low temperature and low light intensity reduced or slowed down the anthocyanin synthesis in stored white asparagus.

Effects on SCC, pH, TA and AA

There were significant ($P \leq 0.05$) linear relationships between SSC and SD (Figure 1C), and pH and SD (Figure 1E) when lemongrass was stored at 10 and 15 ^{o}C. Lemongrass stalk stored at 15 ^{o}C had a lower accumulation of SSC and higher pH compared to other treatments. According Bartz and Brecht (2003) vegetables contain storage reserve such as fructosan or starch which can be degraded into soluble carbohydrates, thus maintaining nearly constant concentrations of soluble sugars for a long period. There was a significant ($P \leq 0.05$) linear relationship between SSC and SD for packaging materials used (Figure 1D), indicating that %SSC were higher when lemongrass was packed with clear plastic bag compared with black plastic bag. According to Cia et al. (2006) SSC increased with storage time, similar to the result obtained in this study. The higher %SSC indicated higher degradation rate of storage carbohydrates of lemongrass. There were no significant effects of SD, P and ST on TA and AA.

Effects on firmness

There were significant linear relationships between firmness and SD of lemongrass (Figure 2). The use of black packaging reduced exposure of the lemongrass to light. According to Sams (1999) light above photosynthetic saturation levels, especially intense exposure, can increase fruit temperature and may result in fruit damage and a loss of firmness. As the storage progressed, firmness of the lemongrass decreased. According to Deng et al. (2005) fruit softening is associated with the disassembly of primary cell wall and middle lamella structures. The changes in cell wall structure and composition result from the composite action of hydrolytic enzymes produced by the fruit, namely, polygalacturonase, pectinesterase, b-galactosidase, pectate lyase, and cellulase.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

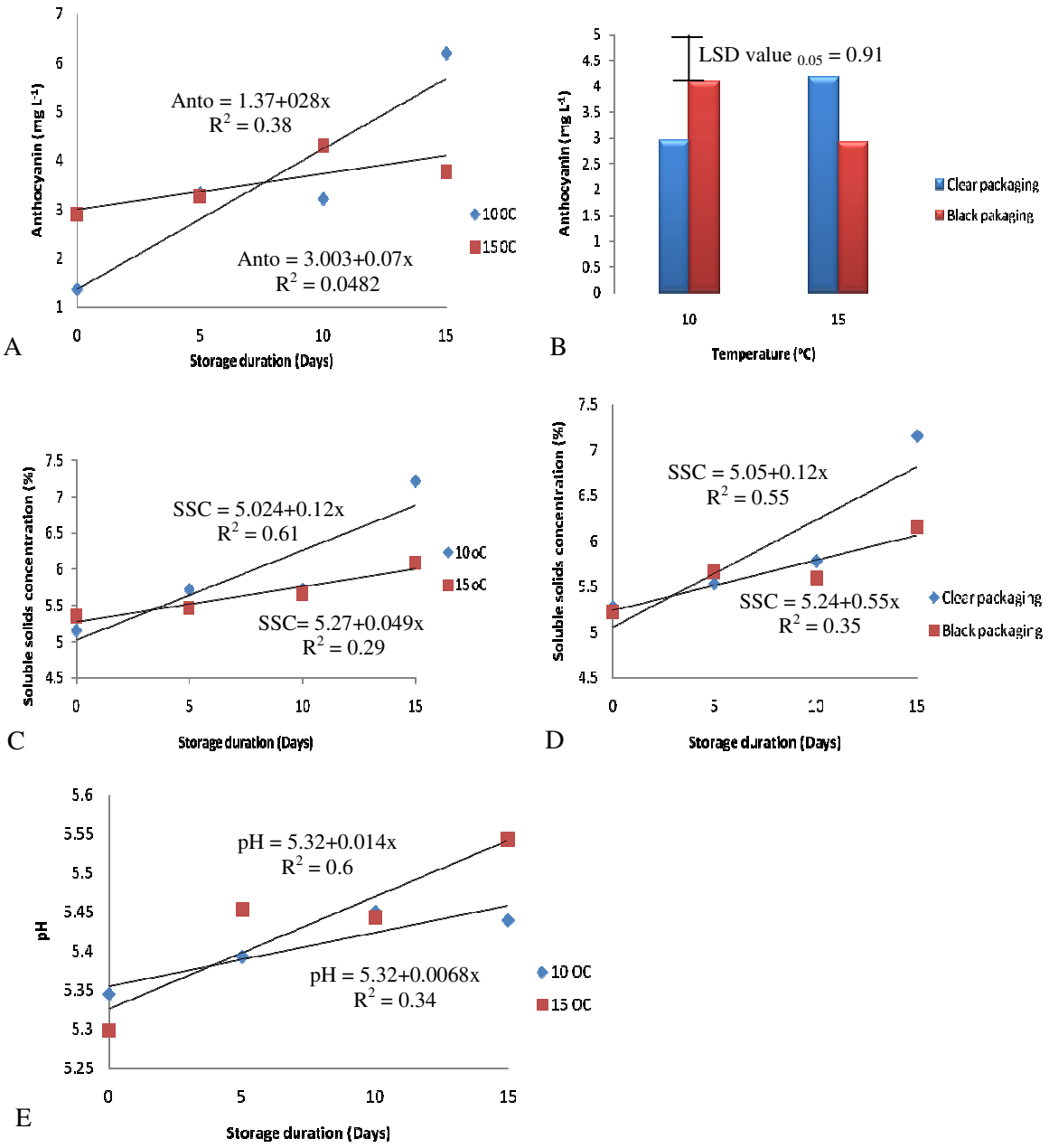

Figure 1. Relationship between chemical characteristics of Hijau lemongrass and storage duration: (A) anthocyanin concentration (Anto) of lemongrass stored at 10 and 15 °C, (B) anthocyanin concentration of lemongrass packed with clear and black plastic bag, (C) soluble solids concentration (SSC) of lemongrass stored at 10 and 15 °C, (D) SSC of lemongrass packed with clear and black plastic bag, and (E) pH of lemongrass stored at 10 and 15 °C.

Table 1: Main and interaction effects of temperature, packaging and storage duration on firmness soluble solids concentration (SSC), titratable acidity (TA), acid ascorbic (AA), pH and anthocyanin.

Factors	Firmness	SSC	TA	AA	pH	Anthocyanin
Storage temperature (ST)						
10 °C	149.84 A[z]	5.95 A	0.17 A	3.04 A	5.41 B	3.52 A
15 °C	147.10 A	5.64 B	0.15 A	3.15 A	5.43 A	3.53 A
Type of plastic packaging (P)						
Clear	149.50 A	5.94 A	0.16 A	3.16 A	5.40 B	3.57 A
Black	147.40 B	5.66 B	0.16 A	3.03A	5.44 A	3.50 A
Storage duration (SD)						
0	167.59 B	5.24 C	0.16 A	2,77 B	5.32 C	2.12 C
5	175.14 A	5.60 B	0.16 A	2.54 B	5.42 B	3.29 BC
10	124.76 C	5.69 B	0.15 A	3.72 A	5.45 B	3.75 AB
15	126.30 C	6.70 A	0.17 A	3.35 A	5.49 A	4.98 A
ST XP	NS	NS	*	NS	NS	*
T X SD	NS	*	NS	NS	*	*
P X SD	*	*	NS	NS	NS	NS
ST X P X SD	NS	NS	NS	NS	NS	*

[z] *For each treatment, means within a column followed by the same letter are not significantly different (P≤ 0.05) by LSD*
*NS, **, * not significant or significant at P≤ 0.01 and P≤ 0.05, respectively*

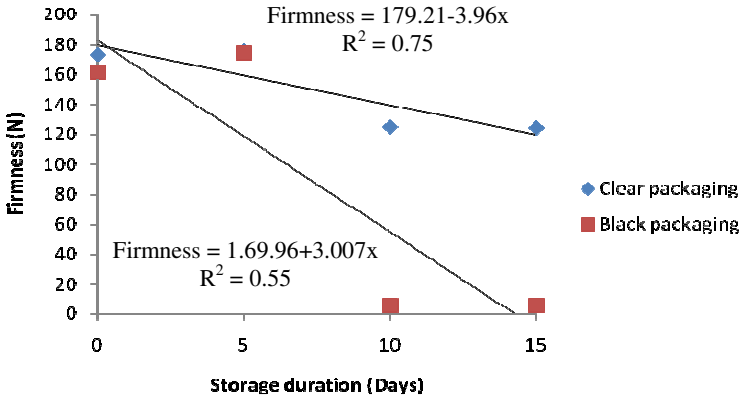

Figure 2. Relationship between firmness and storage duration of Hijau lemongrass packed in clear and black plastic bag.

Conclusions

In this study, the storage temperature, packaging material and storage duration affected anthocyanin content and quality of Hijau lemongrass. The lemongrass stalk packed with black plastic bag and stored at 15 °C had lowest anthocyanin formation compared and to other treatments. By packaging in black plastic bag, light was prevented from penetrating the lemongrass. Therefore, the formation of

anthocyanin was reduced. Postharvest quality characteristics of the lemongrass were also maintained during the 15 days of storage.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 3

ECOPHYSIOLOGY AND STRESS BIOLOGY

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Preliminary Assessment of the Impacts of Recreation Activities on Growth and Chlorophyll Fluorescence of *Tristaniopsis fruticosa* in Mount Tahan

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Introduction

Chlorophyll fluorescence has been used to study the difference of functional levels of photosynthesis where it give insights into the ability of a plant to deal with environmental stresses and into the extent to which those stresses have damage the photosynthetic apparatus (Kate and Giles, 2000). Photosynthesis provides an indicator for the quantitative characterization of states of stress and of functional limitations imposed by environmental factors. Leaf chlorophyll fluorescence probe is a powerful and sensitive intrinsic measurement of the photosynthetic process (Govindjee et al., 1995) that can be used to detect the influence of various environmental stress factors**.** Fv/Fm and Fo are widely use to determine the occurrence of photo inhibitory damage as response to high temperature (Gamon and Pearcy, 1989) and low temperature (Groom and Baker, 1992).

Chlorophyll fluorescence which is widely used as an early stress indicator is also very useful to study the effects of environmental stresses on plants since photosynthesis is often reduced in plants experiencing adverse conditions. Numerous aspects of the photosynthetic process can be analyzed from these parameters. In healthy leaves, the value usually close to 0.8, independently of the plant species studied. A lower value shows that a proportion of PSH reaction centers are damaged. High mountain ecosystem has a sensitive environment where extremely cold and high wind and unstable temperature altogether combine to stress the plants (Dickinson and Murphy, 2007). Human activities such as camping and trampling in mountain areas has been said to create disturbance which can cause changes to the structure of a community in an environment. Based on Dickinson and Murphy (2007), environment which receives low level of disturbance has higher level of environmental stress. This will significantly limit the photosynthetic production and therefore will effects the plant growth.

This study were done with the objective of quantifying the impact of disturbance on photosynthetic rate of *Tristaniopsis fruticosa* in Mount Tahan using chlorophyll fluorescence as an indicator of environmental stresses.

Materials and Methods

Study area

Experiments were conducted in Mount Tahan (2,187 m above sea level) which is the highest point (4°38'N, 102°14'E) in Peninsular Malaysia located within the Pahang national park with an area of 1,677 square miles (4,343 km^2). The elevation ranges from 1800 m above and topography is hilly with slopes of 50 to 70 degrees. Mount Tahan has been a hot spot for local and international climbers since 1960's for its magnificent values of natural flora and fauna.

Experimental design

This study were carried out by following a standard experimental procedures for studying recreational trampling of vegetation proposed by Cole and Bayfield (1993) There are two sites or locations, one designated as the control site and the other as the impact site or site where the activity is hypothesized to have an influence. Measurements of change which are chlorophyll fluorescence potential were compared between controls and impacted sites. In this study, six plots sized 20 m X 20 m representing disturbed and undisturbed were established in each camping (Botak and Summit area) and trampling

(tracking trail) areas. Undisturbed, plots were located about 20 m away from disturbed plots whether on the left or the right side depending on the topography of the selected areas. Data collections were made on three phases which were on February 2008, May 2008 and February 2009. For the purposed of evaluation, the multiple fluorescence values were averaged in order to get the most accurate reading.

Data collection

For each plot, 20 trees of *T. fruticosa* were chosen for chlorophyll fluorescence (CF) measurements. CF was measured using Hansatech Handy PEA. Leaf clips were placed on three leaves per tree with shutter in closed position for dark adaptation.

Data analysis

Data were analyzed and compared using independent T-Test. The statistical analyses were performed using Social Package for Social Sciences version 12.0 and the significance level was set at 0.05. Graph and figures were carried out using CF handy Pea instrument package.

Results

The mean values of chlorophyll fluorescence (CF) parameters were found higher in disturbed plots than in undisturbed plots particularly for all camping and trampling areas. The reading shows that the highest Fv/Fm values were found in Botak campsite area followed by Summit campsite (Figure 1). Fv/Fm value was significantly different between disturbed trail and undisturbed plot. The lowest mean values of CF were found in undisturbed trail area. Furthermore, in contrast, Fo value shows a significant different for disturbed and undisturbed plots in camping area. Figure 2 indicated that height and DBH for *T. fruticosa* in camping areas were found higher in treatment plots compared to control (undisturbed) plots. These scenarios were obtained as a result to the photosynthetic rate in those areas.

Table 1. The values presented are mean and standard error of chlorophyll fluorescence parameters of *T. fruticosa* with significant level at $p<0.05$

Parameters	Camping area			Tracking Trails		
	Disturbed	Undisturbed	F-value	Disturbed	Undisturbed	F-value
Fo	160.71±2.14	176.11±2.36	7.724*	155.19±3.24	155.70±3.04	2.110*
Fm	615.83±13.17	675.22±14.94	2.641*	570.45±19.67	558.04±17.74	1.743*
Fv	455.11±11.67	499.11±13.57	1.517*	415.26±17.82	402.33±15.82	1.376*
Fv/Fm	0.72±0.004	0.73±.004	1.101*	0.715±.007	0.7091±.007	0.005*

Discussion

The reduction of rate in photosynthesis is a symptom of environmental stresses. Percival et al. (2003) indicated that photosynthesis is central to plant biosynthesis that provides an interactive link between the internal metabolism of a tree and the external environment. Alexandrov (1964) has long recognized that changes in photosynthesis provide an earlier indication of stress caused by cold and heat than other criteria of cell stress and damage.

ISBN: 978-967-344-188-4

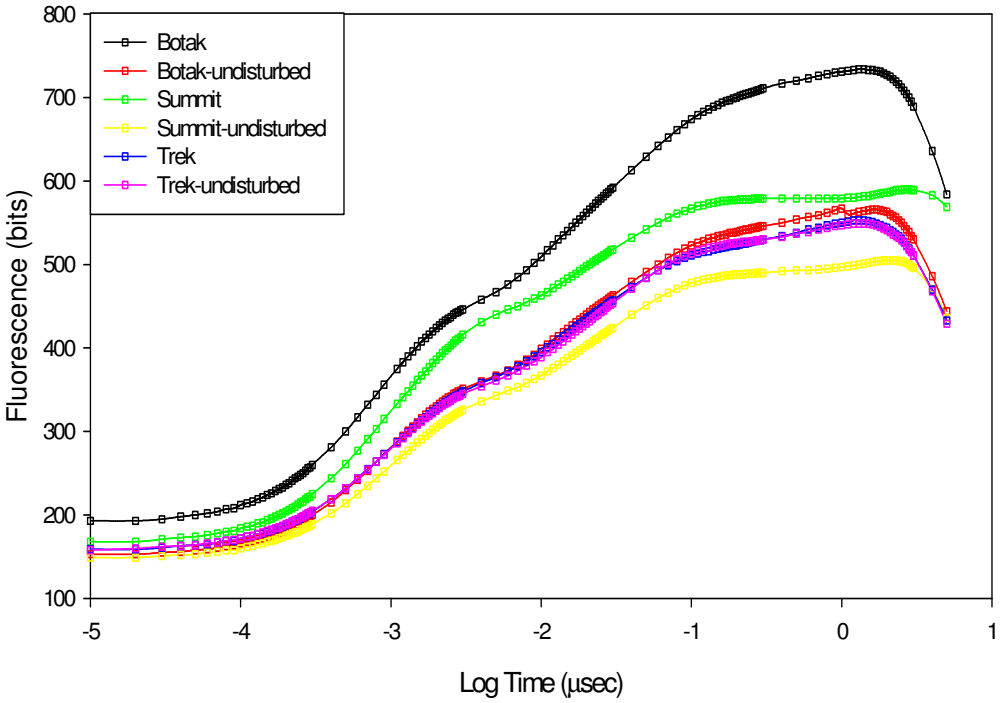

Figure 1. Means of fluorescence in one second measurement of *T. Fruticosa*

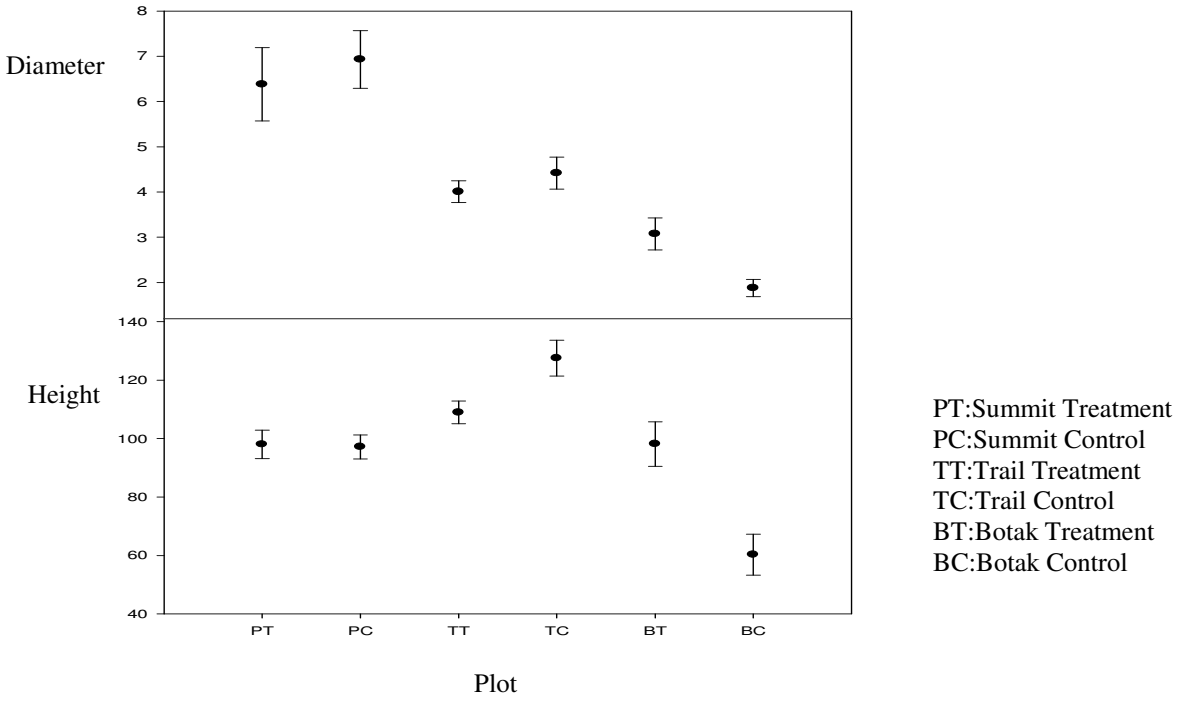

Figure 2. A comparison of diameter and height of *T. fruticosa* between treatment and control plots in both areas. The values presented in the table are mean and standard error with significant level at $p<0.05$.

CF readings were low on impacted areas which are under stresses. Control plot in Botak Hill received direct exposure of sunlight, wind and high intensity of radiation receipt at high altitudes especially on clear summer days. This situation makes the area have higher light intensities and uneven temperature which localized plants under severe water stress. According to Dickinson and Murphy (2007), high

mountain ecosystems have cold and high winds that will combine to stress the plants by coupling low metabolic activity being called physiological drought produced by the desiccating effect of the wind. At temperature too high or too low the photosynthetic yields decrease steadily until CO_2 uptake ceases (Schulze and Caldwell, 1993). Lower value of Fv/Fm indicated that there is a decreasing in PSII production which means that the tree is under stress condition. From our observation, we could see that most tree in control plots especially in Botak Hill and summit seem to have chlorosis and stunting effect where yellowing of the leaves caused by the reducing of chlorophyll synthesis. Leaves seem to be particularly sensitive indicators of mineral deficiency, tending to be reduced in size, abnormal in shape or structure, pale in colour and even develop dead areas on the tips or margins. Figure 2 supported the theories where it shows that tree height and DBH in control plots for camping areas are lower than that of the treated (disturbed) plots.

Soil condition in Botak and Trail control plots are more compact compared to treatment plots. The rock layers in Botak control plots are covered by smaller vegetation. Soil compaction slows tree growth and cause subsequent loss of ground vegetation. If the moisture content of soil is low, then, water absorption by root is difficult and photosynthesis cannot be proceed. Studies have shown that soil compaction reduced shoot growth by restricting the soil volume for root expansion, causing the reduction water and nutrient availability to plants, Taylor and Brar (1991).

In this study, the mean values of CF parameters were found to be higher in the disturbed plots than undisturbed plots for both camping and trampling area (Table 1, Figure 1). CF rate will be low in plants which experienced severe conditions such as stresses. Differences between the results obviously related to the environmental quality factors such as soil quality and mineral contents. Canopy opening and activities done by recreationist (i.e. cooking) created more exposure to disturbance. Nevertheless, higher moisture and soil minerals derived by human waste and disposal in camping areas probably contribute to the contrasting results. The supply of nutrients from the environment varies continuously within time and spaces. The optimum supply of nutrients will allow maximum growth while lack of them will lead to the inhibiting growth. In contrast to that, eroded soil in tracking trails released more soil organic matter, nutrients and minerals downhill. Erosion especially on steep slopes may form gullies which will encourage breakdown and release soil particles contained minerals and nutrients to the lower area. Chemicals dissolved in water flowing through the soil have reacted with the underlying rocks to release the mineral nutrients plants need in which the process called chemical weathering (Allaby, 2004). Furthermore we can see that there is an occurrence of shrubs and grasses in treatment plots which indicate that there is disturbance in those areas**.**

Conclusions

Environmental stress occurs when any factor which tends to reduce the efficiency of functioning of one or more key physiological processes in the organisms occupy in the ecosystem. Growth generally reduced as the effect to low nutrients, to water deficit, or to temperature different from optimum. Anything which reduces the efficiency, accuracy and rate of photosynthesis accumulation is a source of stress. Environmental stresses that occur will significantly limit the photosynthesis production. One of the symptoms of environmental stresses is often showed by the decreasing of photosynthesis rate. These study shows two contrasting result which concluded that human activities (i.e. camping and trampling) is not a major factor of disturbance and growth stresses in mountain areas which are originally a sensitive ecosystem. Some vegetation communities are relatively benefited from these activities which added and decomposed minerals and nutrients availability in those areas. Both disturbance and human activities affect different ecosystems in different ways.

Acknowledgements

This research was funded by RUGS (UPM) competitive grant RUGS/91046. The authors are very grateful to PERHILITAN for the permission to work on Mount Tahan.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Influence of Salinity on the Germination of Iranian Alfalfa Ecotypes

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Introduction

Crop production is seriously suppressed by salinity in more than 800 million hectares of land throughout the world (FAO, 2005). Crop yield decreases markedly with increase in salt concentration, but the threshold concentration and rate of yield decrease depend on species (Keiffer and Ungar, 1997). Seed germination is usually the most critical stage in influencing establishment in arid and semi-arid regions (Khaje-Hossini et al., 2003). Salinity affects germination of seeds by creating an external osmotic potential that prevents water uptake or due to the toxic effects of sodium and chloride ions on the germinating seed affecting the uniformity of plant density with negative effect on yield (Gamze, 2004). Germination and seedling growth are reduced in saline soils with varying responses for species and cultivars (Hampson and Simpson, 1990). Several investigations of seed germination under salinity stress have indicated that seeds of most species achieve their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development (Ghoulam and Fares, 2001; Keiffer and Ungar, 1997). Alfalfa is worldwide forage that originated from Iran, Turkey, Turkmenistan and Caucasus. Alfalfa is moderately sensitive to salt and the ideal soil is that with an EC 0-2 Ms/cm and soils with EC more than 5 Ms/cm are unsuitable for alfalfa cultivation (Peel et al., 2004). This study was carried out to screen alfalfa ecotypes for salt tolerance during germination. The association of salt tolerance with different geographic locations was also evaluated.

Materials and Methods

Seeds of 20 ecotypes of alfalfa were obtained from the Iran Seed and Plant Improvement Institute, in August 2007. The ecotypes were collected from alfalfa growing areas throughout Iran representing various geographical regions. The experiments were carried out during March 2008 at the Seed Technology Laboratory of Universiti Putra Malaysia. Seeds were germinated in sterilized, covered, disposable plastic petri dishes containing Whatman No. 2 filter paper moistened with either distilled water (control), or 50, 100, 150, 200, 250 mM of sodium chloride (NaCl) solution. Three replicates of 25 seeds each were used for all treatments. Seeds were incubated in laboratory condition at 23 - 27^{o}C and were considered germinated when the radical had emerged. Germinated seeds were counted daily until the end of the germination period of 7 days. In order to maintain adequate moisture, 3 ml of the original salt solution was added to each petri dish on the third day. Parameters observed included germination percentage, germination speed and germination index. For germination percentage total seed germinated at the end of experiment was considered and for germination speed the formula: $\sum GT1/T1 + \ldots. + GTn/Tn$ was used where GT is seed germinated per day and T is days during trial. The germination index (GI) was calculated based on the formula $GI = (5 * n1) + (4 * n2) + \ldots + (1 * n5)$ where n1, n2, etc are numbers of seed germinating on days 1, 2, etc. This experiment was a factorial with ecotypes and the concentration of NaCl as factors, and a completely randomized design (CRD) was used. Data were analyzed using the analysis of variance (ANOVA) and treatment means were compared using Duncan multiple range test.

Results and Discussion

To evaluate the effects of salinity on germination, three parameters were calculated namely germination percentages (GP), germination speed (GS) and germination index (GI). Salinity concentrations, ecotypes and interaction between levels of salinity and ecotypes in all parameters (GP,

GS, and GI) were significant at 1% probability level (data not shown). All germination parameters were progressively inhibited by increased NaCl concentrations. The germination percentage at 250 mM NaCl (12.0%) was significantly depressed compared to control with 86.4% germination ($P<0.05$) (data not shown).

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For germination speed there was a consistent linear rate of decline with increasing salinity (Figure 1). The reduction in germination speed was particularly severe from 200 mM to 250 mM NaCl. Germination index can be considered as a perfect measure of tolerance between the ecotypes because the formula takes into consideration time and number of germinated seeds. Germination index showed a linear decline with increasing salinity from 0 to 150 mM NaCl. At 200 mM NaCl, GI was 50% of control while at 250 mM NaCl it was reduced to 10% of control (Figure 2).

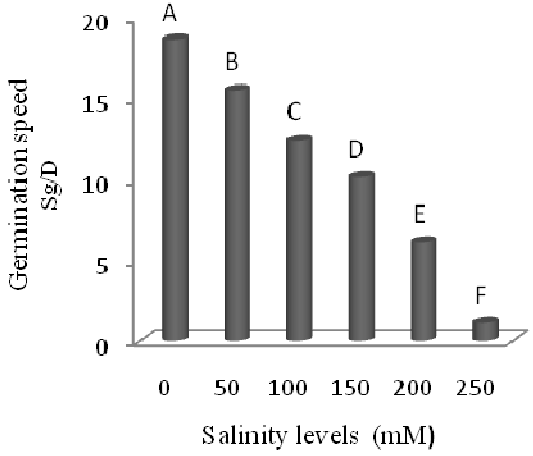

Figure 1: Effect of salinity on germination speed

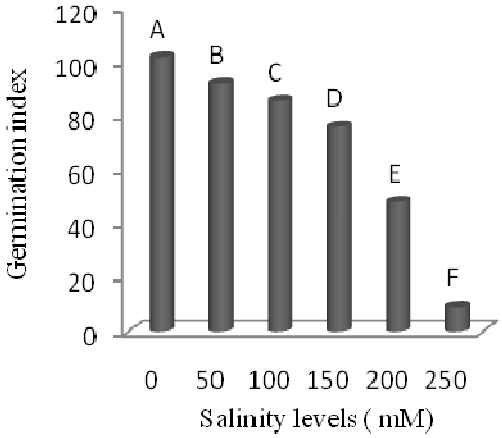

Figure 2: Effect of salinity on germination index

Reactions of ecotypes to salinity levels for all germination parameter (GP, GS, and GI) were significantly different and there was a wide range of salinity tolerance in alfalfa ecotypes throughout Iran (Figures 3 and 4).

The response of ecotypes to increasing levels of salinity varied between ecotypes as shown by two extreme ecotypes (Figures 3 and 4). The gradient of regression line indicates the rate of tolerance so that with increasing slope the level of tolerance to salinity is lower. Ecotypes have been chosen from different climates and there appears to be an association between climate and salinity tolerance. Germination index of ecotypes from Mediterranean climate (Ecotype 6) were the highest compared to ecotypes from the semiarid climate (Ecotype 17). There is a need to do more research on the correlation between cold tolerance and salt tolerance because the lower temperature in Mediterranean climate during winter may make it more salt tolerant than those from the arid and semiarid region. According to the results, there were significant interaction effects between ecotypes and salinity levels relative to all germination parameter.

Conclusions

The response to salinity during the germination stage was variable between ecotypes. It was clear that during germination the ecotypes showed a wide range of variation of salt tolerance. The results showed that up to 100 mM salt concentration, there was a slight reduction in the germination of alfalfa ecotypes but there was a considerable decrease in germination at 200 mM and 250 mM. Increased salinity affected germination speed more than germination percentage so that the seeds grown under high salinity needed more time for germination. There was an association between geographic locations and salt tolerance as ecotypes from the Mediterranean climate showed greater salt tolerance than ecotypes from the arid and semiarid regions. The results should lead to further research to investigate the correlation between cold tolerance and salt tolerance. It was concluded that

the wide range of tolerance among alfalfa germplasms may promote the release of some salt tolerant cultivars of alfalfa.

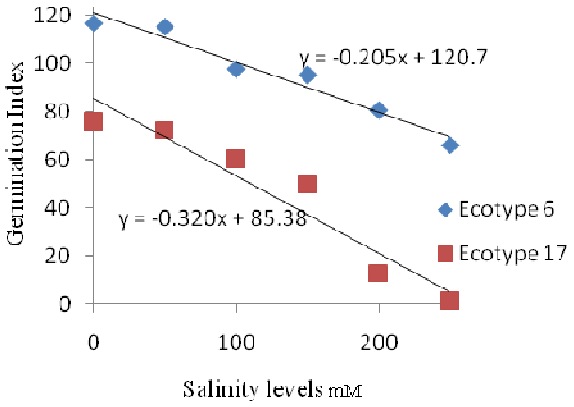

Figure 3: Linear regression between salinity levels and germination index in highest and lowest ecotypes

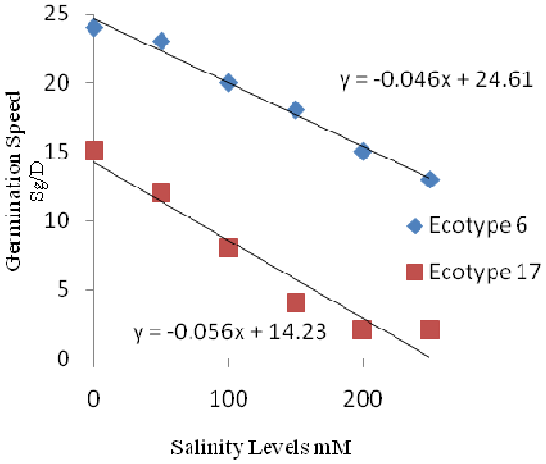

Figure 4: Linear regression between salinity levels and germination speed in highest and lowest ecotypes

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Physiological Responses to Light Stress in the Epiphytes of *Platycerium bifurcatum* (Cav.) C. Chr.

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Introduction

Presently climate change is one of the major environmental issues that gain major concern by the international community. Extreme weather, high light intensity, increasing temperature, floods, drought and expected sea level rise are some impact of climate change. These changes also affect the physiological process of the plants. Plant processes of photosynthesis and growth of plants would change as they adapt and react to the changes in their environment (Schurr et al., 2006). Plants like epiphytes living under plant canopy are usually exposed to harsh environment. Environmental stress like drought and high light intensity may occur in epiphytic plants that live in that environment. The physiological attributes such as rate of photosynthesis, transpiration, and stomatal conductance of such plants will be affected. *Platycerium bifurcatum* (Cav.) C. Chr. is an understorey epiphytic ferns recognized to be used widely as an ornamental plant (Rut et al., 2008). The plant is usually highly exposed to the environmental stress such as high light intensity. Such exposure to extreme condition would affect the epiphytic ferns of *P. bifurcatum* in terms of physiological attributes. Thus, it is vital to determine the responses in physiological aspect of this plant to certain level of light intensity. Therefore, the objective of this study is to explore the effect of light stress on physiological attributes of *P. bifurcatum*.

Materials and Methods

Forty plants of *P. bifurcatum* of similar size and age were selected. This study was conducted in April 2009 at the green house of Faculty of Forestry, University Putra Malaysia, Serdang, Selangor for about four weeks. The plants were subjected to four levels of light intensity; namely 20 μm $m^{-2}s^{-1}$ (T1), 70 μm $m^{-2}s^{-1}$ (T2), 200 μm $m^{-2}s^{-1}$ (T3) and 1500 μm $m^{-2}s^{-1}$ (T4). There were ten plants assigned as ten replicates for each treatment. Plant responses to light treatments were quantified by measuring the photosynthesis rate (A_{net}), intercellular CO_2 concentration (Ci) , stomatal conductance (g_s), transpiration rate (E) and leaf to air vapor pressure deficit calculated based on leaf temperature (D) in the morning by using a Photosynthesis System LI-COR 6400 (LI-COR Biosciences, Inc., Lincoln, NE). Analysis of variance (ANOVA) was used to test for variations in the physiological response of *P. bifurcatum* to light stress using SPSS version 17.0. Further mean separation test by using Tukey's multiple comparisons with $p<0.05$ was also conducted once significant difference between treatments was found.

Results and Discussion

The summary of analysis of variance (ANOVA) for physiological parameters is shown in Table 1. There were significant differences observed for all parameters studied between weeks except for *Ci*.

Table 2 shows the mean values of physiological parameters as affected by light intensity treatments. It was observed that plants subjected to T1 had a lower mean value of A_{net} followed by T2, T4 and T3. In T2, the mean values of g_s and Ci was found lower than the other treatments. For E_L, T4 recorded the highest value whereas T1 showed the lowest value. Same goes to D where T4 recorded the highest mean value for this parameter.

Table 1. The summary of ANOVA for week and treatment responses parameters of *P. bifurcatum* growth and physiological response of on light stress

Source of variation	Parameter	Mean square	F	P
Week				
	A_{net}	74.14	85.27	0.000
	G_s	0.05	43.74	0.000
	Ci	82.67	0.46	0.830
	E_l	0.83	17.62	0.000
	D	10.10	303.34	0.000
Treatment				
	A_{net}	2.40	2.76	0.049
	G_s	0.01	3.76	0.015
	Ci	126.81	1.19	0.319
	E_l	0.08	1.64	0.188
	D	0.32	9.45	0.000

Table 2. The mean values of physiological aspects after treatment at different levels of light intensity

Parameter	Treatment			
	T1	T 2	T 3	T 4
A_{net} (µm co_2 m^{-2} s^{-1})	2.03^a	2.22^{ab}	2.88^b	2.49^{ab}
G_s (µmol h_2o m^{-2} s^{-1})	0.04^a	0.03^a	$0.06a^b$	0.07^b
Ci (µmol co_2 mol^{-1})	261.39^a	239.22^a	242.50^a	259.12^a
E_l (mm h_2o m^{-2} s^{-1})	0.40^a	0.41^a	0.48^a	0.55^a
D (kpa)	1.18^a	1.35^b	1.02^a	1.14^a

Means with the same letter(s) are not significantly different at 5% level of significance

Basically, physiology responses play an important part in the development and enlargement of the plants. *Platycerium bifurcatum* showed a low photosynthesis rate (A_{net}) value in all treatments (Table 1). Ferns, mosses and lichens were basically observed to have a very low A_{net} as compared to other plants especially woody plants due to their thinner assimilation organs (Larcher, 2003). This is supported by Stunz and Zotz (2001) where they found that there were low values of photosynthetic rate for most of the epiphytes species, i.e. an average of 2.6 µmol CO_2 $m^{-2}s^{-1}$. A_{net} in T1 and T2 (Table 2) was the lowest as compared to T3 and T4. The higher A_{net} value observed in the T3 and T4 suggested that they may have reacted to high light intensity and showed high gas exchange capacity. It also reflected that they use this mechanism to overcome and respond to the stress effect. There was significant difference between treatments for stomatal conductance (g_s) throughout the experiment, albeit there was only a slight difference between treatments. The g_s response to environmental condition in this experiment was higher in T3 and T4 than T1 and T2 (Table 2). The lower values of A_{net} and g_s signified that there was a limitation of PAR for photosynthesis under lower light intensity (i.e shaded plants) (Medina et al., 2002). Low g_s in T1 and T2 might reflect stomatal limitation which consequently reduced photosynthesis. This was supported by Franck and Vaast (2009) and Elsheery and Cao (2008) where closure of stomata reduced the diffusion of ambient CO_2 into mesophyll and resulted in the photosynthetic rate reduction (Cornic, 2002). The transpiration rate (E_L) in T1 and T2 was also lower as compared to T3 and T4. Nicolas et al. (2008) previously reported that shade plants showed lower E_L which could be explained by low irradiance received by them. In this study, g_s in T2 was low but the plants had high *D* value in accordance to most cases recorded (Oren et al., 1999; Monteith, 1995).

Conclusions

Plants in the higher light intensities environment (T3 and T4) performed higher mean value of A_{net} and g_s as compared to those treated at lower light intensities (T1 and T2). On the contrary, plants at lower light intensities (T1 and T2) performed higher mean value of D as compared to those treated at higher

light intensity environment (T3 and T4). This reflected that this plant does response through its physiological attributes when exposed to different levels of light intensity. This indicated that light plays an important role that affects the physiological attributes of *P. bifurcatum*.

Acknowledgements

This study was a part of the research project No. 03/01/07/0035RU which was funded by the Research University Grant Scheme (RUGS). The authors would like to thank Mr. Syahrilnazeem, Mr. Ismail, Ms. Syahaneem and Ms. Nur Aisyah for their technical assistance.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 4

PEST AND DISEASE MANAGEMENT

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Sources of Resistance to *Phytophthora palmivora* in Durian

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Introduction

Peninsula Malaysia and Borneo are the major centers of diversity for durian. Durian is classified under the genus *Durio* under the family Bombacaceae. A total of 29 species have been recorded and distributed throughout the Southeast Asian regions extending from Sri Lanka to Myanmar, Thailand, Malaysia, Indonesia, Philippines and Madagascar (Kostermans, 1958; 1992).

Durio zibethinus or the common durian, also dubbed as the 'King of Fruits' is very popular in this region due to the superb, exquisite, delicious and unique taste. Several other *Durio* species are also edible. The common durian is a very popular fruit with high economic value in Southeast Asian countries. Several other *Durio* sp. was also cultivated in certain areas, although on a small scale. *Durio graveolens* and *Durio kutejensis* are just as popular and of high economic importance as the common durian in the states of Sabah and Sarawak. In Brunei, *Durio graveolens* is sought after much more than the common durian.

Durian is one of the most important fruit crop in Malaysia. More than 122,000 hectares of land are being cultivated with durian in 2000 and the acreage has stabilized. Although some of the clones have superior characteristics and qualities, there were also inferior characteristics that need to be addressed. One of the most important problems in durian cultivation is the susceptibility to stem canker caused by *Phytophthora palmivora* (Tai, 1971). This disease can attack any stage of the plant from seedlings until as long as the natural life of the tree. The fungus can attack the trunk, branches, twigs, leaves, flowers, fruit and the underground parts of the stem and roots. The entry of the pathogen is through wounds caused by mechanical injury or through natural openings. The incidence of stem canker in most orchards is high. This disease can result in 10% mortality of plants every year.

The objective of this study is to evaluate the resistance/susceptibility of *Durio* spp. to the stem canker disease caused by *P. palmivora*

Materials and Methods

Phytophthora palmivora isolate used in this experiment was isolated from the bark of a diseased durian tree in MARDI, Serdang, Selangor and the fungus was maintained on corn meal agar. For inoculation of durian trees, fresh culture was prepared on potato dextrose agar and the inoculum was obtained from the actively growing margin of the fungal colony. Inoculation of the durian accessions were conducted on the twigs. Healthy twigs about the size of a pencil and more than 15 cm long were chosen. A small portion of the bark was removed using a 5 mm diameter cork borer. A similar size agar plug from the actively growing region or the advancing margin of the fungal colony was placed onto the wounded portion of the bark, covered with a small piece of wet cotton wool and finally wrapped with parafilm. Sixteen twigs were inoculated for each tree. Measurement of the lesion as a result of the fungal infection was carried out at 5-day intervals by destructive sampling of 4 twigs at each sampling interval. The inoculated twigs were cut off from the tree, the bark removed to reveal the lesion caused by the fungal infection. The length of the lesion above and below the infection court was measured. The inoculated twigs were heat sterilized before disposal.

Results and Discussion

A total of 111 accessions of *D. zibethinus* in Kemaman, 103 *Durio* sp. in Jerangau, including 31 accessions of *Durio lowianus* , 13 accessions of *D. graveolens,* 5 accessions of *Durio kutejensis* grown and maintained at the Fruit Arboretum in MARDI, Serdang were screened for their reaction to the patch canker disease caused by *P. palmivora*. *Durio zibethinus* showed a wide range of variation and 1 accession was rated as resistant based on lesion development per day (Table 1).

Table 1. Patch canker disease rating of *D. zibethinus* accessions on the collection in Kemaman, Trengganu.

Disease rating (lesion spread per day, mm)	Disease category	No. accessions
0 - 1.0	Resistant	1
1.01 – 2.0	Moderately resistant	2
2.01 – 3.0	Susceptible	11
> 3.01	Highly susceptible	92

Accession in Jerangau showed more resistant but their species need to be clarified further (Table 2). *Durio lowianus* exhibited a wide range of reaction to *P. palmivora* based on the development of lesions following inoculation. Results showed lesion length ranges from 3.52 mm to 21.86 mm 20 days after inoculation with an average of 11.48 mm. Durian clone D24, considered as the susceptible control accession, showed lesion development of 13.64 mm.

Durio graveolens similarly exhibited a wide range of reaction to *P. palmivora* based on lesion development after inoculation. Results showed lesion length ranges from a low of 6.61 mm to as high as 14.38 mm, 20 days after inoculation. The length of lesion of all the accessions averages 9.67 mm 20 days after inoculation.

Table 2**.** Patch canker disease rating of durian accessions (*Durio* sp.) on the collection in Jerangau, Trengganu.

Disease rating (lesion spread per day, mm)	Disease category	No. accessions
0 - 1.0	Resistant	34
1.01 – 2.0	Moderately resistant	3
2.01 – 3.0	Susceptible	11
> 3.01	Highly susceptible	66

Based on these results, resistance or susceptibility of *D. lowianus* can be arbitrarily categorized as in Table 3. One accession was categorized as resistant based on lesion development of less than 5 mm at 20 days after inoculation. Another 7 accessions were classified as moderately resistant or moderately susceptible with lesion development ranging between 5.1-10.0 mm after 20 days inoculation. These clones were better than the susceptible control plant with lesion development at 13.64 mm at 20 days after inoculation. A majority of the accessions were considered as susceptible as these 21 accessions showed lesion length of 10.1-15.0 mm at 20 days after inoculation. A further 2 accessions were considered very susceptible with lesion development higher than 15.1 mm and also much higher than the susceptible check plant.

Durio graveolens exhibited much less resistant reaction based on the accessions evaluated. No accession can be rated as resistant. Eight accessions were rated as moderately resistant or susceptible with lesion length between 5.1-10.0 mm at 20 days after inoculation. Another 5 accessions were rated as susceptible with measurable lesion length ranging from 10.1-15.0 mm at 20 days after inoculation. Evaluation of 5 accessions of *D. kutejensis* showed 1 accession rated as resistant, 3 accessions rated as moderately resistant or susceptible and 1 accession as susceptible.

Table 3. Phenotypic reactions of *Durio* species in Serdang to infection by *P. palmivora.*

Lesion length at 20 days after inoculation (mm)	Disease Rating	No. accessions			
		D. lowianus	*D. kutejensis*	*D. dulcis*	*D. graveolens*
0-5	Resistant	1	1	0	0
5.1-10.0	Moderately resistant/susceptible	7	3	1	8
10.1-15.0	Susceptible	21	1	0	5
> 15.1	Very Susceptible	2	0	0	0

Conclusions

In conclusion, resistance is available in the wild durian *D. lowianus* and *D. kutejensis*. Resistance in *D. graveolens* was not detected among the accessions evaluated.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Detection of *Candidatus* Liberibacter asiaticus Causes Citrus Greening Disease Using Surface Plasmon Resonance (SPR)

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Introduction

Citrus greening disease or Huanglongbing (HLB) is caused by the gram-negative bacterium *Candidatus* Liberibacter asiaticus (*Ca.* L. asiaticus) (Garnier et al., 2000) and was confirmed in Malaysia in 1989 (Lim et al., 1989). This disease is vectored by *Diaphorina citri* and *Trioza erytreae*, which colonized the citrus-growing regions in Malaysia. *Diaphorinacitri* acquires the greening bacterium while feeding on infected phloem (Hung et al., 2004). HLB ultimately is fatal to susceptible citrus trees, so early detection and removal of infected trees is important for disease management. Unfortunately, citrus trees often are asymptomatic for years before the common signs of HLB, including yellowing and mottling of leaf veins and misshapen green-colored fruit, are noticeable (da Graça, 1991). Utilization of disease-free planting materials is recommended for new area planting and replacement of infected plants in existing orchards (Ibrahim et al., 2003). Utilization of disease-free planting materials will delay the onset of disease in the field provided a proper management of vector population is practiced to arrest any incoming disease inoculum. Hence, efficient and cheap detection methods need to be developed to detect disease presence in the field and to index planting materials prior to their distribution.

The existing method of *Liberibacter asiaticus* detection involving electron microscopy which uses serial sections and stereomicrographing, inoculation, immunofluorescence, dot-blot hybridization, staining technique using gentisic acid and PCR (Garnier and Bove, 1993; Jagoueix et al., 1996). Currently, PCR is used to detect infection and for indexing planting materials in certain nurseries in Malaysia. Even though the technique is highly efficient but it is laborious and expensive making large scale adoption of the technique not practical.

Immunoassay using SPR is more sensitive and can be used to index large volume of test materials. Antibody against *Ca.* L. asiaticus outer membrane protein (Omp) has been produced and the detection limit of 0.05 mg/mL Omp has been determined in previous research which is ten times lower and more sensitive than ELISA method. Therefore, SPR is developed to be another alternative for *Ca.* L .asiaticus detection in citrus plants.

Materials and Methods

Sample preparation

All samples were obtained from MARDI station, Jerangau (Terengganu, Malaysia). Total proteins were extracted from *Ca*. L. asiaticus infected citrus midribs and healthy citrus midribs using FOCUS^{TM}-Plant proteome extraction kit from GBioscience.

Immobilization of antibody

The exposed gold surface of Autolab sensor was incubated in a solution of 11 mg 11-MUA in 50mL ethanol to get a thiol layer. The gold surface was then stabilized by injecting acetate buffer (pH 4.5) about 30 min until the sensor response became stable. The running buffers were maintained at 50 µL/min throughout the stabilization and immobilization process. When the gold surface has been stabilized, 75 µL of 400 mM EDC and 100 mM NHS were injected onto the sensor surface for 5 min.

After that, 75 µL of Omp polyclonal antibody was injected to create a layer of *Ca*. L. asiaticus binding site. Finally, 75 µL of 1M ethanolamine solution introduced to block the non-specific binding sites on the sensor surface.

Detection of Ca. L. asiaticus in infected citrus plant

With the functionalized Autolab biosensor, total protein samples from infected and uninfected citrus plants were analyzed by injecting 50 µL at 20 µL/s. The response of the Autolab biosensor was represented by the refraction index at which surface plasmon resonance occurred. 1.0 mg/mL of recombinant Omp was loaded as positive control.

Results and Discussion

The citrus industry is interested in detecting the *Ca*. L. asiaticus in citrus plants. To investigate the performance of the SPR biosensor in detecting the infected citrus, the infected plant was selected as a representative sample from Terengganu. Figure 1 shows the sensorgram exhibits the response of protein from infected citrus midribs, healthy citrus midribs and recombinant Omp as positive control. Specific sensor responses were determined to be 345 and 280 millidegrees for 1.0 mg/mL of Omp and infected plant. However, there was no angle shift for healthy plant. Therefore, SPR biosensor manages to detect the *Ca*. L. asiaticus in infected citrus midribs.

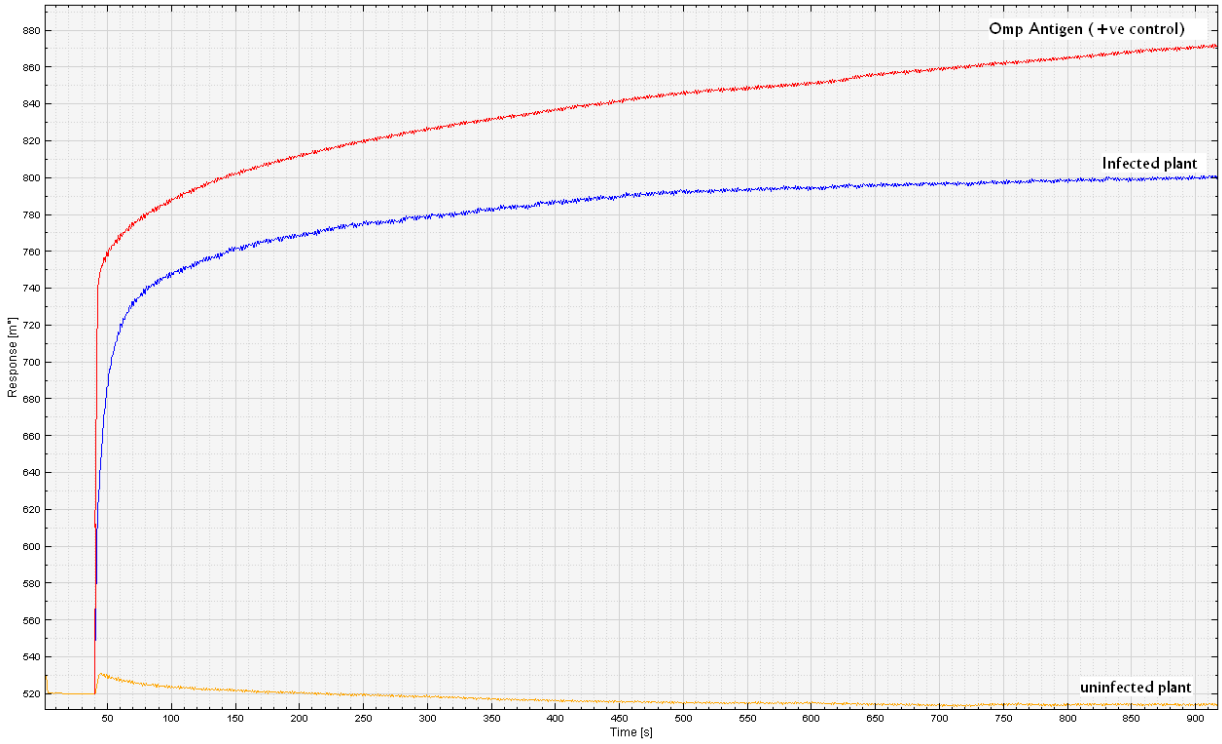

Figure 1. Response of the SPR sensor towards *Ca*. L. asiaticus infected and healthy citrus plants using Omp antigen as positive control.

Conclusions

The SPR sensorgram showed 350 millidegrees angle shift for the positive control (Omp antigen) and 280 millidegrees angle shift for the infected citrus sample but no signal has been detected on the non-infected citrus sample. Therefore, the polyclonal antibody produced from Omp recombinant protein is specific to *Ca*. L. asiaticus and SPR can be a biosensor tool for the detection of Citrus Greening Disease.

Acknowledgements

This work was supported by ScienceFund MOSTI under project code 02-03-08-SF0021. We express gratitude to Dr. Anne Lamers from Autolab for her technical support on SPR biosensor equipment.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Sensitivity Determination of Polyclonal Antibodies against Outer Membrane Protein of *Candidatus* Liberibacter asiaticus using Surface Plasmon Resonance Sensing

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Introduction

Citrus greening disease is one of the most destructive citrus diseases in Africa and Asia, including Malaysia. The pathogen of the disease is a bacterium restricted to the phloem of infected plants and is called a greening organism (GO). These fastidious bacteria causing greening cannot be cultured. They are submicroscopic walled prokaryotes, existing in a sieve tube. The GO has two strains, *Liberobacter africanum* and *Liberobacter asiaticum*. These are transmitted by two psyllid vectors, *Diaphorina citri* and *Trioza erytreae*.

In Malaysia, citrus is one of the major fruit crops although it was more extensively cultivated in the 50s and 60s. Most of the citrus cultivated in Malaysia are susceptible to greening disease and was first confirmed in 1989 (Lim et al., 1989). Pummelo which was long considered to be free from this disease has been found to be naturally infected with the disease first in the East and then in Peninsular Malaysia in 1995. Therefore, greening disease is a major causal factor of the decline in the citrus acreage (Teo, 1995). Utilization of disease-free planting materials is recommended for new area planting and replacement of infected plants in existing orchards (Ibrahim et al., 2003). Utilization of disease-free planting materials will delay the onset of disease in the field provided a proper management of vector population is practiced to arrest any incoming disease inoculum. Hence, efficient and cheap detection methods need to be developed to detect the presence of the disease in the field and to index planting materials prior to their distribution.

The existing method of *Liberibacter asiaticus* detection involving electron microscopy which is using serial sections and stereomicrographing, inoculation, immunofluorescence, dot-blot hybridization, staining technique using gentisic acid and PCR (Garnier and Bove, 1993; Jagoueix et al., 1996). Currently, PCR is used to detect infection and for indexing planting materials in certain nurseries in Malaysia. Even though the technique is highly efficient but it is laborious and expensive thus making large scale adoption of the technique not practical.

Immunoassay is relatively cheaper and can be used to index large volume of test materials. However, immunoassay requires antibodies against the pathogen. The greening causing organism, *Ca.* L. asiaticus is restricted to the phloem of infected plants and could not be cultured, making extracting this antigen from plant samples difficult. Hence, we believe that the use of recombinant protein of the pathogen should be explored to facilitate developing antibody-based detection of the pathogen using ELISA and SPR.

Materials and Methods

Immobilization of antibody

The exposed gold surface of the Autolab sensor was incubated in a solution of 11 mg 11-MUA in 50 ml ethanol to get a thiol layer. The gold surface was then stabilized by injecting acetate buffer (pH 4.5) about 30 min until the sensor response became stable. The running buffers were maintained at 50 µL/min throughout the stabilization and immobilization process. When the gold surface has been stabilized, 75 µL of 400 mM EDC and 100 mM NHS were injected onto the sensor surface for 5 min. After that, 75 µL of Omp polyclonal antibody was injected to create a layer of *Ca.* L. asiaticus binding

site. Finally, 75 µL of 1M ethanolamine solution introduced to block the non-specific binding sites on the sensor surface.

Sample interaction

With the functionalized Autolab biosensor, consecutive measurements were performed using serially diluted recombinant Omp protein in PBS. Each sample was analyzed by injecting 50 µL at 16.7 µL/s. The response of the Autolab biosensor was represented by the refraction index at which surface plasmon resonance occurred. To normalize the response, the baseline refractive index, which was measured with PBS only, was subtracted from the responses.

Results and Discussion

The immobilization process of the antibody showed the response of a MUA-coated SPR sensor to successive EDC/NHS activation, anti-Omp immobilization (1 mg/mL) and ethanolamine deactivation (Figure 1). The binding of anti-Omp causes about a 700-millidegree angle shift, and confirmed successful immobilization of the antibody. The sensorgram of the developed SPR sensor with various concentrations of Omp in PBS buffer showed a rapid increase in the signal (Figure 2). This increase is associated with the specific binding of Omp to anti-Omp molecules on the sensor surface. Specific sensor responses were determined to be 45, 105, 160, 260, 295 and 305 millidegrees for Omp concentrations of 0.05, 0.08, 0.3, 0.5, 0.8 and 1.0 mg/mL, respectively. The detection limit of the sensor is as low as 0.05 mg/mL of Omp.

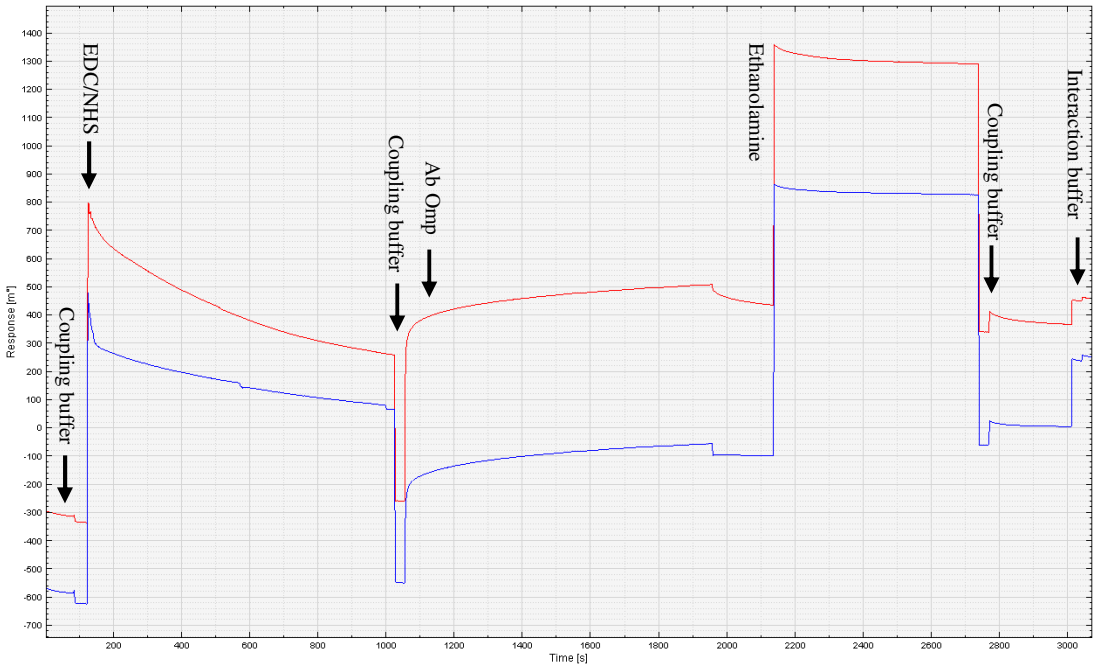

Figure 1. Immobilization process of a capture antibody layer.

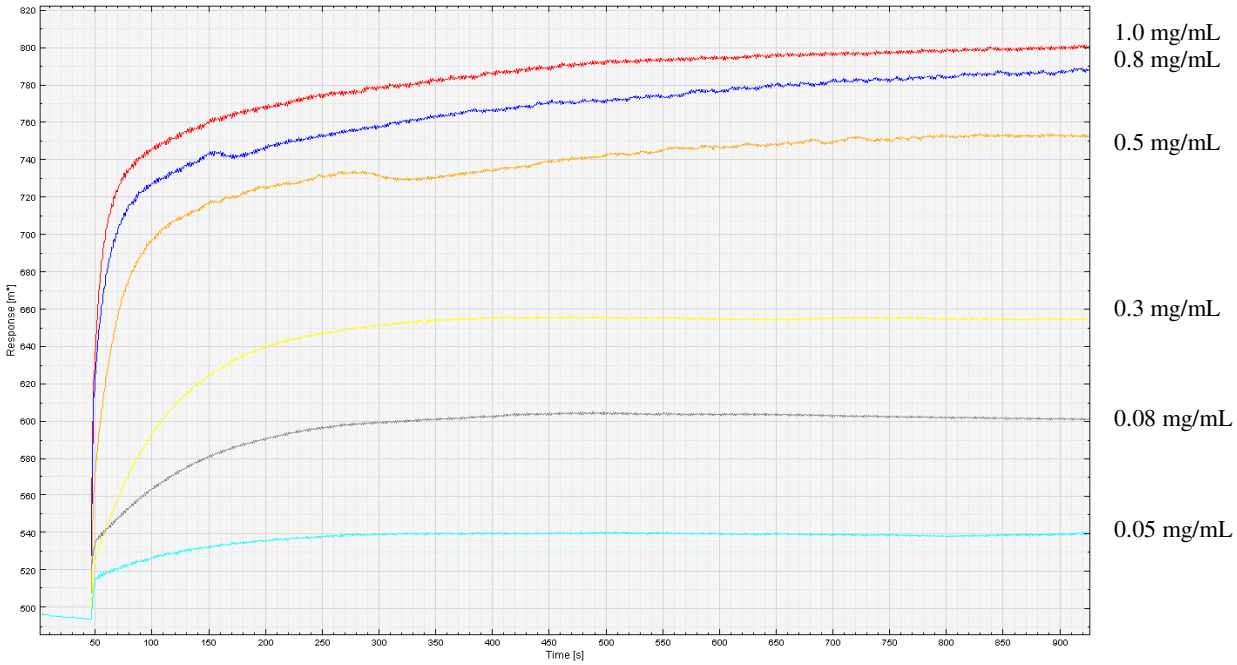

Figure 2. Response of the biosensor to various concentrations of recombinant Omp protein in PBS.

Conclusions

Six serial dilutions of Omp recombinant protein (1 mg/mL, 0.8 mg/mL, 0.5 mg/mL, 0.3 mg/mL, 0.08 mg/mL and 0.05 mg/mL) have been used as ligand to react with the polyclonal antibody. The sensitivity of the produced polyclonal antibody using SPR can detect as low as 0.05 mg/mL Omp antigen. This result can be used in our subsequent studies to do field sample testing using SPR biosensor.

Acknowledgements

This work was supported by ScienceFund MOSTI under project code 02-03-08-SF0021. We express gratitude to Dr. Anne Lamers from Autolab for her technical support on SPR biosensor equipment.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Polyclonal Antibody Purification using Protein A Affinity Chromatography for Detecting *Ganoderma* BSR

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Introduction

Basal stem rot (BSR) is among the major disease infecting many economically important crops such as coconut, rubber and oil palm (Ho and Nawawi, 1985). BSR disease is widely caused by the bracket fungus, *Ganoderma boninense (Ganoderma* BSR*).* The early stage of infection is usually at the underground roots, thus detection of early disease onset is not possible. Infection of roots and stems results in plugging of the cells with mycelium, gums and other materials and collapse and disintegration of cell walls and cells forming gaps and cavities in the tissues. The emergence of fruiting bodies on trunks usually indicates already extensive internal tissue decay and any form of disease control applied at this stage would be futile.

Therefore, the development of a simple, inexpensive and accurate enzyme immunoassay screening method for detecting *Ganoderma* BSR in palms is highly desirable in decision making for appropriate control of *Ganoderma* BSR (Arifin et al., 1989). In this work we have purified polyclonal antibody anti-hyphae using protein A affinity chromatography column for detecting *Ganoderma* BSR using indirect ELISA.

Methodology

Production of polyclonal antibody

New Zealand white rabbits were injected sub-cutaneously with an emulsion consisting 1.0 mg of immunogen (protein from hyphae) dissolved in 0.5 mL of PBS and an equal volume of Freund's complete adjuvant. The injections were repeated three times weekly after the initial injection, substituting Freund's incomplete adjuvant for complete adjuvant. A booster injection was given one month after the initial injection and was repeated at monthly intervals thereafter. The rabbits were bled for antibody titer determinations two weeks after each boost. Antisera for *Ganoderma* (hyphae) direct ELISA development were prepared from a single bleed in each case.

Purification of polyclonal antibody against hyphea (H28 @ EG41) using protein A sepharose affinity column

Anti-hyphea (1:10) antisera were precipitated with saturated ammonium sulphate, centrifuged, dialyzed and eluted through a protein A sepharose affinity column chromatography. Fractions giving the highest absorbance reading at OD_{280nm} were collected and freeze dried (IgG stock). IgG titers for this antibody eluted from Protein A sepharose column was determined by indirect ELISA method. This antibody was also used in detecting of *Ganoderma* BSR in infected oil palms.

Indirect ELISA method for sample determination

Microtitre plates were coated with samples by adding 200 µL of the infected oil palm extract and incubated overnight at 4°C. The plate was emptied and washed three times with PBS-tween 20 (200 µL/well). Unoccupied sites on the polystyrene well surface were blocked by treating with 0.1% (w/v) Bovine Serum Albumin (BSA) in PBS (200 µL/well) for 30 min at room temperature. Then, the plate was again emptied and washed three times with PBS-Tween 20 (200 µL/well). Purified antibodies

(antibodies against hyphae from protein A column) at 0.01 mg/ml concentration were added and the plate was incubated for 2 hours at room temperature. The plate was emptied and washed three times with PBS-Tween 20 (200 µL/well). Goat anti-rabbit IgG secondary antibody conjugated with alkaline phosphatase was diluted in PBS (1:1000) and was added (200 µL/well) to the plate. The plate was incubated for 30 min at room temperature. The plate was emptied and washed three times with PBS-Tween 20 (200 µL/well). PNPP (p-Nitrophenyl Phosphate, Disodium salt) substrate was added to the plate (200 µL/well). The reaction was stopped after 30 min by adding 50 µL/well of 3M H_2SO_4 and the absorbance at 405nm was measured using Dynex Microplate Reader. The intensity of the color was proportional to the amount of antigen present.

Results and Discussion

Purification of polyclonal antibody against hyphea (H28 @ EG41) Using protein A sepharose affinity Column

Anti-hyphae antibody with high yields, purity and specificity of antibody was obtained using Protein A affinity chromatography. Sensitivity of anti-hyphae antibody was improved using a Protein A column when compared with DEAE 52 ion-exchange column. The titers for the antibody eluted from DEAE 52 ion-exchange (Figure 1) and Protein A affinity (Figure 2) columns were $1{:}10^6$ and $1{:}10^{10}$ respectively. Antibody eluted from protein A showed high antibody titer but lower yield. At working dilution concentration (0.001 mg/mL), the absorbance reading at 405nm for the antibody eluted from Protein A column showed higher absorbance (1.2) when compared with that eluted from DEAE column (1.0). The increase in absorbance reading of the antibody eluted from Protein A column showed higher purity antibody. Therefore, IgG protein (anti-hyphea antibody) binds specifically to Protein A column and thus, increased the sensitivity of anti-hyphae by excluding other contaminating proteins, while the DEAE column binding is only based on protein charge, thus a possibility of protein contamination was potentially high and the antibody sensitivity decreased. Therefore, this new batch of improved antibody (anti-hyphae from Protein A column) was used in the second analysis of detection of *Ganoderma* BSR infection in oil palm plants by indirect ELISA method. The polyclonal antibody against hyphae using the ELISA method showed promising detection of *Ganoderma* BSR infection in oil palm samples from various locations.

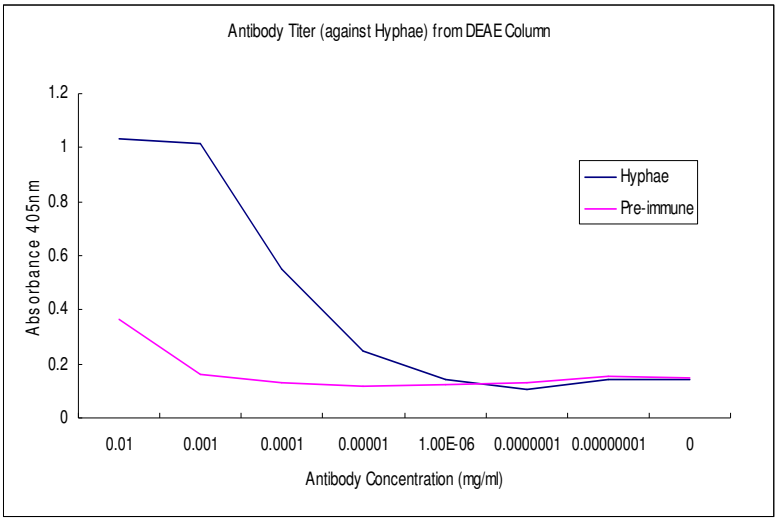

Figure 1. Titer of antibody purified using DEAE 52 column chromatography

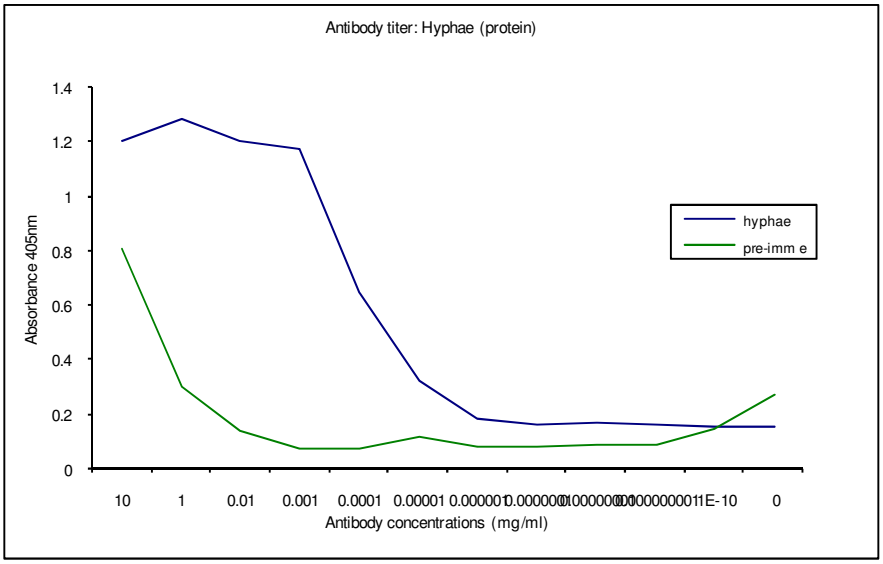

Figure 2. Titer of antibody purified using Protein A affinity chromatography

Conclusions

Polyclonal antibody against *Ganoderma* BSR hyphae with high yield, purity and specificity has been obtained using Protein A affinity chromatography. The sensitivity of polyclonal antibody via Protein A column chromatography was increased. Based on the outcome of the above work, a prototype ELISA kit for the early detection of *Ganoderma* BSR infection in oil palm is being developed. Validation of the results obtained from ELISA will require comparison with other method such as PCR.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Ultrastructural Investigation of *Azadirachta excelsa* Seedlings Infected by *Rigidoporus microporus* White Root Disease

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Introduction

Rigidoporus microporus is considered the most destructive pathogen of *Hevea brasiliensis* worldwide (Geiger et al., 1986). This pathogen has also been reported to kill fruit trees around the world (Wood and Lass, 1985; Ann et al., 2002). Like other white rot fungi that secrete a wide range of hydrolytic and oxidative enzymes (Blanchette. 1991), it is capable of degrading wood actively.

Recently, *R. microporus* was also reported as a major cause of tree mortality in forest plantations in Peninsular Malaysia (Mohd Farid et al., 2005). The pathogen produces similar below and above-ground symptoms on forest trees as on *H. brasiliensis*. Until now, the infection processes associated with this pathogen in forest tree species remain unknown. Therefore, the present study was undertaken to gain further insight into the role of *R. microporus* in the development of white root disease on selected forest plantation tree species. Seedlings of *Azadirachta excelsa* (sentang) were selected as the host for investigation of the infection process.

Materials and Methods

Preparation of seedlings

Twenty healthy *A. excelsa* seedlings of approximately five months-old were used. They were grown in polybags measuring 30 cm height x 20 cm diameter filled with a well mixed soil media (forest top soil, sand and peat at 3:2:1 ratio). Prior to inoculation, the uniformly sized seedlings were divided equally into two treatment blocks where each treatment block consisted of ten seedlings inoculated with *R. microporus* or uninoculated control, respectively.

Preparation of inoculum blocks

The pathogen inoculum was prepared using a well colonized rubber wood block measuring approximately 5 cm long x 1 cm diameter according to the method described by Lee and Sikin (1999). *Rigidoporus microporus* (FRIM 590) which was isolated from *H. brasiliensis* was used as the inoculum. The inoculated blocks were incubated in the dark at room temperature (28±2^{o}C) for a month until they were fully colonized by the pathogen. For inoculation, a wood block was placed in contact with the taproot of the seedling approximately 5 cm below the soil surface. For the control, seedlings were inoculated with uninoculated blocks. During inoculation care was taken that roots of all the *A. excelsa* seedlings were not injured. The seedlings were watered once daily in the morning. Appearance of above ground-symptoms was monitored weekly for five weeks.

Harvesting and microscopic observations

Two seedlings with foliar symptoms were randomly harvested from each treatment each week over five weeks to study the colonization process. The roots were washed thoroughly and cut into approximately 1 cm^3 fragments. The infection process of roots inoculated with the pathogen was studied histologically under a transmitted light microscope and transmission electron microscopy (TEM).

Results

Histology of A. excelsa seedling root disease infection by R. microporus

One week after inoculation a thin layer of fungal mycelia was observed growing on the root surface of the seedling (Figure 1). A cross-section of the root revealed that the hyphae had already infected the epidermis cells. However, at this stage, hyphae were not observed in the cortex and parenchyma cells. Three weeks after inoculation, masses of fungal mycelia had grown and adhered to the root surface of the seedling. Mycelia were also observed to fully colonize some areas in the epidermis layer and some of the hyphae had begun to advance into the cortex cells. Closer observation revealed that the hyphae had invaded the root cells, especially in the epidermis (Figure 2).

Five weeks after inoculation, colonization of the epidermis cells by the pathogen was even more extensive, causing some of the heavily colonized periderm and epidermis cells to rupture (Figure 3a). At some penetration sites, the hyphae had begun to penetrate into the parenchyma cells. By this time, fungal hyphae were also observed to extensively penetrate and colonize the cortex cells (Figure 3b). However, the fungal hyphae did not cause any cell disruption in the cortical cells.

Ultrastructural observations on hyphal penetration and cell wall degradation of A. excelsa seedlings inoculated with R. microporus

Root tissues of healthy uninoculated control seedlings were observed to be free from fungal infection throughout the study period. TEM observations revealed that the cells of healthy plants have clean, thick and smooth walls without any deformation (Figure 4a). In addition, no fungal hyphae or extracellular materials were observed in the cells. In contrast, TEM observations of *R. microporus* inoculated *A. excelsa* roots five weeks after inoculation revealed the presence of fungal hyphae in the root cells. These hyphae penetrated the cells intercellularly and intracellularly (Figure 4b). In the cells, two types of hyphae were observed, primary hyphae (thick-walled hyphae) and secondary hyphae (thin-walled hyphae). The two types of hyphae are shown in Figures 5a and 5b. During penetration, the thick-walled primary hyphae pierces the cell wall by mechanical force and the piercing tip of the hyphae constricts while forcing its way through the cell wall (Figure 5a). Once penetrating through the wall, the hypha enlarges to form it normal dimension. However, in between the cell walls, the hypha was observed to be sharply constricted due to invagination of the cell walls at the edges of the penetration site (Figure 5b).

In contrast to penetration by thick-walled hyphae, penetration by thin-walled hyphae appears to be via enzymatic action. This is shown by localized degradation of cell wall and also the presence of extracellular materials in the cells (Figure 6a). The present study also showed that thick-walled hyphae were present in the thinned cell walls (Figure 6b). In more advanced stages, cell walls were extensively degraded with large amounts of extracellular materials present in front of thin-walled hyphae (Figure 7).

Discussion

Results from this histopathological study have provided some information on the mechanism of infection of *A. excelsa* seedlings by *R. microporus*. During pathogenesis, three stages were involved, the penetration and colonization of hyphae into the host cells, and subsequent degradation of host cell walls. Cross-sections of the infected root tissues provided evidence that *R. microporus* was capable of both penetrating and colonizing *A. excelsa* roots within a short period of time. The pathogen could penetrate the host root cells within one week after inoculation. This shows that the infection process was rapid and this could be due to the fast growth of the white fungal rhizormorphs on the root surface as observed in this study. This was accompanied by the rapid development of above-ground symptoms on the inoculated seedlings.

Although the development of crown symptoms was clearly observed due to the fungal infection of the root, the mode of entry of the pathogen into the root was unclear. The white rhizormorphs on the root surface were easily detached especially during embedding of the root sections. Subsequently, preparation of root sections with attached rhizomorphs was difficult and a clear observation of the means of external hyphal penetration into the root system could not be observed. However, we found that this pathogen could enter the root system of the inoculated seedlings despite the lack of injury to the roots. Thus, this suggests that the pathogen is either able to enter the root system through natural openings such as lenticles or via direct penetration. These results are similar to those previously reported for *A. excelsa* seedlings inoculated with *R. vinctus, P. noxius* and *Phellinus* sp. (Mohd Farid et al., 2001). Further studies should be conducted to confirm the mode of infection of *R. microporus*.

The thick-walled and thin-walled hyphae found in root cells of *R. microporus* inoculated *A. excelsa* seedlings were similar to those observed by Nicole and Benhamou (1991) on *H. brasiliensis* infected by the same fungus. According to Mendgen et al. (1996) the thin-walled hyphae often branched from the thick-walled primary hyphae. This suggests that the thin-walled hyphae observed in the present study could have developed from the thick-walled hyphae as well. In addition, cytochemical studies have shown that a considerable decrease in the wall thickness of a hypha occurs when hyphae of *R. microporus* become infecting structures (Nicole et al., 1987; Nicole and Benhamou, 1991). However, further histological studies are required to clearly reveal the connection between these hyphae.

The thick-walled hyphae present in the root cells of *A. excelsa* seedlings inoculated with *R. microporus* were often associated with cell wall penetration. According to Nicole et al. (1986) these thick-walled hyphae appeared to be specialized for root penetration. The constriction of the thick walled hyphae observed in this study indicated that thick-walled hyphae penetrated the cell wall by mechanical force rather than by enzymatic action. Other studies have also reported that penetration of cell wall by this kind of hyphae involve a combination of mechanical and enzymatic actions (Nicole et al., 1982; 1986; 1987; Peries and Irugalbandara, 1973). However, the action of enzymes released during the penetration process seemed to be limited only to the area around the thick-walled hyphae as indicated by degradation of cell wall near the hyphae. This is in agreement with a report on penetration of *H. brasiliensis* cell walls by *R. microporus* (Nicole et al., 1986).

The thin-walled hyphae developed by *R. microporus* were often associated with cell colonization and degradation. In fact, degradation of cell walls was often found some distance away from the thin-walled hyphae. This suggests that degradation of cell walls was mainly due to enzymatic action and that the enzymes are able to diffuse in advance of the hyphae. Enzymatic action in degrading cell walls was also reported by several researchers (Nicole et al., 1985; 1987; 1992; and Nicole and Benhamou, 1991) while working with *R. microporus.* Although no study was conducted *in-vivo* to determine the types of enzymes involved in cell wall degradation, previous studies revealed that cell wall degradation by *R. microporus* was mainly concerned with the enzyme laccases (Galliano et al., 1988; Nicole et al., 1982; 1992; Nicole and Benhamou, 1991). These reports suggest that *R. microporus* used in the present study may produce similar enzyme to degrade the root cell walls.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

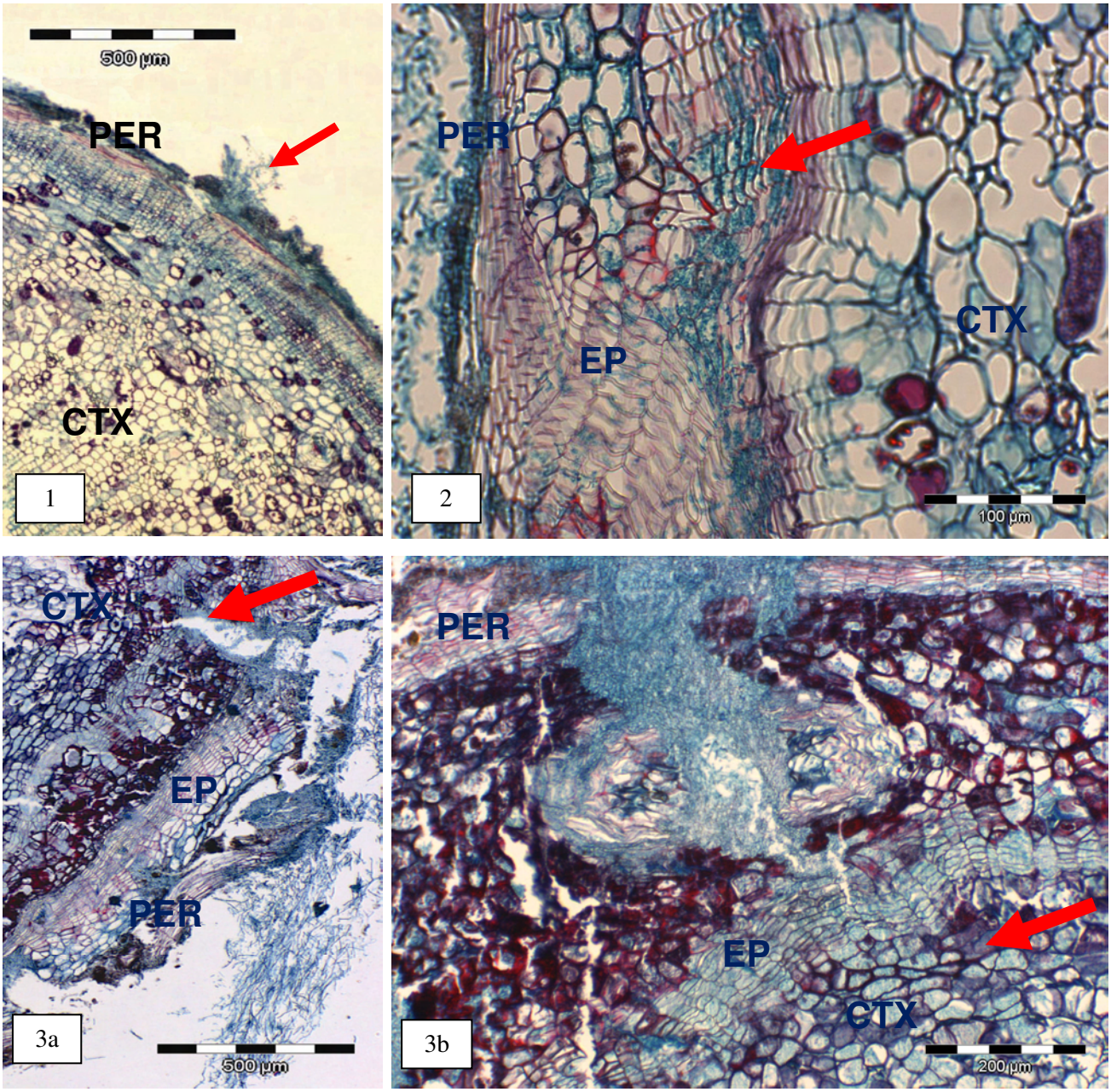

Figure 1. Cross-section of *A. excelsa* seedling root inoculated with *R. microporus* showing a thin layer of fungal hyphae (arrows) growing on the root surface 1 week after inoculation (10x mag.).

Figure 2. Cross-section of root showing masses of mycelia in epidermis cell (arrow) (40x mag.).

Figure 3a. Thin root section 5 weeks after inoculation showing *R. microporus* hyphae extensively colonizing and rupturing the epidermis cells to form an opening (arrow) (10x mag.).

Figure 3b. Fungal hyphae colonize cortex cells (arrow) indicating advanced stages of root disease infection by the pathogen (40x mag.). P=Parenchyma; CTX=Cortex; EP=Epidermis; PER=Periderm

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

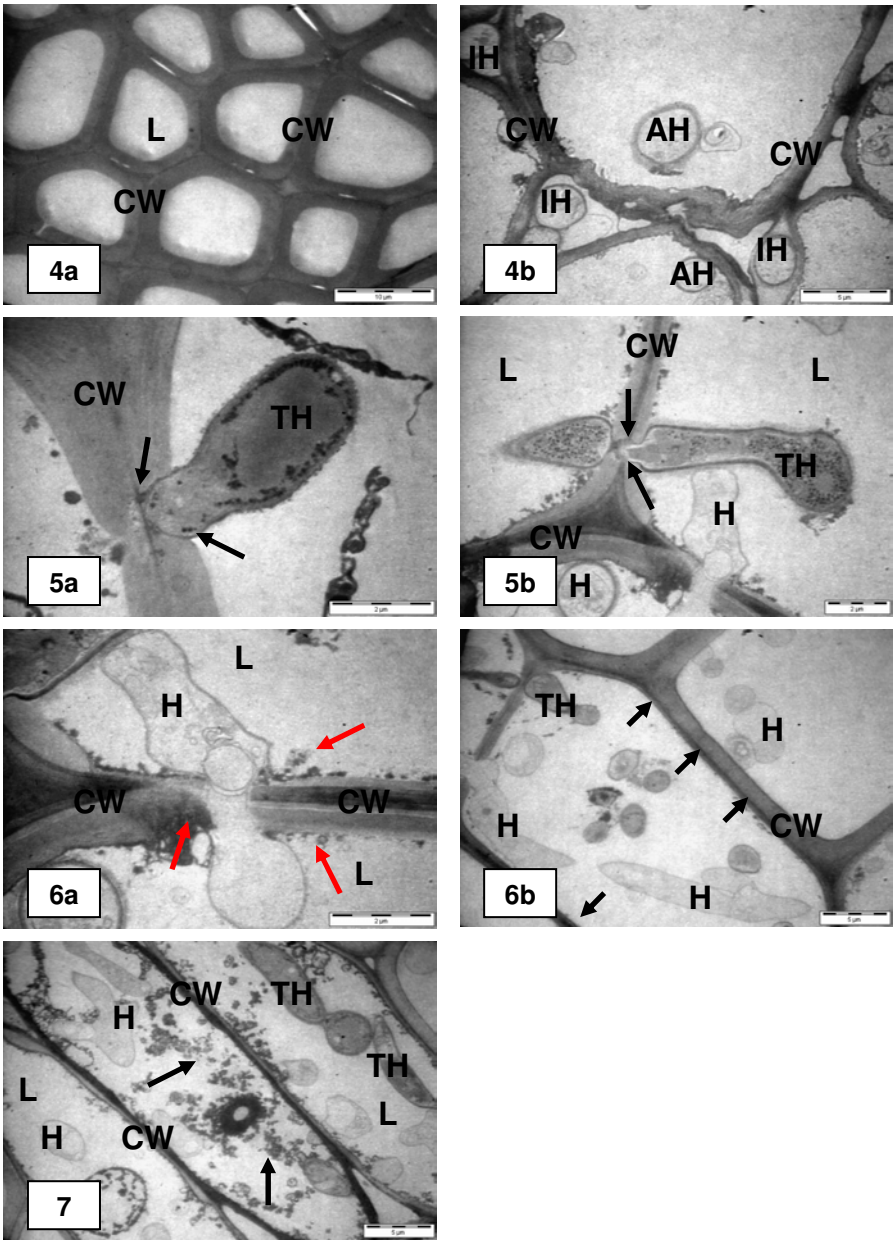

Figure 4a. TEM observation of root section of uninoculated *A. excelsa* seedling showing intact cells free from fungal infection at week 5.

Figure 4b. Intercellular (IH) and intracellular (AH) hyphae in *A. excelsa* root during active penetration by *R. microporus*.

Figure 5a. Cell wall penetration by a thick-walled primary hypha showing constriction at the tip (arrow) while forcing its way into the cell wall.

Figure 5b. Cell wall penetration showing thick-walled hypha sharply constricted (arrows) during penetration through the wall but resuming normal dimension on emergence into the lumen of the neighbouring cell.

Figure 6a. Localized cell wall degradation around thin-walled hypha (black arrows) of *R. microporus* during intercellular hypha penetration; note the presence of extracellular materials (red arrows) in the root cells indicating an enzymatic alteration of the wall.

Figure 6b. Thinning of cell walls (arrows) occur in cell heavily colonized by hyphae of *R. microporus*.

Figure 7. Advanced stage of root infection by *R. microporus* showing extensive cell wall degradation, presence of numerous hyphae and abundant extracellular materials (arrows) present within the host cells. IH= Intercellular; AH= Intracellular; CW= Cell wall; L= Lumen

Conclusions

This study provided a better insight into the mechanism of *R. microporus* white root disease infection of *A. excelsa* seedlings and the role of the fungal hyphae in pathogenesis. Based on root symptoms observation, the presence of white fungal strands on root surface was the first sign of white root disease infection. This observation suggests that the infection process starts with the development of white fungal strands on the root surface. The pathogen can infect the host root system within a week after inoculation although no wounds were made. The fungal hyphae penetrates the root epidermis, colonizing the cells and subsequently breaks down the cell wall. Based on ultrastructural observations, two types of hyphae, namely, thick-walled and thin-wall hyphae were observed in root cells of the infected seedlings. The thick-walled hyphae were mainly involved in initial penetration of cell walls by mechanical force, whereas the thin-walled hyphae, were mainly involved in degradation of cell walls through their enzymatic action. Overall, the development of above-ground symptoms on the inoculated *A. excelsa* seedlings was mainly due to the destruction of root cells wall caused by *R. microporus* white root disease.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Ultrastructures of *Candidatus* Liberibacter asiaticus and its Damage in Huanglongbing (HLB) Infected Citrus

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Introduction

Huanglongbing (HLB) is a destructive disease of citrus plants. HLB or citrus greening disease is caused by a sieve tube restricted bacterium, named liberibacters, which are transmitted by citrus psyllid insect vectors *Diaphorina citri* in Asia and America, and *Trioza erytreae* in Africa (Teixeira et al., 2005a; Halbert and Majunath, 2004). HLB has been threatening citrus industry in the world. America used to be free from HLB, but in March 2004 and August 2005, symptoms of the disease were recognized and detected by PCR, respectively in the State of São Paulo, Brasil, and in Florida, USA, two of the largest citrus growing regions in the world (Texeira et al., 2005a). HLB has destroyed an estimated 60 million trees in Africa and Asia (Timmer et al., 2003). Typical symptoms of HLB disease on citrus leaves of infected trees include reduced size, yellowing, blotchy mottle or variegated type of chlorosis with small upright leaves, followed by leaf drop and twig dieback at later stages. Some disease symptoms caused by HLB are similar to the symptoms caused by nutritional deficiencies (Bové et al., 1974).

HLB is an uncultured phloem- limited bacterium that was first characterized in 1994 with the 16S rDNA sequence and shown to be a new genus in the α-Proteobacteria (Jagoueix et al., 1994). PCR method is as specific and at least as sensitive for the detection of different Liberibacter species (Hocquellet et al., 1999). Conventional PCR based on the amplification of 1160 bp fragments of liberibacter 16S rDNA (Jagoueix et al., 1996) by primer pair OI1/OI2c is able to amplify the rDNA of both liberibacter species. Lately, detection of *Ca.* Liberibacter spp. is based mainly on conventional polymerase chain reaction (PCR) and quantitative real-time PCR with species-specific primers developed based on 16S rDNA and β-operon. *Candidatus* Liberibacter asiaticus can also be detected with an electron microscope (Garnier and Bove, 1983). Elongated sinuous rod like structures, 0.15-0.25 μm in diameter and length were detected. These can be seen by electron microscopy in the sieve tubes of infected trees (Moll and Martin, 1973). The aim of this study was to confirm the presence of *Candidatus* Liberibacter asiaticus pathogen using PCR and transmission electron microscopy (TEM), to measure the HLB bacteria size and to identify the cell wall modifications of the phloem.

Materials and Methods

Sample collection and HLB detection using conventional PCR

Samples were collected in an area of Kuala Berang, Terengganu. The state of Terengganu overlooks the South China Sea on the east side of Malaysia. It has a strong tropical monsoon climate, with relatively uniform temperature within the 21 °C to 32 °C range. The weather in January to April is dry and warm, with humidity in the lowlands being consistently high, between 82-86 percent annually. Terengganu's average rainfall is 2,032 mm to 2540 mm per year; with most rain falling between November to January. Samples were collected in an orchard of *Citrus reticulata* cv. Limau Madu which was cultivated in 2000. Citrus leaves with typical symptom of HLB were collected. Typical symptom of HLB is blotchy mottling and midrib yellowing. DNA was extracted from the midribs of leaves by modified method which was described by (Hung et al., 2000). DNA extracted from the citrus tissues following the method by grinding up the tissue in a mortar and pestle using liquid nitrogen until no large pieces of tissue remained. DNA was extracted from HLB-infected tissue using

cetyl trimethyl ammonium bromide (CTAB). The pellets were washed with 70% ethanol, dried and resuspended in 100 µl TE buffer. Then PCR was performed using 25 µl of reaction mixture containing 20mM Tris-HCl (pH 8.0), 50 mM KCl, 4 mM $MgCl_2$, 0.2 mM of dATP, dTTP, dCTP and dGTP, 50 ng forward primer, 50 ng reverse primer, 0.75 units of Taq DNA polymerase and 200ng genomic DNA. The thermal cycle condition was: one cycle at 95 °C for 2 min. 35 cycles at 95 °C for 40 sec, 60 °C for 1 min and 72 °C for 1 min followed by a 72 °C extension for 10 min. Specific primer pair, composed of the forward primer of OI1 (5'-GCG CGT ATG CAA TAC GAG CGG CA-3') and reverse primer of OI2c (5'-GCC TCG CGA CTT CGC AAC CCA T-3'), was used to amplify the 16S ribosomal DNA fragment. Amplification of DNA was determined by electrophoresis on 1.2% agarose gel for about 30-45 min and visualized by ethidium bromide staining.

HLB detection, Candidatus Liberibacter asiaticus size measurement and cell modification of HLB infected citrus using transmission electron microscopy (TEM)

Terminal shoots of *C. reticulata* trees with typical symptoms (blotchy mottled and midrib yellowing) were collected and washed. Midrib were taken from the leaves and chopped into 2-5 mm pieces, similar to the TEM techniques described by (Aubert, 1990). The samples were fixed into 5% glutaraldehyde buffered with 0.1 M phosphate buffer saline (PBS) pH 7.4. The samples were then vacuumed in an oven at 60°C for two days. After that, the samples were washed with 0.1 M sodium cacodylate (SC) buffer three times in changes of 30 min, respectively. Subsequently, samples were post-fixed with 1% osmium tetroxide for one day at 4 °C. The samples were then washed again three times with SC buffer. After dehydration process with a series of ethanol concentrations (35, 50, 75, 95 and 100%) for one hr, respectively, the samples were infiltrated and embedded in Epon 812. After polymerization, ultra thin sectioning (60-90 nm) was carried out by using diamond knife and ultra microtome. Golden sections were double stained with uranyl acetate and lead citrate for 15 and 30 min, respectively. The samples were then examined under transmission electron microscope (30-100 Kv magnification) for the detection and identification of *Candidatus* liberibacter asiaticus. Size of 9 *Candidatus* Liberibacter asiaticus isolates was measured.

Results and Discussion

PCR confirmation of HLB diagnosis

For PCR detection of the *Candidatus* Liberibacter asiaticus, blotchy mottled and midrib yellowing leaves were used. One pair primers, f-OI1 and r-OI2c, for amplification of 16S rDNA was used. Line 3 of Figure 1 shows weak band of blotchy mottling symptoms after PCR amplification while line 5-7 shows strong band of midrib yellowing symptoms. It is true to state, as it is sometimes written, that it is difficult to detect liberibacters. In Brazil, most PCR reactions gave negative results, even though leaves with strong symptoms of blotchy mottle were used (Texeira et al., 2005b).Therefore, HLB infection was confirmed by PCR amplification in the typical symptoms. A polymerase chain reaction (PCR) with specific primers OI1/OI2c and A2/J5 were used for detection and it produced specific band of 1160 bp and 703 bp, respectively in a study in Thailand. These were amplified from diseased leaves where no product from healthy citrus plants could be obtained. HLB was detected in Peninsular Malaysia using OI1 and OI2c primers (Ahmad Khirolmazmi et al., 2008). It was also detected in 15 citrus species in this country (Hajivand et al., 2009).

TEM detection and ultrastructures of Candidatus Liberibacter asiaticus

Electron micrographs of sieve tube elements of infected *C. reticulata* leaf at high magnification (30-100 kV) were observed. In Figure 2, two shapes of *Candidatus* Liberibacter asiaticus were present; spherical and rod shape. In 1984, a long rod-shaped gram negative organism from African greening-infected citrus leaf mid-ribs was isolated (Garnier et al., 1984). The ultrastructure of this organism was described as similar to that of the organisms observed in greening-infected citrus, periwinkle, and insect vectors (Ariovich and Garnett, 1984). On a solid medium, it formed small round colonies with predominantly long rod-shaped cells near the edges, but rounder cells in the oldest parts. In this

ISBN: 978-967-344-188-4
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experiment, length and width of the HLB isolate from Terengganu was also measured (Table 1). The length of the bacteria ranged from 594.57 to 1368.16 nm (mean 930.09 nm) and its width ranged from 201.68 to 811.15 nm (mean 410.61 nm). Bové (2006) concluded that: using a transmission electron microscope, observed a "mycoplasma-like organism" in citrus phloem tissue infected with citrus greening disease. The organisms were about 2000 nm long and 100-200 nm in diameter. Similar bodies were soon observed in both vectors of the citrus greening disease, *T. erytreae* (Moll and Martin, 1973) and *D. citri* (Bove, 2006). Results showed abundant bacteria cells damaging the cell wall in sieve tube cell at 30-70 kV magnification (Figure 2). TEM detection of HLB in Asia and African has shown that the number of bacteria in sieve tubes is higher in leaves with strong mottle than in those with mild mottle (Bove, 2006). According to these results, the organisms are seen surrounded by a cell wall (Figure 3).

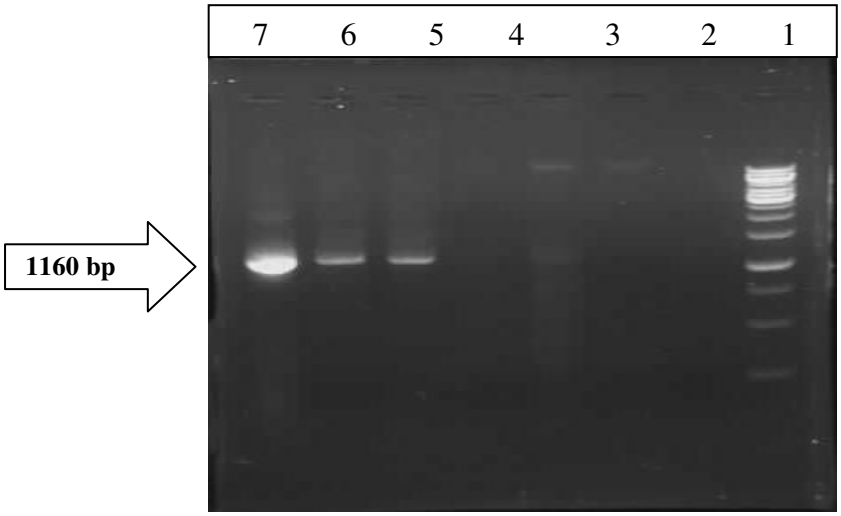

Figure 1. 16s rDNA fragments with molecular weight of 1160 bp were successfully amplified from the infected samples; (M): Marker (100 bp invitogen); (line 1): Water; (line 2): Negative sample; (line 3): Blotchy mottling symptoms; (line 4): Negative sample; (line 5-7): Midrib yellowing symptoms

Table 1. Size of *Candidatus* Liberibacter asiaticus isolates

Number of Bacteria	Length (nm)	Width (nm)
1	879.15	403.70
2	782.55	267.12
3	1155.08	316.09
4	1134.2	225.50
5	803.34	796.84
6	723.67	811.15
7	594.57	201.68
8	1368.16	315.33
9	930.09	358.10
Mean	930.09	410.61

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

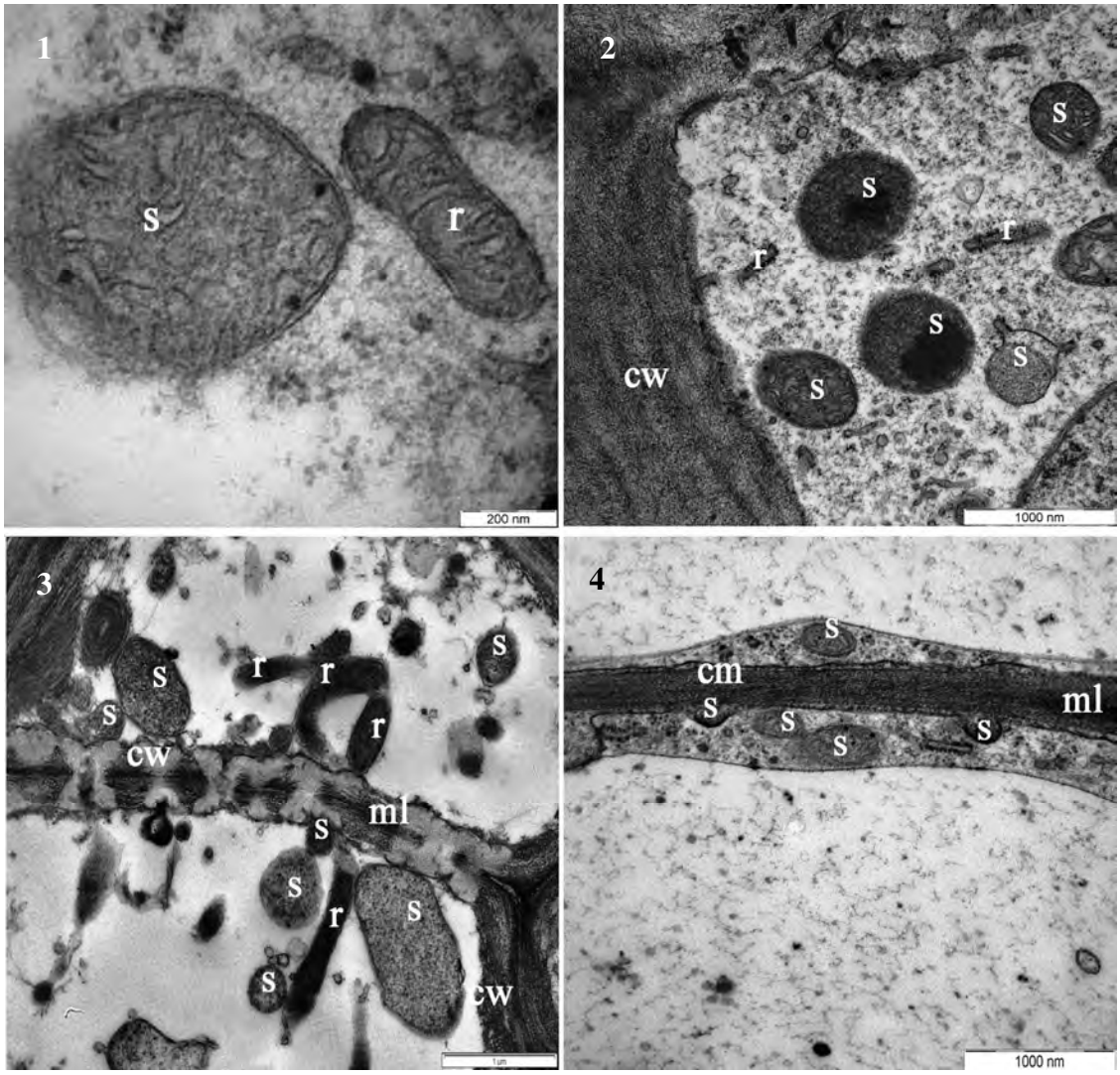

Figure 2. Electron micrographs of the vascular system (1) of *C. reticulata* showing sieve tube cells containing spherical (s) and rod shape bacteria (r) from the infected midrib with blotchy mottling symptoms of HLB disease at high magnification (100 kV). Cross section of sieve tube (2–4) showed abundant bacteria cells damaging the cell wall (cw) and middle lamella (ml) in sieve tube cell (2- mag. 30 kV, 3- and 4- mag. 70 kV).

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

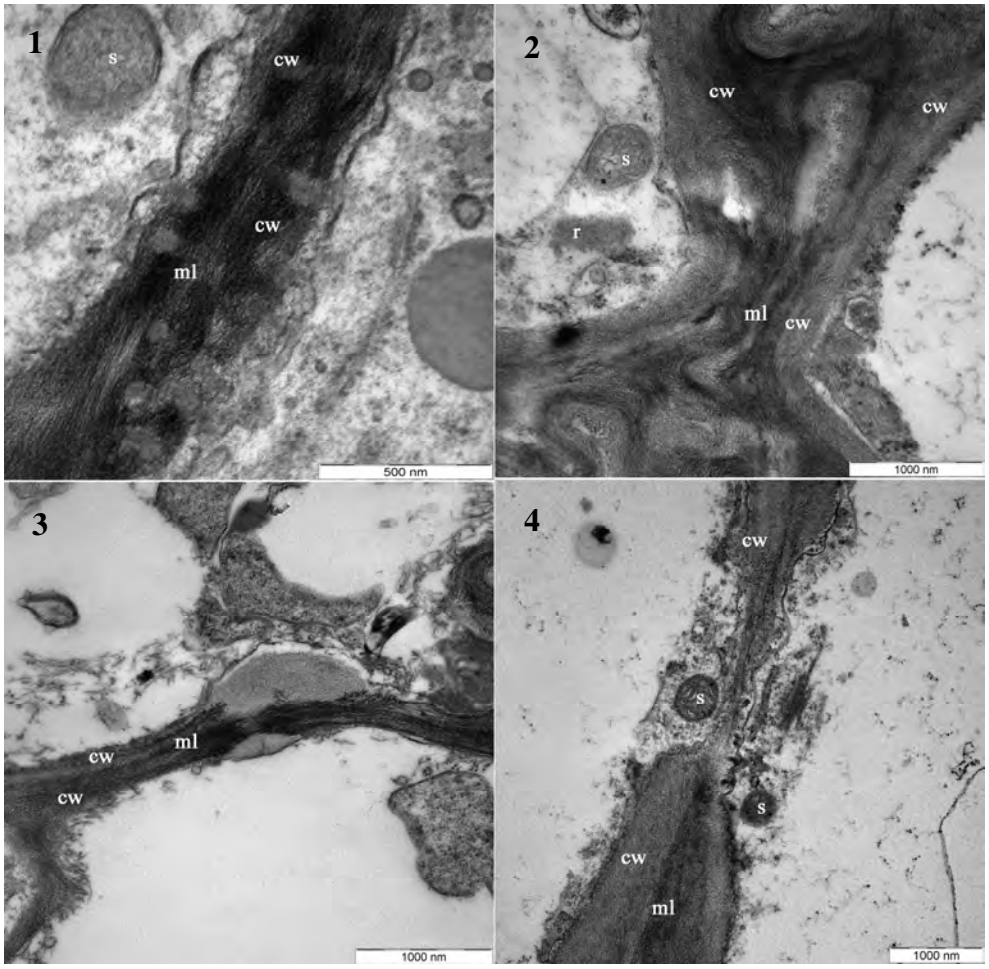

Figure 3. Electron micrographs of the sieve tube of *C. reticulata* leaf; section (1 and 2) showing the fusion confluent of middle lamella (ml) and cell wall (cw) structure (1-mag at 70 kV and 2-mag at 30 kV magnification). Cross section of sieve tube (3 and 4) showing damaged cell wall (cw) and middle lamella (ml) caused by *Candidatus* Liberibacter asiaticus penetrating through the cells (3-mag at 30 kV and 4-mag at 20 kV magnification).

Conclusions

Base on this result it can be concluded that the *Candidatus* Liberibacter asiaticus strain in Terengganu observed were spherical and rod shaped in the sieve tube cells. Damaged caused by this pathogen to the cell wall and cell membranes. Cell wall and cell membranes were irregular in shape and of different thickness. Damage was caused by *Candidatus* Liberibacter asiaticus penetrating through the cell wall and their movement between the cells.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 5

BEST PRACTICES AND CURRENT TECHNIQUES

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Effects of Different Soil Conservation Practices on Soil Chemical Properties in a Sloping Land Oil Palm Plantation

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Introduction

Proper soil conservation practices are needed to maintain and improve soil fertility and oil palm productivity on steep lands. Organic mulches and silt pit are among the reliable conservation practices to reduce surface runoff and improve soil chemical properties which are important in maintaining soil fertility. Empty Fruit Bunches (EFB) of oil palm, Eco- Mat and palm fronds, are organic mulches prevailing in Malaysia. EFB is the major waste of oil palm fresh fruit bunches after oil mill process, and Eco mat is a carpet made from EFB so that its weight is lighter than EFB, and therefore, its transportation and field application are easier. Silt pit is like a trench and helps to reduce the length of slope and trapping running water and sediment, on sloping lands, results in conserving soil nutrients and water.

Both EFB and Eco-Mat are rich in nutrients and organic carbon which can be released during decomposition process, and improve soil physical and chemical properties. The positive effects of EFB on several soil chemical properties have been documented (Kheong, 2008; Lim and Zaharah, 2002; Lim and Zaharah, 2000; Rosenani and Wingkis, 1999; Wingkis, 1998; Ortiz et al., 1992).

Information on the effects of Eco-Mat and silt pit on soil properties is limited. However, a study by Ibrahim (2006) showed that mulch mat increases soil moisture and temperature, plant growth and N, P and K uptake by acacia plant. Soon and Hong (2002) found that silt pit decreased surface runoff and soil loss on a sloping land. In an experiment carried out in India, George et al. (2003) showed that silt pit was effective in conserving soil and N, P and K. Lim also (as cited in Lim et al., 1995) reported 23% reduction in soil loss due to silt pit in an oil palm plantation on sloping lands. From the literature review, it can be inferred that most of the researches were focused on the effects of EFB, Eco-Mat, palm fronds and silt pit on soil properties separately. Therefore, the objective of this experiment was to compare the effects of these conservation practices on several soil chemical properties.

Materials and Methods

A field experiment was conducted at Balau Estate near Semenyih, Selangor in Malaysia with a slope gradient of 6˚ (2˚55′57″ N and 101˚52′56″E). Effects of four conservation practices, EFB, Eco Mat, palm fronds and Silt pit on soil chemical properties of a sloping land cultivated with 8-year old oil palm trees were examined. The experimental layout was a split plot in time and space arranged in a completely randomized block design with three replications. The conservation practices (CP) including EFB, Eco-Mat, silt pit and palm fronds as a control were allocated to the whole plots, soil sampling times (T) and soil depths (D) were considered as sub- and sub-sub plot respectively. The silt pit dimension was 4 × 1 × 0.5 meter in length, width and depth respectively. EFB applied as 1000 kg per plot according to the rate proposed by Chan et al. (1980) and four pieces of 1 × 2 m Eco-Mat having 2 cm thickness were placed on the soil surface between the trees. Soil samples were taken at 0-15, 15-30 and 30-45 cm depths and analyzed for pH, CEC, total C and N, available P, exchangeable K, Ca, and Mg. Soil C:N ratio was also calculated. The data was analyzed by SAS and means separation test was done by LSD.

ISBN: 978-967-344-188-4
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Results and Discussion

The effect of CP×T×D on soil total C, P and Ca, and the CP×T effect on soil CEC, exchangeable K, Mg and C:N ratio was significant (Table 1). Furthermore, in terms of soil pH, exchangeable K and Mg, not only the effect of CP×T, but also the CP×SD effects were significant. There was no significant effect due to conservation practices on soil total N. It may be due to low N-release pattern of the mulching materials and high mobility of N in soil, which can accelerate the leaching of N from the soil profile. Zaharah and Lim (2000) did not detect any EFB-N releasing during the 10 months of decomposition.

Table 1. Statistical analysis of the soil chemical properties.

	Soil Property								
Source of Variation	C	CEC	pH	N	C:N	P	K	Ca	Mg
CP	ns	ns	*	ns	ns	ns	**	ns	**
T	**	**	**	**	*	**	**	**	**
SD	**	ns	**	*	ns	ns	ns	**	*
CP×T	ns	*	*	ns	*	ns	**	*	**
CP×SD	*	ns	**	ns	ns	**	*	**	**
T×SD	**	ns	**	*	ns	**	ns	**	**
CP×T×SD	*	ns	ns	ns	ns	**	ns	*	ns

Remark: The effect of CP × T for pH was significant at 0.06 % level.

Changes in soil pH, and exchangeable K and Mg due to different conservation practices over time were also observed. Soil pH, exchangeable K and Mg was higher in EFB than the other conservation practices throughout the observation after field application of the treatments (Figure 1). However, there were no significant different in soil pH, exchangeable K and Mg among Eco-Mat, silt pit and control. Average soil pH, exchangeable K and Mg in EFB were 5.72, 1.15 cmol/kg and 0.23 cmol/kg, respectively. In comparison to control, EFB increased soil pH, and exchangeable K and Mg by an average of 28.9%, 304.38 and 297.29%, respectively.

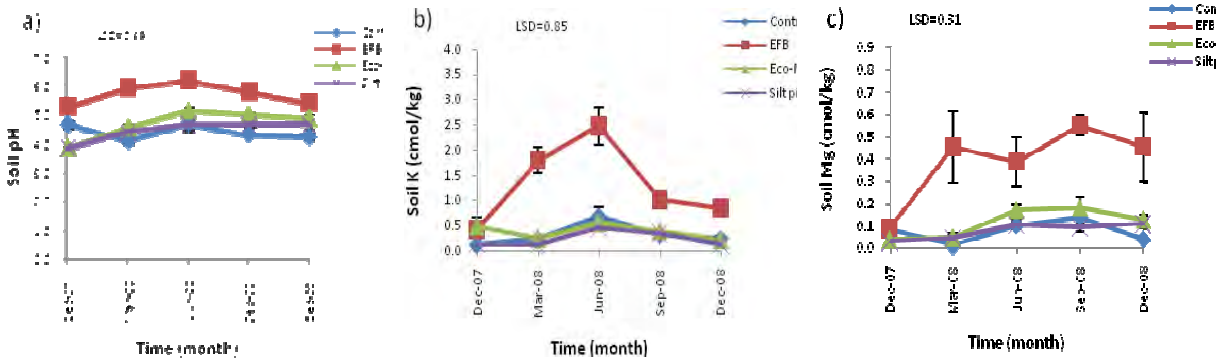

Figure 1. Changes in mean soil pH (a), exchangeable K (b), and exchangeable Mg (c) over time.

Soil total C was significantly higher in EFB than other conservation practices in nearly all of the times (Figure 2a). The average of total C at 0-15 cm soil depth for EFB, Eco-Mat and silt pit were 2.20, 1.72 and 1.72%, respectively. In comparison to control (1.68), EFB increased soil total C by 30.40% which is significantly higher than the increasing rates for both Eco-Mat and silt pit (1.90%). In general, there was no significant difference in soil C content among Eco-Mat, silt pit and control at all soil depths.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

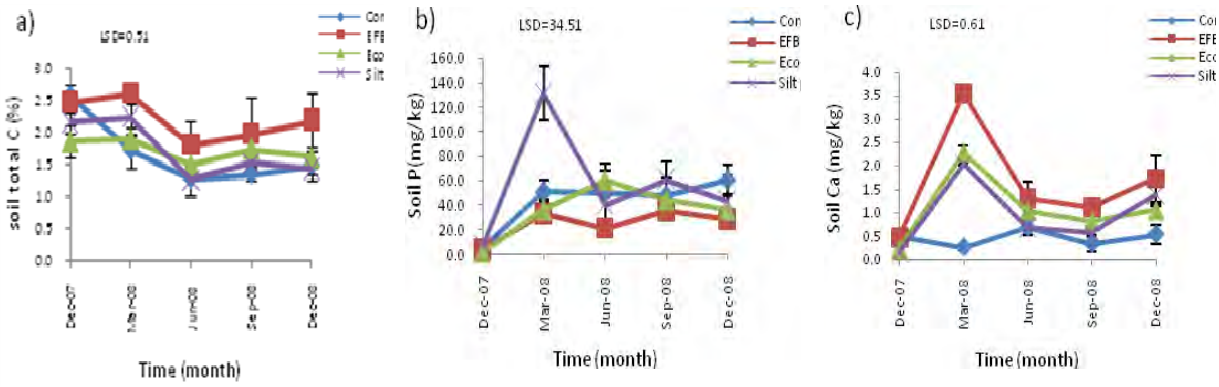

Figure 2. Changes in soil total C (a), available P (b), and exchangeable Ca (c) for 0-15 cm soil depth over time.

The most changes in soil P due to the conservation practices had occurred at 0-15 cm soil depth (Figure 2b). At this depth, silt pit was significantly different from the other conservation practices in increasing soil phosphorous at only three months after field application of the treatments (March). Thereafter, it was not different from Eco-Mat, control and EFB. Furthermore, comparing between the average soil available P across the times (Table 2) showed that at all soil depths, the highest P was due to silt pit. Soil Phosphorous content in Eco-Mat and EFB were not generally different from control. Therefore, these practices were not as effective as silt pit in increasing soil P. The higher P in silt pit may be contributed to the low mobility of phosphorous in soil and therefore, more movement through surface water rather than deep percolation.

Table 2: Soil available P for different conservation practices at different soil depth

	Soil depth (cm)		
Conservation practice	0-15	15-30	30-45
Control	43.49 ab	17.68 b	17.07 b
EFB	25.08 c	18.84 b	18.63 ab
Eco-Mat	35.91 bc	27.44 a	18.29 ab
Silt pit	57.09 a	29.12 a	26.29 a

As shown in Figure 2b, generally, EFB, Eco-Mat and silt pit increased soil exchangeable Ca significantly different from the control. However, there was no significant difference among EFB, Eco-Mat and silt pit. While at 0-15 cm depth, the effects of EFB, Eco-Mat and silt pit on soil Ca were similar the same, EFB increased average soil Ca significantly different from others across the times. In comparison to control, EFB, Eco-Mat and silt pit increased the average soil exchangeable calcium by 247.44, 131.89 and 106.78%, respectively. In the other soil depths, there was no significant difference among the conservation practices. Comparing the means by LSD showed that generally, there were no significant differences in soil CEC and C:N ratio among the conservation practices.

Conclusions

From the results, it can be concluded that soil pH, total C, K, Ca, Mg and P were significantly affected by the conservation practices. However, soil total N, C:N ratio and CEC were not affected. EFB significantly increased the soil pH, exchangeable K and Mg and in fact significantly higher than the other practices at all of the soil depths and total C at 0-15 cm depth for all the times. EFB increased the average soil exchangeable Ca at 0-15 cm soil depth and significantly higher than the other practices. Eco-Mat was effective in increasing the average soil pH and Ca at only the soil surface and at the second rank after EFB. Silt pit was effective in increasing soil available P which was significantly higher than EFB and Eco-Mat at only surface soil. In conclusion, EFB which is not only

a native and natural environmental friendly resource, but also a good source of basic essential nutrients and organic matter, is recommended to improve most of the soil chemical properties related to soil fertility in the sloping lands oil palm plantation.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Soil Water Content under Several Soil Water Conservation Methods in an Oil Palm Estate

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Introduction

Oil palm (*Elaeis guineensis*) is one of Malaysia's major agricultural crops. According to the Malaysian Palm Oil Board (2006), oil palm plantation covered approximately 4.1 million hectares or 13.20% of land in Malaysia, and it increases every year. The expansion of oil palm plantation is now being limited to marginal soils. One of these marginal lands includes those in hilly, steep land areas. These areas are prone to soil and water loss erosion during heavy rainfall through run-off.

Therefore, soil and water conservation practices on steep lands are essential so as to maintain water and soil nutrients. This is because these areas are steep, which are exposed to water erosion via heavy run-off. In order to reduce water run-off and retain soil water, the use of empty fruit bunches (EFB), Ecomat (Wan Rasidah and Wan Asma, 2007; Yeo, 2007) and silt pit are some of the recent conservation methods practiced. The objective of this study was to determine the effect of these conservation methods on the soil water profile compared with the control (palm frond heaps) for about a year beginning March 2008.

Materials and Methods

The experiment was conducted at Balau Estate (02º55'57'' N; 101º52'56'' E) managed by Boustead Plantation near Semenyih, Selangor, with a slope of 6º. The soil is classified as a Typic Paleudult (Rengam series), which has a sandy clay texture (37% clay and 56% sand). This area was cultivated with 8-10 year old oil palm trees planted with 8 x 8 m spacing.

The field layout (Figure 1) was arranged in three blocks, where each block was located at a different hill elevation. Each block was divided equally into four plots, and the treatments randomly assigned to the plots where each plot measured 8 x 8 m and with a gap of 8 m between two plots. The treatments were control (normal field practice where pruned fronds were arranged on the soil surface), empty fruit bunches (EFB), Ecomat and silt pit. In the middle of each EFB treatment plot, empty fruit bunches (1000 kg EFB $palm^{-1}$ $year^{-1}$) were heaped as a single layer on the ground. Likewise, four Ecomat carpets (2 m x 2 m and 0.02 m thick) were arranged in a single layer on the ground. The silt pits were constructed by digging a trench, measuring 1 m wide, 4 m long and 0.5 m deep along the hill contour.

From the middle of each treatment plot, water content was measured using AquaPro-Sensor (Aquatic Sensors, Nevada), and rainfall data was collected using a portable weather station (Watchdog Model 700, Spectrum Technologies Inc., Illinois). Daily soil water content between treatments was analyzed using one way ANOVA to determine the difference in the soil water content between the four treatments. This study had a large sample size. The number of levels for treatment was four, with three replications, seven soil depths and 166 number of days soil water content was measured. Therefore, the sample size was nearly 14,000. In addition, a precise day-to-day comparison of soil water content between treatments was not required. In this paper, it was sufficient to determine if the daily soil water content, averaged across the whole period of study, would differ significantly between treatments.

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a)

b)

c)

d)

Figure 1. The four soil water conservation methods in this study: a) Control, b) EFB, c) Ecomat, and d) Silt pit

Results and Discussion

Figure 2 shows the total soil water for treatments within the 13 months (March 2008 to March 2009) study period. Due to a fault in soil profile probe, the data for the soil profile was collected for certain periods and the graph were plotted from 7th to 27th March 2008, 14th April to 5th June 2008, 8th September to 25th October 2008, and 2nd January to 25th March 2009. Figure 3 shows the total rainfall and, as expected, soil water content increased after each rainfall period.

ISBN: 978-967-344-188-4
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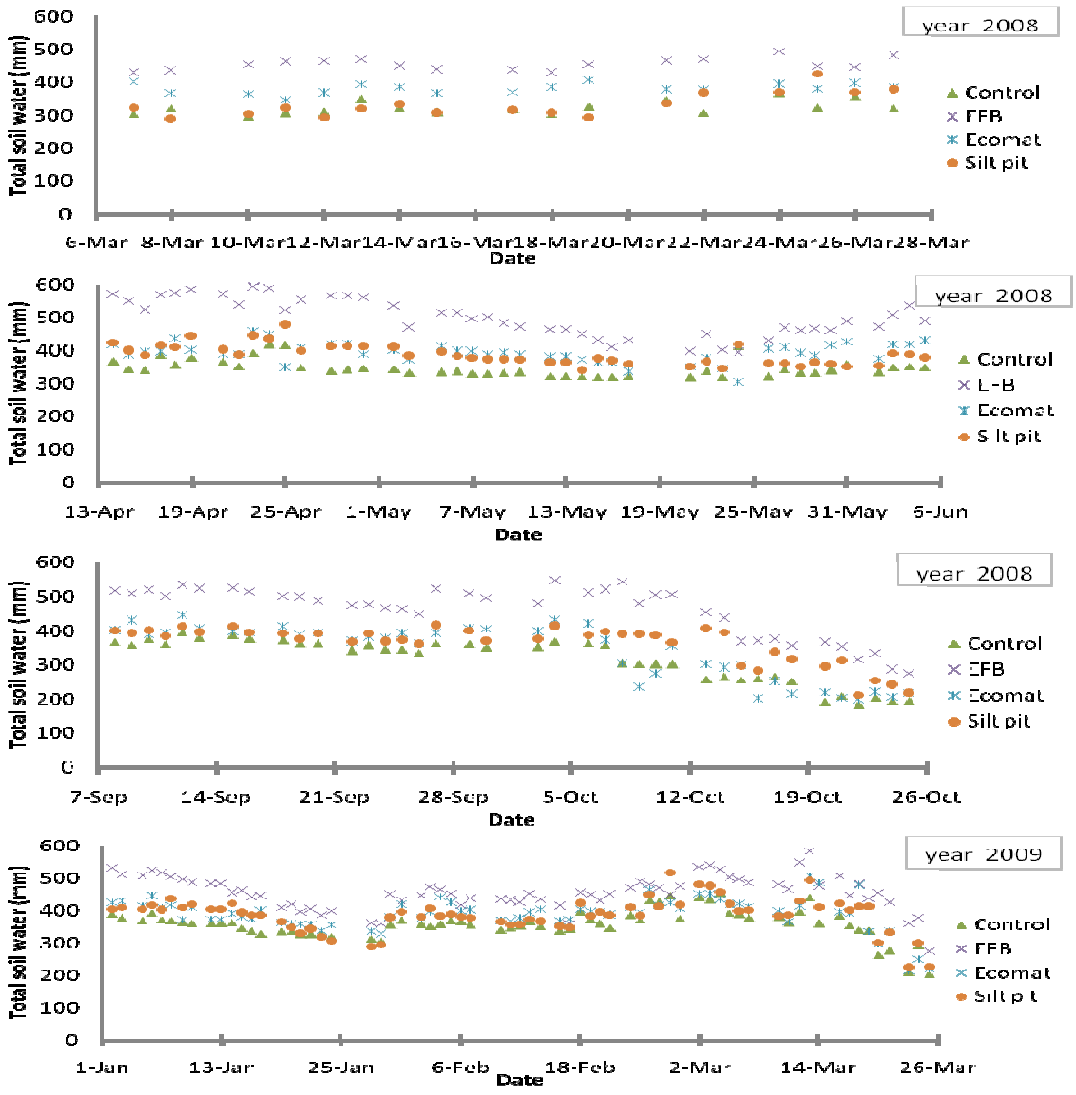

Figure 2. Total soil water for the year of 2008 and 2009

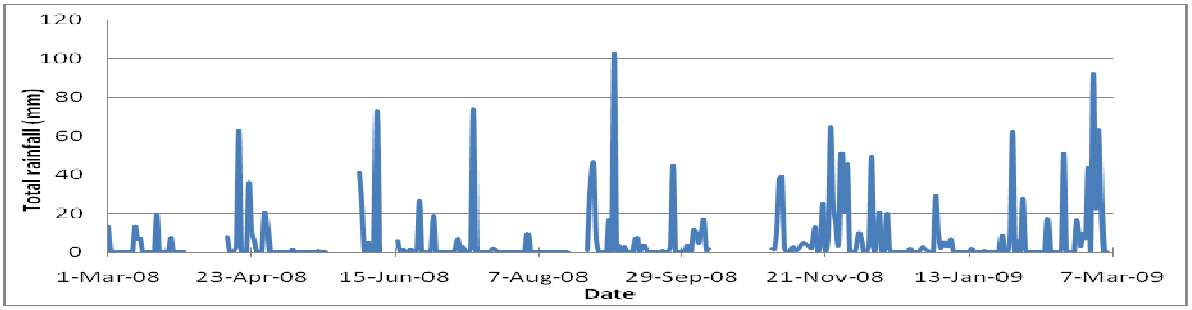

Figure 3. Total daily rainfall from March 2008 to March 2009

ANOVA shows that there were significant differences between treatments (Table 1).

Table 1. ANOVA of the four treatments on total soil water content

	Sum of Squares	df	Mean Square	F	Sig.
Treatments	1467408.459	3	489136.153	163.430	< 0.001
Error	1975334.394	660	2992.931		
Total	3442742.853	663			

In order to compare the means, Duncan multiple range test (DMRT) was conducted (Table 2).

Table 2. Average daily soil water content

Treatment	Mean
Control	340.41c
Silt pit	374.20b
Ecomat	376.55b
EFB	467.14a

Means with the same letter are not significantly different from each other according to DMRT at 5% level.

The result shows that EFB had the highest amount of soil water content, and the least was control. Silt pit and Ecomat had statistically similar soil water content. EFB had 37.23% higher soil water content than control. Ecomat and silt pit have the similar capability in retaining soil water, which were 10.62% and 9.93% respectively, higher than control.

Conclusions

EFB was found to be the best water conservation method to retain soil water among the four treatments in this experiment, which was 37.23% higher than control. Both Ecomat and silt pit were better than control in conserving water at 10.62% and 9.93% higher, respectively.

Acknowledgements

The authors would like to thank Mr. Subra at Balau Estate for his co-operation in this study.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Scanning Electron Microscope of *Syzygium campanulatum* Leaf and Stem Treated with Uniconazole

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Introduction

Syzygium campanulatum belongs to the family Myrtaceae and is native to Malaysia. This species naturally grows at the coastal areas of Terengganu, Kelantan, Pahang and Johor. It grows well in the urban areas as hedge plant, topiary or single planting. Besides its attractive reddish young flushes, *S. campanulatum* is a fast growing species which needs frequent trimming to maintain its shape and the aesthetic value.

Plant growth regulators (PGRs) are compounds which in small amounts promote, inhibit or qualitatively alter growth and development of a plant. PGRs are always used for improving quality, increasing yields, or facilitating harvesting (Barret, 2001; Bruner et al., 2001; White, 2003; Ahmad Nazarudin et al., 2005). These compounds interfere with the biosynthesis of gibberellins (GAs), thus inhibiting stem elongation (Fletcher et al., 2000). The most evident plant growth response to triazole treatment is reduced stem elongation, hence reducing the plant heights (Nie et al., 2001; Kim et al., 1999; Mike et al., 1999). However, the dosage required and the plant responses may vary between plant species or cultivars. These compounds also reduce leaf area, but increase epicuticular wax, width and thickness (Gao et al., 1987). Some species will produce wrinkled leaves or severe retardation growth due to overdose of PGRs. Uniconazole is one of the PGRs that have been used for the above mentioned purpose. It has been widely recorded as one of the most persistent triazoles in controlling the vegetative growth of a wide range of angiosperms (Fletcher et al., 2000; Ahmad Nazarudin et al., 2005). The objective of this study was to investigate the effects of uniconazole on the leaf and stem anatomy of a vigorous landscape plant, *S. campanulatum*.

Materials and Methods

This study was carried out at three-month after the plants were treated with uniconazole (0, 10, 20 and 30 mgL^{-1}). All treatments were replicated four times. The first three fully matured leaves and the second internodes of stem in each plant were collected and processed for Scanning Electron Microscopy (SEM) viewing (Zakaria and Razak, 1999). The specimens were then viewed under the JOEL 6400 SEM at an acceleration voltage of 15 kV.

All data were subjected to ANOVA using Statistical Analysis Software (SAS) and the treatment means were then compared by using Tukey‘s studentized range (HSD) test to detect significant difference among treatments.

Results and Discussion

The palisade mesophyll cells and the spongy parenchyma of treated plants were closely arranged as compared to the untreated control plants (Figures 1 and 2). The palisade parenchyma thickness of treated plants was increased, however there was no significant difference in spongy parenchyma thickness (Table 1). Microscopic examination of stem cross-sections found that the thickness of xylem tissue in the treated plants was reduced (Figures 3 and 4; Table 1). These effects could be due to the decreased size of the leaf and stem. Nie et al. (2001) reported that uniconazole inhibited shoot length, leaf area and specific leaf area but increased leaf thickness and mesophyll density. Restricted

water and nutrient supplied might occur, hence it could be partially responsible for the slow development of the plants (Wang and Gregg, 1989).

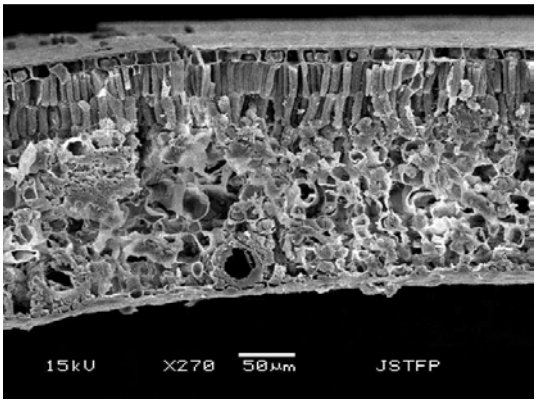

Figure 1. Cross section of leaf lamina (untreated control plant)

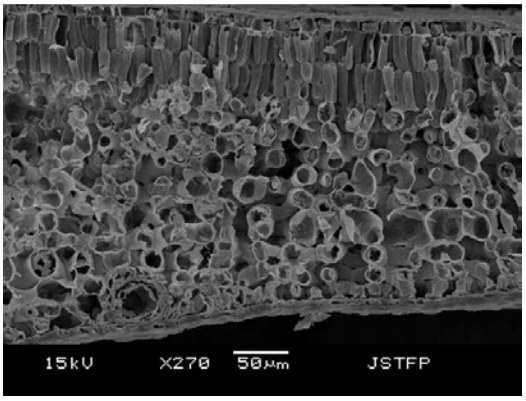

Figure 2. Cross section of leaf lamina (treated with 30 mgL^{-1} uniconazole)

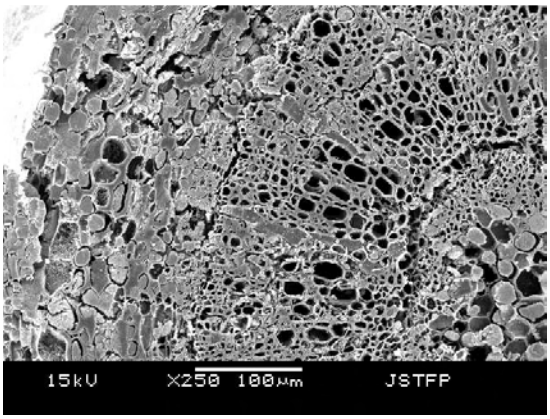

Figure 3. Cross section of stem (untreated control plant)

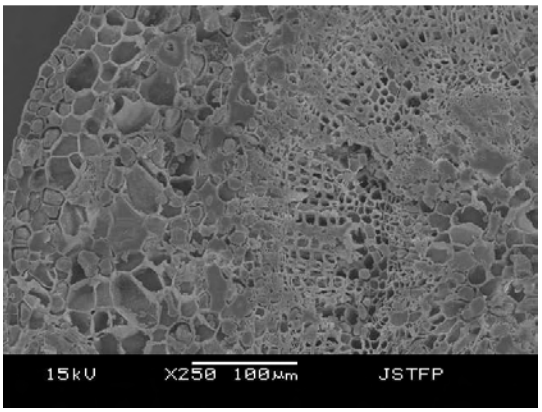

Figure 4. Cross section of stem (treated with 30 mgL^{-1} uniconazole)

Table 1. Palisade parenchyma, spongy parenchyma and xylem thickness of *S. Campanulatum.*

Uniconazole, mgL^{-1}	Palisade parenchyma thickness (µm)	Spongy parenchyma thickness (µm)	Xylem thickness (µm)
0	43.63b	98.35	141.78a
10	64.18a	116.78	97.60b
20	66.45a	125.38	94.53b
30	69.88a	107.15	85.95b

Means followed by the same letter(s) do not differ (P<0.05) by Tukey's studentized range test.

Conclusions

The application of uniconazole reduced the leaf size, hence affected the palisade mesophyll cells. This growth regulator also reduced the proportion of xylem which is responsible for the inhibition of the plant growth. As a consequence, the leaf area was smaller and the stem was shorter.

Acknowledgements

This study was funded by the Malaysian Government (IRPA 01-04-01-0074 EA001).

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 6

PLANT PRODUCTION IN CHANGING ENVIRONMENTS

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Understorey Light Variations in Chronosequence Rehabilitated Forest Stands

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Introduction

Rehabilitation is a process of returning the land in a given area to some degree of its former state, after some process (industry, natural disasters etc.) has resulted in its damage. Many projects and developments will result in the land becoming degraded, for example mining, farming and forestry. Large areas of the world's forest have been lost or degraded and landscapes everywhere are being simplified by current land-use practices (Lamb and Gilmour, 2003). Land use practices are moving away from simplification of the landscape into the complex and often uncharted territory of landscape rehabilitation and rainforest restoration (Erskine et al., 2005) While it is rarely possible to restore the land to its original condition, the rehabilitation process usually attempts to bring some degree of restoration. Modern methods have in many cases not only restored degraded land but actually improved it, depending on what criteria are used to measure 'improvement'. However, most of forest rehabilitation projects focus on technical aspects and pay scant attention to socio-economic viability, acceptability and sustainability of the system. Then there is vital need to monitor and learn from past and ongoing rehabilitation efforts, and understand the ecological and socio-economic factors that have contributed to success and failure.

Today, it is seen in the concept of co-evolution of climate and life (Scheider et al., 2004) and more recently in the recognition that climate changes mitigation strategies must include an integrated assessment of terrestrial feedback on climate (Betts, 2007). Climate also changes over shorter timescale (Mann and Jones, 2003; Jones and Mann, 2004; North et al., 2006; Jansen et al., 2007). The coupling of ecosystems and climate occurs over a continuum of timescales from minutes to seasons to thousands of years (Bonan, 2008). At short timescale, the seasonal emergence and senescence of leaves alters the absorption of radiation, the dissipation of energy into latent and sensible heat, and carbon dioxide uptake. The development of tree crown in the planted trees will shades the areas and reduces the amount of solar radiation reaching the ground. The changes in the microenvironment will also influence the physiological processes of the trees, regeneration and the growth of the trees in the rehabilitated forest. Therefore, it is necessary to monitor and consider the changes in the microenvironment so that survival and growth rate of the trees can be increased.

Light plays a major role in regulating survival and growth of tropical forest plants (Chazdon, 1988; Press et al., 1996). In forest understorey, photosynthetically active radiation affects the growth and survival of tree regeneration (Kozlowski et al., 1991; Lieffers et al., 1999). Forest understorey light regimes are determined in large part by seasonal and diurnal variations in the sun position, weather, topographic position, and forest canopy structure (Rich et al., 1993).

The rationale for this study is to provide growth and development information in order to facilitate restoration efforts with understorey light variations. The objective of this study was to investigate the understorey light variations in the different age stands in the rehabilitated forest.

Materials and Methods

Site description

The study site was located in Bintulu, about 600 km North-east of Kuching, Sarawak. The study was conducted on a 47.5 ha area in the campus of Universiti Putra Malaysia (UPM), Bintulu branch, Sarawak (latitude 3˚ 12' N, longitude 113˚ 05' E) at altitude 50 m above sea level. The site receives annual rainfall of 290 - 443 cm and annual temperature of 26.4 - 27.8 ˚C (Mohd Zaki, 1992). Most of the rain falls during the North-East monsoon (November to January annually). The average relative humidity is 80% throughout the year (Yusuf and Abas, 1992). According to Peli et al. (1984), the soil at the site is of Nyalau and Bekenu Series, which are well drained. Between July 1991 and 2005, about 350,000 trees from 126 species had been planted successfully. The planting concept use of multiple species and planting technique was 3 planted trees in 1 meter per square (Mohamad Azani, 1994).

The stands selected were from different ages (19, 16, 10 and 4 year old) and used as a chronosequence. A plot of 30 m x 30 m was established in each 3 ages stands (19, 16, 4 year) while a 20 m x 20 m plot was established for 10 year stand. In 30 m x 30 m plots there were 16 grid points while 20 m x 20 m plots had 9 grid points. In each plot a grid of 10 m x 10 m was established and marked with 2 m height of PVC pipes with measurements done using WacthDog Model 2475 Plant Growth Station. The mast was erected in the middle of the stand. Measurements were conducted on 25th, 26th, and 27th May 2009 from 9.00 am to 3.00 pm. Mean values of relative light intensity (RLI) and photosynthetically active radiation (PAR) were obtained from the measurement recorded from each grid points. Measurements were taken from 16 grid points for 3 age stands (19, 16, 4 years) and 9 grid points for 10 year old stand.

Results and Discussion

Mean photosynthetically active radiation (PAR) in the 4 year old stand was the highest at the averaged of 465.8 µmolm^{-2} s^{-1} while mean (PAR) in 19 year old stand was the lowest with 43.6 µmolm^{-2} s^{-1} (Table 1). Mean Relative Light Intensity (RLI) in the 4 year old stand showed the highest value, 64.78%, whilst 19 year old stand was the lowest with 2.25%.

Table 1. Differences between mean photosynthetically active radiation (PAR) and mean values of relative light intensity (RLI) across the age of stands

Age (Year)	19	16	10	4
PAR (µmolm^{-2} s^{-1})	43.6	100.5	341.0	465.8
RLI (%)	2.25	4.91	17.53	64.78

An older stand has low RLI and PAR value compared to the younger stands. The value between relative light intensity (RLI) and photosynthetically active radiation (PAR) that estimate were varying according to the ages of stands and physiognomic structure (such as light, canopy opening etc.). The occurrence of lower light availability will increase as ages of stands become older. The spatial distribution of forest canopy structure influences both the total understorey light availability and its spatial distribution (Nicotra et al., 1999).

Conclusions

As conclusion, under storey light variations was affected by different ages of stand in rehabilitation forest. Furthermore, the recognition of spatial dependence variability of light conditions is important in developing spatially explicit models to predict forest dynamics. The results of this study have direct implication for forest rehabilitation management and used to measure 'improvement'. Clearly the nature of this kind of light can play an important contributing role in maintaining growing survival in

light-limited forest rehabilitated structure. Change in community composition is generally accompanied by changes in the environment caused by plants themselves.

Acknowledgements

We are greatly indebted to the following people who helped us in the research project, Prof. Dato' Dr. Nik Muhamad Ab Majid (Chairman of COE), Assoc. Dr. Mohamad Azani Alias and the other staff of Forest Health Unit (COE), UPM. This Research was sponsored by Mitsubishi Corporation.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Determination of Pesticide Residues in Organically and Conventionally Grown MRQ74 Rice

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Introduction

Organically grown produce has become the choice for health and environment conscious consumers. Organic production system avoids the use of chemical fertilizer, pesticides and growth regulators (Sullivan, 2003). Despite of none synthetic pesticide use in organic production system, analysis of pesticide residue in United States revealed that certain organic produce were found to contain pesticide residues (Baker et al., 2002). This could be due to contamination of spray drift from nearby conventional farms. Certain farms which were formerly adopting pesticide application as means to control pest attack, might cause some types of pesticides to accumulate in organic crops through root uptake, if the pesticide is very persistence and water-soluble.

The study compares pesticide residue content between organically and conventionally grown rice. One of the objectives of the study was to investigate whether there was any presence of pesticide residues in organic rice from organic rice production in Kampung Ewa, which has a history of none or very little use of pesticides.

Materials and Methods

The agronomic practices of organically and conventionally grown rice are summarized in Table 1. MRQ74 variety, which is a type of fragrant rice variety introduced by MARDI in 2005, were planted in the two rice production areas.

Table 1. Summary of agronomic practice of organically and conventionally grown rice

	Organic Practice	Conventional Practice
Location	Kg. Ewa, Langkawi, Kedah	Kg. Padang Kandang, Kubang Kerian, Kelantan
Starting Period	August 2008	August 2008
Seeding Method	Transplanting	Direct seeding
Pest Control	Insect control: Neem and serai wangi Weed control: Flooding and hand weeding	Insect control: Carbofuran, cypermethrin Weed control: Paraquat, propanil
Harvest Period	January 2009	December 2008
Duration per season	~ 6 months	~ 5 months

Paddy was harvested from both rice production areas using a mechanized harvester. Harvested paddy was sent to MARDI, Bukit Raya, for milling and/or storage. The milling process dehusks the paddy to produce milled rice or white rice. Milled rice was subjected to aging process where the milled rice was kept in a rice sack for storage at room temperature for duration of six months before the rice was marketed. Paddy or pre-milled rice samples were kept also in similar condition as milled rice so that comparison could be made in term of pesticide residue content.

For the purpose of the study, pre-milled and milled rice were sampled prior to storage, 3 and 6 months after storage. The sampling for pre-milled and milled rice of each storage period was done in duplicate. Rice samples were sent to Pesticide Residue Laboratory, MARDI, Serdang for

determination of pesticide residues. Quenchers-based method (Anastassiades and Lethotay, 2003) was used as the analytical method.

Results and Discussion

The summary of results is shown in Table 2. No pesticide residue was detected in any of the samples except pre-milled sample before storage. The concentration detected were the lowest that the method could quantify, which was 0.01 $mgkg^{-1}$. Endosulfan is very persistent in nature (Mohana and Satyanarayana, 1980) and was formerly used as an insecticide in rice production area before it was banned by The Pesticide Board in 2005. The presence of endosulfan in the samples could be due to uptake of endosulfan from soil or spray drift from nearby farm although no record of endosulfan use was found in the selected conventional farm for this study. Pesticides listed in Table 1 were also not detected in all the samples from conventional practice. The pesticide residues could have dissipated or degraded to less than Limit of Quantification, 0.01 $mgkg^{-1}$.

Table 2. Pesticide residue concentrations ($mgkg^{-1}$) in rice samples.

Sample	Pesticide Residue Concentration ($mgkg^{-1}$)	
	Organic Practice	Conventional Practice
Pre-milled rice		
0 month storage R1	< LOQ	0.010* ± 0.005
0 month storage R2	< LOQ	0.010* ± 0.005
3 month storage R1	< LOQ	< LOQ
3 month storage R2	< LOQ	< LOQ
6 month storage R1	< LOQ	< LOQ
6 month storage R2	< LOQ	< LOQ
Milled rice		
0 month storage R1	< LOQ	< LOQ
0 month storage R2	< LOQ	< LOQ
3 month storage R1	< LOQ	< LOQ
3 month storage R2	< LOQ	< LOQ
6 month storage R1	< LOQ	< LOQ
6 month storage R2	< LOQ	< LOQ

LOQ : Limit of Quantification = 0.01 mg kg^{-1}
** Endosulfan*

Conclusions

It can be concluded that organic rice from Kampung Ewa was free from any pesticide residues. Traces of endosulfan at very low level (0.01 $mgkg^{-1}$) found in pre-milled rice samples from conventionally grown rice were probably due to long persistency of endosulfan and/or spray drift from possible misused of endosulfan. The milling process seems to remove pesticide residue which resided in risk husk.

Acknowledgements

This project was funded under Science Fund Project (05-03-08-SF0020). The authors wish to thank Siti Rahmah, Jamiah Jaafar, Khairatul Azmah Mohamed and Catherine Baun Dudong for their supports.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Organic Carbon, Nutrients and pH from Drained and Undrained Areas of Pekan Forest Reserve

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Introduction

Wetlands have long been utilised for human activity (Lundin, 1994) and Peat Swamp Forest (PSF) in Malaysia is not an exception. The first thing needed to be done before any activity could be carried out would be lowering the water table either to allow transportation access into the forest or preparing the soil for agriculture by drainage (Inubushi, 2003). Draining of excessive water in PSF which is high in organic matter would result in carbon dioxide emissions (Inubushi, 2003). Effects from drainage also causes increased decomposition of organic material causing subsidence of the soil (Inubushi, 2003) and increase the vulnerability to forest fires (Hooijer, 2006). Drainage also contributes to the loss of nutrients and dissolved organic carbon (DOC). This loss will further reduce the nutrient and carbon pool within the ecosystem thus may affect the livelihood of the organisms within the PSF.

This study was quantified carbon and other nutrient in surface water from drained peat swamp forest. The nutrients analysed are nitrogen (N), Phosphorus (P), Potassium (K), Magnesium (Mg), calcium (Ca) and dissolved organic carbon (DOC). Mean rainfall from the nearest point of sampling was also used to look at the correlation of nutrient loss with changing patterns of rainfall. pH was also measured on site.

Materials and Methods

Sampling areas

Pekan forest reserve is located within the South-East Pahang Peat Swamp Forest (SEPPSF), the largest remaining undisturbed PSF in Malaysia. This study was carried out at different locations mainly characterized by the drainage. Drained areas consist of three plots which are Ramin plantation (RP), cutting regime (CR) and grassland (GS), while a control site is an undrained area marked as virgin jungle reserve (VJR).

Sampling method

Water sampling is carried out at the selected plot basis from April 2008 till May 2009. Surface water is taken a 1 km drain/line at 100 m intervals. Physical parameters (pH, temperature and electrical conductivity) are taken at each point of sampling. Samples stored in sampling bottles (50 mL) are quickly kept in a cool box at 4 ºC for transport. Upon arrival, the sample is stored in a freezer before being filtered for further analysis.

Chemical analysis

Chemical analysis is done by multiple methods after the water sample is filtered. Nitrogen determination is carried by using the Kjedahl digestion method and distillation process. Organic carbon is determined by using the Wakley and Black's rapid titration method. P, K, Ca and Mg are directly analyzed using an Inductive Couple Plasma Spectrophotometer (ICP)

Statistical analysis

ANOVA was carried out to test the significance of results against rainfall.

Results and Discussion

The chemical analysis showed varied concentrations of nutrients for each site during the sampling period. The minimum, maximum and mean of the chemical analysis are shown in Table 1.

Table 1. Minimum, maximum and mean values of elements in surface water by plot

Element		Plot			
		CR	GS	RP	VJR
N (ppm)	Minimum	0.420	0.700	0.420	0.560
	Maximum	9.800	10.500	9.100	11.200
	Mean	3.102	3.615	3.064	3.294
P (ppm)	Minimum	0.001	0.001	0.001	0.004
	Maximum	0.704	0.202	0.269	0.210
	Mean	0.066	0.056	0.068	0.069
K (ppm)	Minimum	0.020	0.014	0.006	0.101
	Maximum	2.890	2.490	2.253	1.760
	Mean	0.318	0.669	0.236	0.522
DOC (ppm)	Minimum	1.140	6.070	0.500	4.420
	Maximum	106.750	102.250	121.030	254.300
	Mean	45.220	48.700	49.040	51.860
Ca (ppm)	Minimum	0.100	0.440	0.009	0.160
	Maximum	14.140	7.900	4.310	11.000
	Mean	0.980	2.155	0.761	1.002
Mg (ppm)	Minimum	0.190	0.450	0.100	0.094
	Maximum	0.722	5.866	0.670	0.830
	Mean	0.349	1.498	0.376	0.371
pH	Minimum	3.550	3.250	3.500	3.570
	Maximum	4.180	3.900	5.520	4.050
	Mean	3.779	3.528	3.845	3.779

Mean Ca, Mg and K leaching were highest in the GS plots while the other three sites had almost similar levels. This observation supported the findings reported by Laiho et al. (1998). N and P levels were between 3 ppm and 0.06 ppm respectively in these sites were comparable between all these sites. This indicated that N and P leaching was not due to the drainage.

GS is currently being cultivated with pineapple. And hence liming activities had been conducted. Runoff and leaching of Ca and Mg from the soil treatment practice of liming and also from fertilizer may add up to the high concentration of the element in the surface water. While the other two sites, CR and RP no fertilizers were applied although these sites are also drained.

A characteristic of a PSF the tea colour of water in the ecosystem which high in DOC. DOC leaching was the highest in the VJR, which was double the level of the drained sites (Table 1). This is explained by the fact that VJR has the highest soil organic content. No significant differences between the drained and un-drained areas were shown.

The influence of rainfall on nutrient leaching was examined. Significant correlation was noted between rainfall and leaching of P (0.05) and K (0.01) (Table 2). Transport of P in surface water occurs in 3 ways which are 1) dissolved in surface runoff, 2) attached to sediments in surface runoff

and 3) dissolved in leaching water (Baker, 2001). Low concentration levels of P detected in surface water suggest that peat soil have a high adsorption capacity for the element on the sediment. Previous studies also showed that gravimetric concentrations of P in peat soil increases from top to bottom layer after drainage compared to undrained peat soil (Laiho, 1998). With all sites are based on the same soil type, the differences might not be evident for P. P concentration is a surface water issue which is mostly determined on the surface runoff (Lory, 1999). Main factor for surface runoff is rainfall. Correlation of P with rainfall (Table 2) showed a positive significance. This indicates the effect by runoff on P concentration to surface water from drainage or topmost soil layer concentration of P. With the topmost layer gravimetric concentration of P is highest compared to deeper layer peat soil (Laiho, 1998), more rainfall can further causes an increase in the concentration of P in the surface water.

Though rainfall events on saturated peat may lead to faster run off (Beheim, 2006), the concentration of DOC in surface water decreases as rainfall increases. During storm events, water volume increase, DOC concentration on surface water is further diluted by the rainfall and run off (Clark et al., 2006). This negative correlation of DOC and rainfall may not actually give significant change towards the DOC flux during a storm event (Clark et al, 2006).

Table 2. Pearson correlation between pH, rainfall, nutrients and DOC

	Rainfall	pH	N	DOC	P	K	Ca
pH	-0.017						
N	-0.082	-0.132					
DOC	-0.226	0.134	-0.231				
P	0.149*	-0.055	-0.109	0.075			
K	0.184**	-0.367	0.273	-0.113	0.062		
Ca	-0.100	-0.530	0.027	0.155**	-0.088	0.305	
Mg	0.076	-0.516	0.243	-0.151**	0.023	0.569	0.347

**Significant relationship (p<0.05)*
*** Significant relationship (p<0.01)*

Conclusion

Drainage caused the leaching of Ca and Mg while the N and P levels were the same in all these sites. Leaching of P and K were influenced by rainfall.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 7

BIOTECHNOLOGY – TISSUE CULTURE, PLANT GENETICS

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Cloning and Construction of Chilli *Pds* and *Zds* Genes in Plant Expression Vector

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Introduction

MT1 tomato is a lowland variety developed by MARDI that can be easily grown in lowland areas with promising yield. This variety was identified as bacterial wilt resistant and was found to be suitable for sauce and puree production due to its high total solids content which is about 5.6 to 6.1% (Melor and Ramli, 1988; Seed Catalog MARDI, 1990). However, MT1 tomato fruit only achieved tangerine red colour at maturity and lack the attractive red colour of a highland tomato.

Red colour of tomato is due to the accumulation of carotenoids (Thorup et al., 2000). Hence, the limited red pigment in tomato MT1 is believed to be due to the malfunction of one of the carotenoid synthesizing genes. Cloning of the correct genes will therefore allow a complete flux into the biosynthetic pathway and consequently sufficient carotenoid pigment will be synthesized to display the attractive red colour.

Carotenoids are formed from the biosynthesis of tetraterpenes with the involvement of at least eight sequential genes; geranylgeranyl pyrophosphate synthase (GGPPS), phytoene synthase (*psy*), phytoene desaturase (*pds*), ς-carotene desaturase (*zds*), lycopene β-cyclase (*crtL*), β-carotene hydroxidase (*crtZ*), zeaxanthin epoxidase (*ze*) and capsanthin capsorubin (*ccs*).

Phytoene synthase which acts as a branching enzyme that directs substrates irreversibly to carotenoids has been targeted as key enzyme in several genetic manipulation studies. Besides, manipulation of desaturation activity in plants has also been reported in producing transgenic plants with increased carotenoids contents (Naik et al., 2003). In this study, the plant expression vector containing *pds* and *zds* genes have been constructed.

Materials and Methods

Plant materials and RNA extraction

Local red chilli pepper fruits of Kulai variety were obtained from a local market. Total RNA was extracted from the outer layer of the fresh chilli fruits and used as template to synthesize first strand cDNA using MMLV reverse transcriptase (Gibco-BRL) and oligo dT as primer. The PCR amplification was then carried out using the first strand cDNA synthesis product as template and specific primer based on 5'- and 3'-ends of *Pds* and *Zds* genes.

Subcloning and restriction enzyme analysis

PCR products of the appropriate size were cloned into pCR vector (Invitrogen) and transformed into *E. coli* XL-1Blue. After verification by PCR analysis, the fragments of interest were digested with *Bam*HI and *Sal*I separately prior to subcloning into pPJK vector to obtain 35S promoter and poly A terminal and the recombinant plasmids were then transformed into *E. coli* XL-1Blue. After verification by PCR and restriction enzyme analysis, the *Pds* and *Zds* genes together with 35S promoter and nos-terminator cassette were cloned into plant expression vector pCAMBIA2300 separately and transformed into *Agrobacterium tumefaciens* strain LBA4404.

Results and Discussion

The *pds* and *zds* genes were obtained by RT-PCR amplification of the total RNA isolated from outer layer of the fresh chilli fruits. A single DNA fragment of *pds* gene with approximate size of 1.7 kb was amplified by RT-PCR (Figure 1a). The amplified fragment was then analysed with *Eco*RI restriction enzymes digestion giving three fragments with the sizes of 0.1 kb, 0.5 kb and 1.1 kb (Figure 1b). As for *zds* gene, a single DNA fragment with a size of approximately 1.75 kb was obtained (Figure 1a) and confirmed with *Nhe*I restriction enzyme digestion giving two fragment with the sizes of 0.75 kb and 1.0 kb (Figure 1b). Data analysis showed that the amplified fragments have equivalent sizes to the *pds* and *zds* genes reported in GeneBank databases, indicating that the amplified genes were most likely the right genes.

Subsequently, the fragments were cloned into pCR vector and transformed into *E. coli* XL-1Blue cells separately. The inserts were confirmed by carrying out of PCR colony screening. To subclone the *pds* and *zds* gene fragments into pPJK, the pCR vector with insert and pPJK were respectively digested with *Bam*HI and *Sal*I. The *pds* and *zds* gene fragments were then ligated into pPJK and transformed into *E. coli* XL-1Blue cells. After confirmation by PCR colony screening, the plasmids were isolated for verification with restriction enzyme analysis. Cut fragments were obtained with the expected sizes of 1.7 kb and 1.1 kb when digested with *Bam*HI and *Sal*I, and *Eco*RI for *pds* gene; and 1.75 kb and 1.0 kb when digested with *Bam*HI and *Sal*I, and *Nhe*I for *zds* gene, respectively. These indicate that the *pds* and *zds* gene fragments were successfully cloned into pPJK.

The gene cassettes with CaMV35S promoter and nos-terminator at 5'- and 3'-ends of *pds* and *zds* genes (Figure 2) respectively were obtained and subcloned into plant expression vector, pCAMBIA2300, separately. These vectors were then transformed into *Agrobacterium tumefaciens* strain LBA4404. The PCR screening of the colonies showed that the transformed *Agrobacterium* contained the genes; and thus ready for subsequent plant and cell culture transformation.

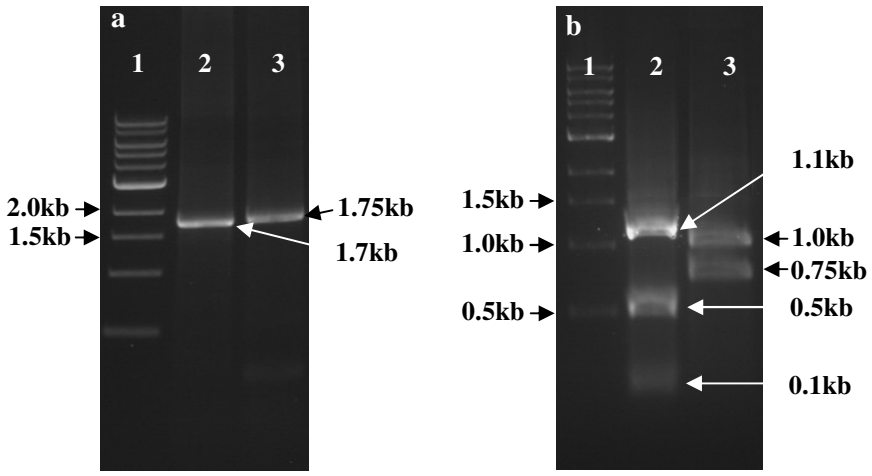

Figure 1. (a) Single DNA fragment amplified by RT-PCR. Lane 1:1 kb DNA Ladder, Lane 2: *pds* gene and Lane 3: *zds* gene.
(b) Restriction enzymes analysis. Lane 1:1 kb DNA Ladder, Lane 2: *pds* gene fragment digested with *Eco*RI and Lane 3: *zds* gene fragment digested with *Nhe*I.

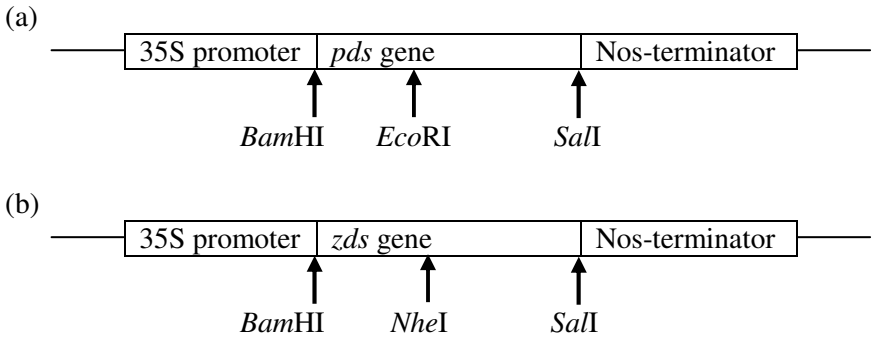

Figure 2. Gene cassettes with CaMV35S promoter and nos-terminator at 5'- and 3'- ends of the (a) *pds* and (b) *zds* genes, respectively.

Conclusions

The *pds* and *zds* genes were successfully isolated from the local chilli pepper of Kulai variety and subcloned into plant expression vector. The constructs were transformed into *Agrobacterium* strain LBA4404 and ready for future plant and cell culture transformation.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Surface Sterilization of *Aquilaria malaccensis* Young Leaf and Nodal Segment Explants

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Introduction

Aquilaria malaccensis is a member of the family Thymelaeaceae. This tree species is distributed throughout the country and confined mainly to plains, hillsides and ridges up to a height of 750 m in both primary and secondary forests (Whitmore, 1972). It is one of a few species of tropical trees and is the principle source of gaharu which is one of the most highly valuable forest products currently traded internationally (Barden et al., 2000). In Malaysia, *A. malaccensis* is the best known species in producing gaharu besides *Aquilaria agallocha* and *Aquilaria crassna*. Gaharu is the most expensive wood in the world valued in many cultures for its distinctive fragrance and used in religious function, medical and aromatic preparations. Top grade gaharu can be sold for over USD 1000/kg due to decreasing supply and high demand especially from Middle Eastern and Asian markets (Barden et al., 2000). The international demand for gaharu and resins has increased at an alarming rate i.e. from 68, 970 kg in 1998 to over 5-fold in 2003 (Abdul Rahman et al., 2005). The high demands of the multipurpose properties of these species have often made them being overexploited through indiscriminate harvesting from natural forests, thus making them threatened and critically endangered (Ng et al., 1997; Adelina, 2004). Currently, this tree species is categorized as vulnerable according to IUCN Red List Categories.

One way to overcome the loss of *A. malaccensis* and to reduce the pressure of gaharu being harvested from natural forest is to encourage or promote forest plantation of this species. Establishment of forest plantation is possible with adequate supply of planting materials which could be achieved through micropropagation which is preferred over conventional methods of propagation. It forms the most viable methods of propagating thus ensuring continuous supply of planting material (Mohan Ram, 1997). Besides that, this tree species produce recalcitrant seed that are sensitive to changes in moisture content which caused poor viability and storage problems thus hindering the production of planting material (Kundu and Kanchari, 2000). Surface sterilization is the first and crucial step in micropropagation since contamination hampers the establishment of truly aseptic plants and therefore influenced the success of micropropagation (Leifert and Cassells, 2001). Therefore, this study is conducted to examine the suitable sterilization procedures of different explants.

Materials and Methods

Plant samples

Explant sources were obtained from 2-year old seedlings raised in the nursery of the Faculty of Forestry, UPM. The explants used in this study comprised of two types of vegetative tissue namely young leaf and nodal segment.

Sterilization of young leaf and nodal segment explants

Explants were washed under running tap water for 15 min and presterilized with 70% (v/v) ethanol for 2 min. Both of the explants were then treated in 5 to 30% (v/v) commercial clorox solution (5.25% sodium hypochlorite) plus 2 to 3 drops of Tween-20® emulsifier respectively for 5 to 30 min. Nodal segment explants were then treated with 0.2% (w/v) mercuric chloride for 5 to 20 min. These were

followed by 3 to 5 washes in sterile distilled water before young leaf explants were cut to 10 x 10 mm and nodal segment explants were cut into 10 to 15 mm long prior to culturing.

Both explants were cultured in borosilicate test tube containing 10 ml Murashige and Skoog (1962) medium without any hormone. Contamination was evaluated after two weeks.

Results and Discussion

Tissue contamination was visually detected within two weeks after sterilization treatment was given. Results were merely based on observation of the morphological appearance due to contamination. Contamination was evaluated according to five ranks i.e. 0 for no contamination without growth, 1 for no contamination with growth, 2 for 25 % contamination, 3 for 50 to 75% contamination, and 4 for more than 75% contamination (Tables 1 and 2).

In this study, the results indicated that young leaf explants achieved 100% aseptic cultures when sterilized with 10 to 20% clorox for 5 to10 minutes (Table 1). For effective disinfection of plant material, hypochlorite solutions should be used at pH 6 to 7. The bactericidal action of hypochlorite solutions is due to both hypochlorous acid (HOCI) and the OCI^- ion with the former being more active so that the disinfecting efficiency of chlorine is best in slightly acidic hypochlorite solutions.

Eliminating contamination in nodal segment explants was more difficult compared to young leaf explants. Mercuric chloride ($HgCl_2$) has been used as sterilant solution to reduce contaminations in these tissues. Contamination of nodal segment explants could be eliminated completely from 75% when treated with mercuric chloride. The results indicated that the use of 25% clorox for 15 min combined with 0.2% mercuric chloride for 5 to 20 min was the most consistent treatment in producing 100% aseptic cultures (Table 2).

Table 1. Contamination assessment of young leaf and nodal segment treated with commercial clorox of different concentrations for various duration two weeks after sterilization

Clorox (%)	Time (min) and explant types											
	5		10		15		20		25		30	
	YL	NS	YL	NS	YL	NS	YL	NS	YL	NS	YL	NS
5	3	4	4	4	3	4	2	4	4	4	4	3
10	1	4	1	4	3	2	4	4	4	4	4	3
15	1	4	1	4	3	4	4	4	3	4	4	4
20	1	4	1	3	2	4	3	4	0	4	3	4
25	4	4	4	3	4	4	4	2	3	4	4	4
30	4	4	4	4	4	3	4	4	4	4	4	4

Note: *YL – young leaf*
NS – nodal segment
0 – no contamination (no growth)
1 – satisfactory sterilization without killing the explant (with growth)
2 – 25% contamination
3 – 50-75% contamination
4 – more than 75% contamination

Higher contamination in nodal explants could be due to the explants being protected from any contact with the disinfectants through protrusions of waxes and trichomes together with gulleys and carvens that were formed by cell junction and stomata (Leifert et al., 1994). It could also be caused by endophytic contaminants that were hard to be eliminated by common methods used for the initial disinfection of explants (Marino et al., 1996).

Table 2. Contamination assessment of nodal segment treated with combination of commercial clorox of different concentrations and 0.2% mercuric chloride for various duration two weeks after sterilization

Clorox (%)	Time (min)	5	10	15	20
	10	3	3	3	3
20	15	3	1	1	1
	20	1	2	3	3
	10	2	2	1	1
25	15	1	1	1	1
	20	4	4	3	1
	10	1	1	1	4
30	15	2	1	2	1
	20	1	2	1	1
	10	1	1	1	1
35	15	1	1	0	0
	20	0	0	0	0

Note: 0 – no contamination (no growth)
1 – satisfactory sterilization without killing the explants (with growth)
2 – 25% contamination
3 – 50-75% contamination
4 – more than 75% contamination

Conclusions

In this study, treatment with 10 to 20% commercial clorox for 5 to 10 min was found to be an effective sterilant for young leaf explants while nodal segment explants required a stronger sterilant of 0.2% mercuric chloride soaked for at least 5 min to produce 100% aseptic culture.

Acknowledgements

The authors are grateful for the financial support provided by Faculty of Forestry, UPM, under FRGS – 5523017 and Graduate Research Fellowship Scheme. We are also grateful to the staff of Agrobiotechnology Lab, Faculty of Agriculture, UPM, for their technical assistance.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Optimizing a Protocol for Sterilization and *In Vitro* Development of Shoot Bud from Safed Musli

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Introduction

Safed Musli (*Chlorophytum borivilianum*) is a traditional medicinal plant which belongs to the family Liliaceae. This species is valuable for the dried fasciculate storage roots (Nayar and Shastry, 1988). These are reputed to have aphrodisiac properties and form an important ingredient of herbal tonics prescribed in the Ayurvedic system of medicine in India (Kirtikar and Basu, 1975). Tuberous roots of *C. borivilianum* possess immunomodulatory and adaptogenic properties and are used to cure impotency, sterility and enhance male potency. In Malaysia, Safed Musli was newly introduced due to its cultivation prospect and its potential. The natural regeneration of this herb is through tuberous roots that have become scarce in nature. However, its seeds have poor germination percentage (11 to 24%), low viability and long dormancy period (Rizvi et al., 2007). So to fill the gap of demand and supply, and to provide genetically uniform planting material from a known source, micropropagation is one of the most desirable options. Tissue culture techniques have been utilized for the conservation and multiplication of several medicinal plants. Considering Safed Musli is an endangered species and the availability of planting material is scarce, the use of tissue culture technique provides a rapid method to mass produce the plants.

Materials and Methods

Shoot bud explants were placed under running tap water for 30 min and then soaked in 5 g/L Benlate for 2 hours. Next shoot buds were immersed in 70% alcohol for 6 min, (20%, 30% and 50%) Clorox for 20 min, 0.1% aqueous mercuric chloride (10 min, 15 min and 20 min) and rinsed three times with sterile distilled water. Then the shoot bud explants were excised and cultured into MSO media supplemented with 30 g/L sucrose and 3.9 g/L gelrite. The treatments were replicated 10 times. The single factor experiment was arranged in a Randomized Complete Block Design (RCBD).

For development shoot multiplication system, shoot bud explants were excised and then cultured into MS media supplemented with 30 g/L sucrose, 3.9 g/L gelrite and BAP at (0, 1.0, 3.0, 5.0, 10.0, 15.0 mg/L). The treatments were replicated 20 times. The single factor experiment was arranged in a Randomized Completely Block Design (RCBD).

Results and Discussion

Highest percentage of clean shoot bud cultures (100%) was observed in treatment using 50% chlorox and 0.1% aqueous mercuric chloride (15 min) followed by the treatment containing 30% chlorox and 0.1% aqueous mercuric chloride (15 min) which resulted with 70% clean culture (Table 1). Treatment with high concentration of Clorox and long immersion of aqueous mercuric chloride showed the lowest percentage in clean culture due to explants dying after one week of culture (Figure 1).

ISBN: 978-967-344-188-4
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Table 1. Effect of different sterilization protocol on growth percentage of shoot bud explants after 4 weeks of culture

Treatment	Concentration of clorox (20 min) and immersion time in 0.1% $HgCl_2$	Percentage of shoot bud explants survived (%) ± sd	Percentage of field grown shoot tip explants contaminated by fungi and bacteria (%) ± sd
T1	20 % Clorox + 10 min in $HgCl_2$	20^{cd}±4.2	80±6.3
T2	30 % Clorox + 10 min in $HgCl_2$	20^{bcd} ±4.8	80±10.4
T3	50 % Clorox + 10 min in $HgCl_2$	50^{bcd} ±5.2	50±10.2
T4	20 % Clorox + 15 min in HgCl2	40^{bcd} ±5.1	60±9.9
T5	30 % Clorox + 15 min in $HgCl_2$	70^{ab}±5.1	30±9.9
T6	50 % Clorox + 15 min in $HgCl_2$	100^{a}±0	0
T7	20 % Clorox + 20 min in $HgCl_2$	60^{abc} ±5.1	40±9.6
T8	30 % Clorox + 20 min in $HgCl_2$	20^{cd} ±4.2	80±9.3
T9	50 % Clorox + 20 min in $HgCl_2$	10^{cd} ±3.1	90±7.9

Means followed by the same letter(s) ± standard deviation (sd) in the same column are not significantly different using Duncan new multiple range test (DNMRT) at p=0.05

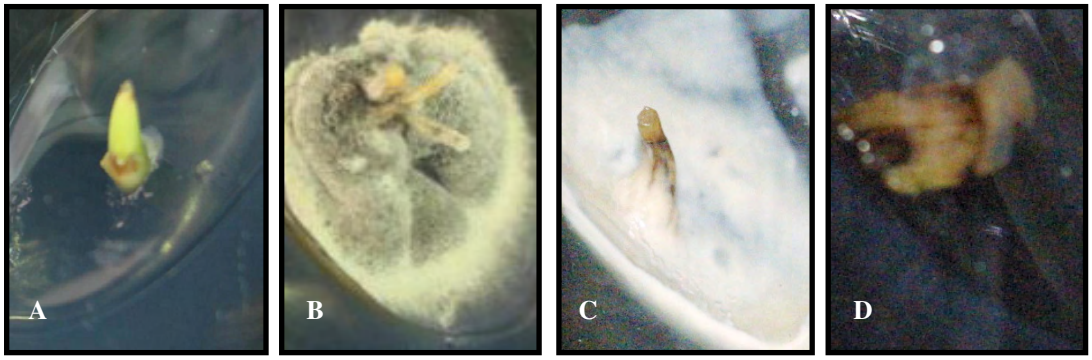

Figure 1. Appearance of explants in different conditions. Shoot bud of *C. borivilianum* survived explant (A), Contaminated explants caused by fungus (B). Contaminated explants caused by bacteria (C), Dead explants after one month of culture in treatment with high concentration of Clorox and long immersion of aqueous mercuric chloride (D)

All treatments containing BAP showed significant differences on a mean number of shoots produced per explants as compared to the control (Table 2). Shoot bud explants, in treatments containing 3.0 mg/L BAP produced the highest percentage of shoot regeneration (100%) with a mean number of 18.85 shoots produced per explants followed by treatment with 5.0 mg/L BAP with a mean number of 13.45 shoots produced per explants after 8 weeks of culture (Figure 2). In this study, the most suitable range of BAP for shoot multiplication from shoot bud explants of *C. borivilianum* ranged from 3 mg/L BAP to 5 mg/L BAP.

Table 2. Effect of different levels of BAP on percentage of shoot bud explants producing means number of shoots per explants obtained after 8 weeks of culture

Treatments BAP (mg/L)	Percentage of explants producing shoots (%) ± sd	Mean number of shoots produced per explants ± sd
MSO	$95^{a} \pm 0$	$6.88^{c} \pm 1.18$
1 BAP	$100^{a} \pm 0$	$12.45^{b} \pm 1.40$
3 BAP	$100^{a} \pm 0$	$18.85^{a} \pm 1.41$
5 BAP	$100^{a} \pm 0$	$13.45^{b} \pm 3.03$
10 BAP	$98^{a} \pm 0$	$11.78^{b} \pm 1.39$
15 BAP	$98^{a} \pm 0$	$11.63^{b} \pm 1.16$

Means followed by the same letter(s) ± standard deviation (sd) in the same column are not significantly different using Duncan new multiple range test (DNMRT) at p=0.05

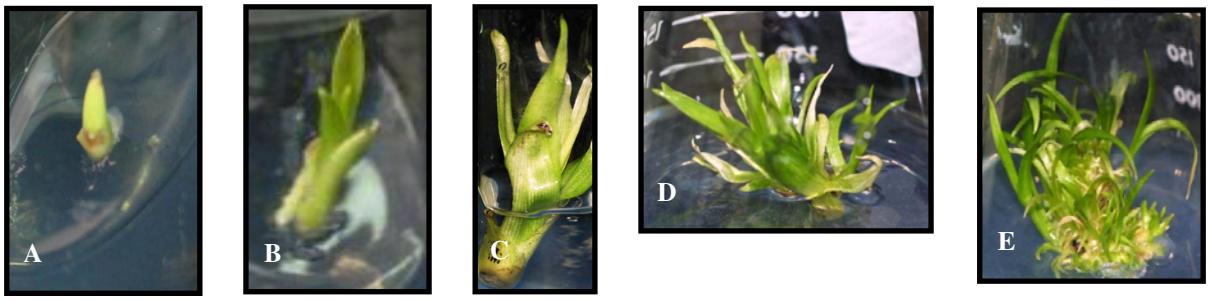

Figure 2. Development stages of shoot multiplication from shoot bud explants. Shoot bud explants in the 1st week of culture (A), at the 2nd week of culture (B), Shoot bud at the 4th week of culture (C), after the 1st subculture shoots were proliferated (D), Shoots multiplication after 8 weeks of culture (E)

Conclusion

A protocol for sterilization and *in vitro* development of shoot bud has been optimized for safed muesli.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Development of Plant Regeneration System from Immature Embryo of Eksotika Papaya (*Carica papaya* L.)

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Introduction

Papaya (*Carica papaya L.*) is considered one of the important fruit crops in many tropical and subtropical countries. It has great nutritive and commercial value. Eksotika papaya (*Carica papaya Linn.* var. Eksotika) has been introduced by MARDI (Malaysia Agriculture Research and Development Institute) in 1987. In Malaysia, this variety is one of the most popular crops for international and local market because of the special characteristics in terms of the shape and taste that fulfill the market demand. Due to the high potential market of papaya, research was conducted to overcome this issue. Papaya is conventionally propagated by seeds and has the disadvantage of heterogeneity as a result of cross-pollination (Bhattacharya and Khuspe, 2001). The need for *in vitro* explants arose because the establishment of cultures from explants obtained from outdoor is difficult due to severe contamination problems (Bhattacharya and Khuspe, 2001). Tissue culture techniques could offer a valuable alternative and reliable procedure for mass propagation of homogenous and uniform plants for both commercial and research purposes. This method could also facilitate the rapid propagation of superior selected lines. An efficient *in vitro* regeneration protocol is also imperative for papaya improvement through recombinant DNA technology. Most of the reports on papaya micropropagation using mature explants are conflicting. Somatic embryogenesis system using hypocotyl (Fitch, 1993) or immature zygotic embryos (Fitch et al., 1990) has been a successful *in vitro* regeneration method. This method is efficient and provides a large quantity of somatic embryos in a relatively short time, and is considered the best system for papaya transformation and regeneration. However, there were only a few studies reported so far on papaya Cv. Eksotika (Vilasini et al., 2000). Therefore, attempts were made in this study to improve a procedure for embryogenic callus indcution and plant regeneration system of Eksotika papaya with modification by using different hormones and handling to encountere those problems and successful regeneration of the plants.

Material and Methods

Explant sterilization and preparation

Immature green papaya fruits from the mid position of fruit bunch were selected as plant material. The fruits were thoroughly washed with the running tap water and sprayed with ethanol. Under aseptic condition, the fruits were cut half and the white immature seeds were taken out. Seeds were then surface sterilized using ethanol (70%) for 1min followed by treatment with Clorox (30%) containing 2 drops of Tween 20 for 10 min in the laminar air flow cabinet. The seeds were rinsed four times with sterile distilled water and blot dried on the sterile filter paper. By using forceps and scalpel, the testa of each seed was removed by cutting the seed at one side and then the immature seed was pressed at the mid portion to take out the immature zygotic embryo.

Induction of somatic embryogenesis

Excised immature zygotic embryos were inoculated on petri dishes containing two types of basal medium (MS and ½ MS) supplemented respectively with two concentrations of sucrose (30 g/L and 60 g/L), 400 mg/L glutamine and varying concentrations of 2, 4-D (8-14 mg/L) (Table 1),

combinations of NAA (0.5-2.0 mg/L) and Kin (0.2-0.8mg/l) (Table 2), 50 mg/L myo inositol and 4 mg/L gelrite. The pH was adjusted to pH 5.7-5.8. The experiment followed a completely randomized design with 10 zygotic embryos for each treatment and each treatment was replicated 5 times. The culture was incubated at 25 °C ± 2 °C in the dark.

Table1. Callus induction with different basal medium and concentrations of sucrose and 2, 4-D

2,4-D (mg/L) / Basal medium	Sucrose (g/l)	8 mg/L	10 mg/L	12 mg/L	14 mg/L
½ MS	30	A	B	C	D
	60	E	F	G	H
MS	30	I	J	K	L
	60	M	N	O	P

Table 2. Combination of selected concentrations of growth regulators NAA and Kin

Basal medium	Sucrose (g/L)	NAA (mg/L) / Kin(mg/L)	0.5	1.0	2.0
MS	60	0.2	Q	R	S
		0.5	T	U	V
		0.8	W	X	Y

Shoot regeneration from embryogenic callus

Several creamy white to light yellow thread like embryos appeared at the induction medium. The clumps of embryogenic callus were then transferred into full strength MS medium containing 30 g/L sucrose, 400 mg/L glutamine, 50 mg/L myo inositol and 4 g/L gelrite with different concentrations of Kin (0.2-0.8 mg/L), GA_3 (0.5-2.0 mg/L), and NAA (0.5-1.0 mg/L) (Table 3). All cultures were maintained in the growth room at 25^0 C with light (12/12 day/night) provided by cool-white florescent lamps.

Table 3. Combination of selected concentrations of growth regulators Kin, GA_3 and NAA

NAA (mg/L)	0			0.5			1.0		
Kin (mg/L) / GA_3 (mg/L)	0.2	0.5	0.8	0.2	0.5	0.8	0.2	0.5	0.8
0.5	R1	R4	R7	R10	-	-	-	R11	-
1.0	R2	R5	R8	-	R12	-	-	-	-
2.0	R3	R6	R9	-	-	-	-	-	-

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by mean differentiation test by Duncan's multiple range test using SAS (Statistical Analysis System) software.

Results and Discussion

Induction of embryogenic callus

Based on the result, formation of callus was obtained in all medium tested. For callus induction, ½ MS supplemented with 60 g/L sucrose and 14 mg/L 2, 4-D (Table 4) gave 92% callus induction which was the highest among all treatments. Meanwhile, the highest mean number (0.734) of embryogenic callus produced was shown in ½ MS supplemented with 60 g/L sucrose and 10 mg/L 2,4-D (Table 4). Although the percentage of callus was higher in ½ MS medium supplemented with 14 mg/L 2,4-D, induction of embryogenic callus was higher in ½ MS medium containing 10 mg/L of 2,4-D even in the same basal medium and concentration of sucrose. Medium supplemented with high concentration of 2, 4-D is important to enhance cell division and callus formation (Yusnita, 2004). Bhojwani and Razdan (1989) also reported that 2,4-D was the effective hormone for induction of embryogenic callus. Fitch (1993) found that an increase in osmoticum of sucrose resulted in a simultaneous increase in the percentage of somatic embryogenesis in 'Kapoho Solo' hypocotyls.

Table 4. Effects of different media supplemented with different concentrations of sucrose and 2, 4-D on percentage callus formation and mean number of embryogenic callus production

2,4-D (mg/L) / Basal medium	Sucrose (g/L)	8 mg/L	10mg/L	12mg/L	14mg/L
½ MS	30 g/l	65.5^{cde}(0.554^{ab})	77.5^{abc}(0.565^{ab})	80.5^{abc}(0.576^{ab})	88.5^{ab}(0.61^{a})
	60 g/l	88^{ab}(0.366^{bc})	74^{abcd}(**0.734^{a}**)	80^{abc}(0.313^{bc})	**92^{a}**(0.412^{abc})
MS	30g/l	90.5^{a}(0.298^{bc})	85^{ab}(0.504^{ab})	53.76^{def}(0.602^{ab})	84.5^{ab}(0.314^{bc})
	60g/l	36^{f}(0.58^{ab})	60^{cde}(0.564^{ab})	38^{ef}(0.08^{c})	42^{ef}(0.286^{bc})

Number in parenthesis presented the mean number of embryogenic callus produced
Means having same letter in the same column are not significantly different at 5% level of significance.

Besides using 2,4-D, combinations of NAA and Kin were also tested for embryogenic callus induction. MS supplemented with 60 g/L sucrose, 2.0 mg/L NAA and 0.2 mg/L Kin produced the highest percentage of callus (92%) and the mean number of embryogenic callus (1.956) (Table 5). Addition of NAA was good for callus growth. It showed that auxin was critical for the initiation and subsequent growth of callus. Among the 3 auxins tested, NAA was the most effective one for this purpose (Da silva et al., 2007). Litz and Conover (1983) indicated that addition of cytokinin to medium supplemented with NAA increased the growth of callus. There were different types of callus formation between media supplemented with 2, 4-D and combination of NAA and Kin (Figure 1) regarding the response of callus growth to hormone. The time required for the induction of callus in medium containing 2,4-D was relatively long as compared to the medium containing NAA and Kin. However the embryogenic callus produced by using 2,4-D was more friable as compared to that in the medium containing NAA and Kin. These types of embryogenic callus are preferred for transformation in the next study because of the availability to regenerate as compared to non friable callus.

Table 5. Effects of combination of different concentrations of NAA and Kin on callus formation and embryogenic callus production

Treatment	Growth regulator (mg/L)		Callus formation (%)	Mean number of embryogenic callus
	NAA	Kin		
Q	0.5	0.2	76[a*]	0.5880[d]
R	0.5	0.5	80[ab]	1.5140[b]
S	0.5	0.8	62[b]	0.7980[d]
T	1.0	0.2	62[b]	0.7130[d]
U	1.0	0.5	76[ab]	0.9200[cd]
V	1.0	0.8	76[ab]	0.7460[d]
W	**2.0**	**0.2**	**92[a]**	**1.9560[a]**
X	2.0	0.5	74[ab]	1.2420[bc]
Y	2.0	0.8	86[a]	1.4640[b]

Shoots regeneration from embryogenic callus

The embryogenic callus produced green shoots and plantlets when cultured in regeneration medium. All treatment combinations resulted in the formation of shoots from embryogenic callus. The highest percentage of shoot development was observed at the medium MS supplemented with combination of 0.2 mg/L Kin and 1.0 mg/L GA_3 (90.5%) (Table 6). The percentage of shoot development was significantly the highest as compared to the other treatments. GA_3 at an optimal level (1.0 mg/L) and low concentration of Kinetin in medium promoted the callus to shoot development. The results also showed that medium supplemented with NAA had decreased shoot development. Medium MS supplemented with 0.5 mg/L GA3, 0.1 mg/L Kin and vitamin Morel and Wetmore produced the highest number of plantlets according to Damayanti et al. (2007).

Table 6. Effect of varying concentrations of Kin, GA_3 and NAA on shoot development

Treatment	Growth regulator (mg/L)			Shoots development (%)*
	Kin	GA_3	NAA	
R1	0.2	0.5	0	45[cd*]
R2	**0.2**	**1.0**	**0**	**90.5[a]**
R3	0.2	2.0	0	34[de]
R4	0.5	0.5	0	34[de]
R5	0.5	1.0	0	6.5[g]
R6	0.5	2.0	0	18.5[fg]
R7	0.8	0.5	0	53.5[bc]
R8	0.8	1.0	0	9.5[fg]
R9	0.8	2.0	0	65[b]
R10	0.2	0.5	0.5	18.5[fg]
R11	0.5	0.5	1.0	33[de]
R12	0.5	1.0	0.5	21.5[ef]

**Values in the same column followed by the same letter are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test*

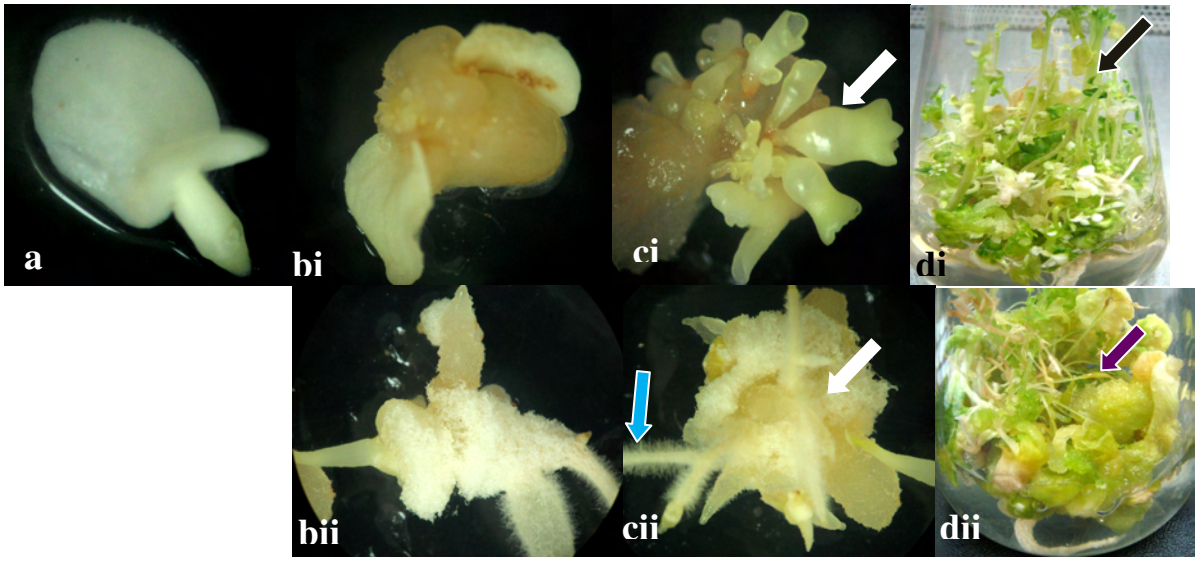

Figure 1. Regeneration of eksotika papaya from embryogenic callus in different medium. a) Immature embryos of papaya; bi) Callus induction in medium ½ MS+60 g/L sucrose+10 mg/L 2,4-D; ci) Embryogenic callus in medium ½ MS+60 g/L sucrose+10 mg/L 2,4-D; di) Shoot formation in medium MS+30 g/L sucrose+0.2 mg/L Kin+1.0 mg/L GA_3; bii) early callus induction in medium MS+60 g/L sucrose+2.0 mg/L NAA+0.2 mg/L Kin; cii) Full callus induction in medium MS+60 g/L sucrose+2.0 mg/L NAA+0.2 mg/L Kin dii) Shoot formation in medium MS+30 g/L sucrose+0.2 mg/L Kin+1.0 mg/L GA_3 from callus in medium MS+60 g/L sucrose+2.0 mg/L NAA+0.2 mg/L Kin. i) White colour arrow: Embryogenic callus; ii) blue colour arrow: root development; iii) black colour arrow: complete plantlet; iv) purple colour arrow: Abnormal shoots

Conclusions

The study showed that immature embryo had high ability to develop into embryogenic callus and shoots. There were differences in terms of embryogenic callus formation between medium containing 2,4-D and combination of NAA and Kin due to the structure of callus and time required for embryogenic callus production. For shoot development, callus that were obtained from 2,4-D developed into complete plantlets. However, callus cultured in medium supplemented with NAA and Kin showed abnormal shoot production. Histology study is necessary for determination of the right callus to be used for genetic transformation.

Acknowledgements

This study was supported by Research University Grant under the UKM-GUP-KPB-08-33-136 grant.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Molecular Cloning and Characterization of UDP-Glucose Pyrophosphorylase cDNA Clone from *Eucheuma denticulatum*

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Introduction

UDP-glucose pyrophosphorylase (UGPase; EC 2.7.7.9) represents an important enzyme for the biosynthesis of UDP-glucose which can be used in the synthesis of cell wall polysaccharides (Kleczkowski et al., 2004) and in production of the carbohydrate moiety (Flores-Diaz et al., 1997). This enzyme was mainly found in eukaryotic and also prokaryotic organisms. In plant, several UGPase have been isolated and characterized in *Cucumis melo* (Dai et al., 2006) and *Oryza sativa* (Chen et al., 2007). The occurrence of UGPase enzyme in red algae was first demonstrated in *Porphyra perforata* (Su and Hassid, 1962). Recently, Lluisma and Ragan (1999) demonstrated the nuclear gene of UGPase from *Gracilaria gracilis*. Herein we report the isolation of cDNA clone which encode UGPase from the marine red alga *Eucheuma denticulatum*.

Materials and Methods

Plant materials

Eucheuma denticulatum was purchased from Marine Borneo Research Institute, Universiti Malaysia Sabah and Lembaga Kemajuan Ikan Malaysia (LKIM) Sabah.

Isolation of UGPase full-length cDNA clone

Total RNA from *E. denticulatum* was extracted using the method described by Lopez-Gomez and Gomez-Lim (1992). 5'-RACE technique was performed to obtain the 5' ends of the putative EdUGP. The 5' first strand cDNA was obtained and amplified using the SMART RACE cDNA Amplification Kit (Clontech). cDNA sythesized from 1 µg total RNA of *E. denticulatum* was used as template for amplification of 5' cDNA fragments using the adapter and the gene specific primers with the following PCR program: 40 cycles at 94 ^{o}C for 30 s, 68 ^{o}C for 30 s and 72 ^{o}C for 3 min. Full-length cDNA clones were amplified using primers designed against the 5' and 3' untranslated region (UTR) of EdUGP cDNA and Advantage^{TM} 2 polymerase mix (Clontech) with first strand cDNA as template.

Sequencing and sequence analysis

Sequencing was carried out using a PCR-based dideoxynucleotide terminator protocol and an ABI PRISM Automated DNA Analyzer (Applied Biosystems). Alignment of deduced amino acid sequences of EdUDP with other UGPase encoding sequences were obtained using the ClustalW program. A phylogenetic tree was generated using the GeneBee TreeTop phylogenetic tree prediction server based on a cluster algorithm (Brodsky et. al. 1995). The default mode was used with PHYLIP and rooted parameters. Three-dimensional structure of this protein was generated using SWISS-MODEL by aligning the target sequence with the template found in this database (Arnold et al., 2006).

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Results and Discussion

Cloning of UGPase full-length cDNA clone EdUDP was obtained by 5'-RACE and LD-PCR approaches. The full-length EdUGP cDNA sequence was found to comprise of 1509 bp ORF (Figure 1). Phylogenetic analysis using Neighbor-joining method showed that *E. denticulatum* UGPase was closely related to UGPase isolated from algae such as *G. gracilis* and other eukaryotic tissues (Figure 2).

Multiple alignment analysis using ClustalW revealed that the deduced amino acid showed high identity with other plant species and the putative amino acid sequence of EdUGP contained five possible highly conserved Lys residues forming the active site of UGPase (Figure 3). 3D structure of UGPase exhibited the nucleotide and sugar binding loop of this enzyme (Figure 4), which might play an important role in the function of this protein.

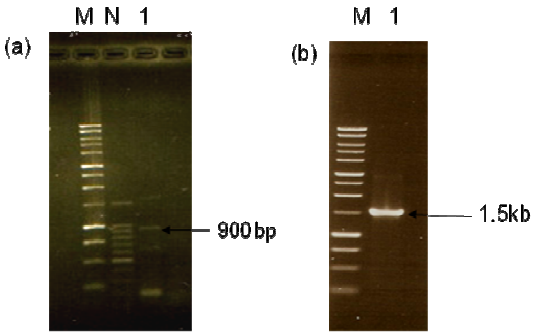

Figure 1. Agaros gel electrophoresis (1%) of (a) Lane 1: 5'-RACE product. (b) Lane 1: LD-PCR product; lane M: 1kb DNA ladder; lane N: 100bp DNA ladder

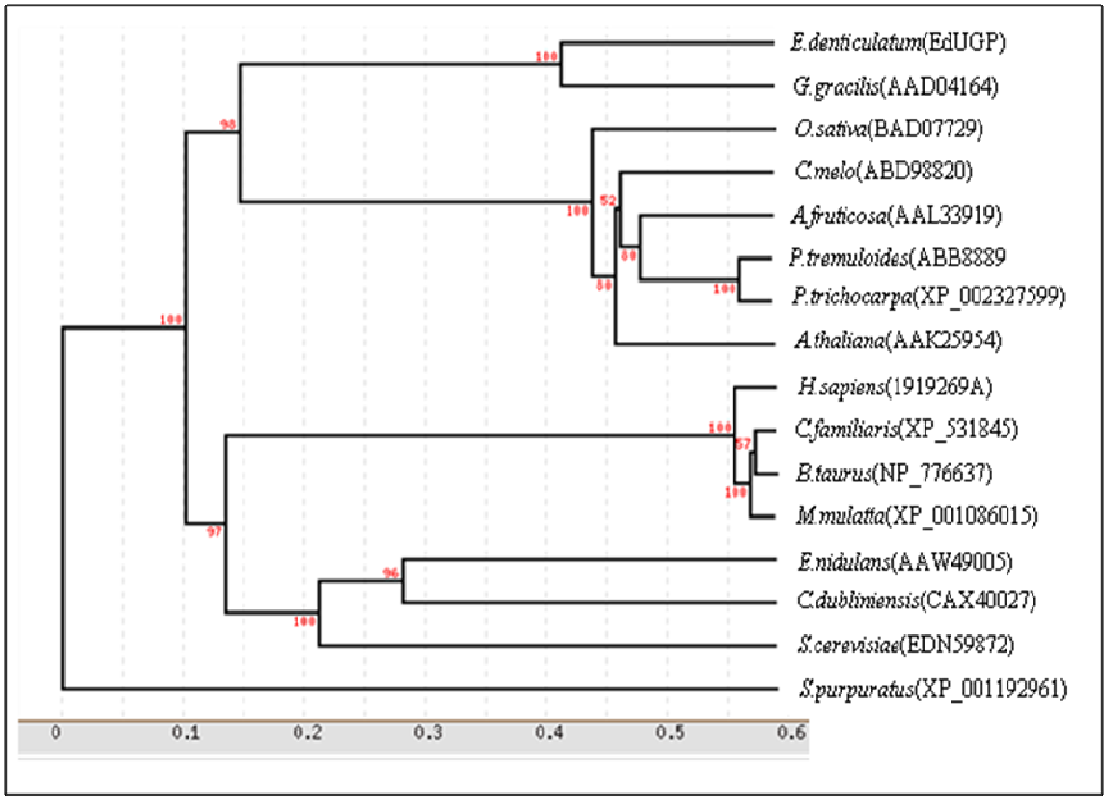

Figure 2. Phylogenetic analysis using Neigbour-Joining

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Trans. Malaysian Soc. Plant Physiol. 18

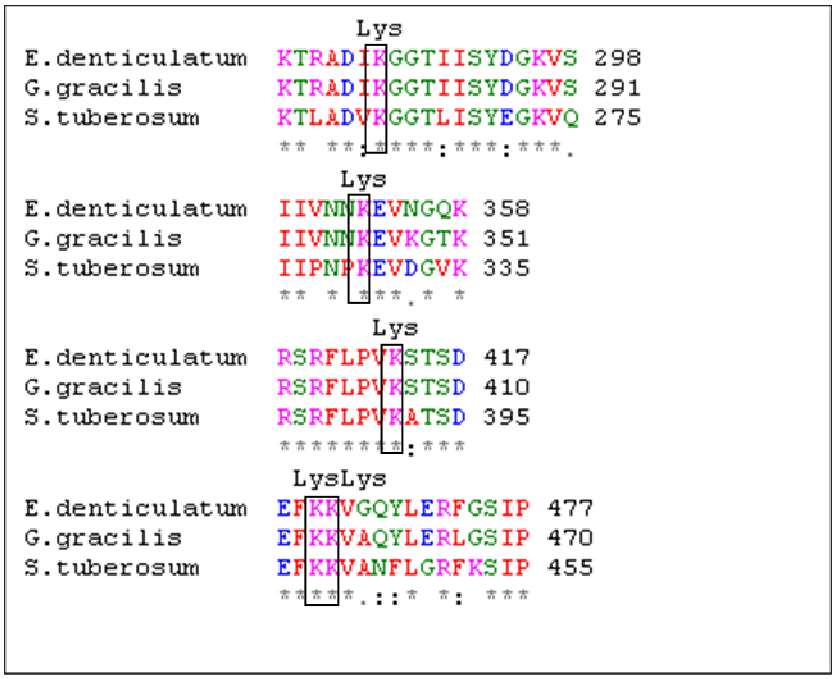

Figure 3. Amino acid alignment of EdUGP with other plant UGPase proteins using ClustalW. The conserved Lys residues forming the active site of UGPase are boxed.

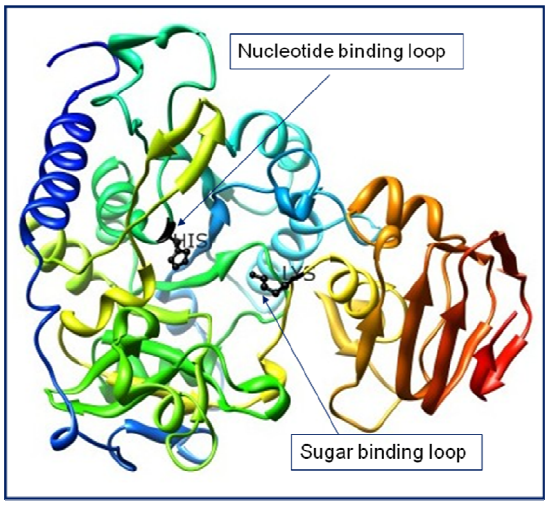

Figure 4. 3D structure of UGPase from *E. denticulatum* using comparative modeling strategy

Conclusions

A full-length cDNA clone encoding UGPase, EdUDP, with the size of 1.5 kb was successfully isolated. It shared high amino acid sequence identities with other plant UGPases and contained conserved Lys residues believed to play important roles for catalysis and substrate binding.

Acknowledments

This work was supported under the 02-01-02-SF0586 Science Fund grant.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Expression of a Mesocarp-Specific Subtilase Gene from Papaya

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Introduction

Plant subtilases which appear to be the largest class of proteases have been grouped into the pyrolysin family, which is characterized by a large insertion between the stabilizing Asn and the reactive Ser (Siezen and Leunissen, 1997). Subtilases occur in distinct parts of plant ranging from seeds to fruits in various plant species such as melon (Yamagata et al., 1994), lily (Kobayashi et al., 1994), *Alnus glutinosa* (Ribeiro et al., 1995), *Arabidopsis thaliana* (Neuteboom et al., 1999), and soybean (Nelsen et al., 2004).

Materials and Methods

Plant material

Papaya (*Carica papaya* L. cv. Eksotika) fruits were purchased from a private farm in the state of Selangor, West Malaysia. The samples were harvested at different stages of development (immature green, mature green) and ripening (5%, 25%, 50%, 75%, 100%), frozen in liquid nitrogen and stored at -80 °C. Other parts of plant (seed, leaves and flower tissues) were similarly frozen and stored.

Total RNA extraction and full length cDNA CpSUB1 cloning

Mesocarp at different ripening stages were ground in liquid nitrogen and the powder was subjected to RNA extraction using Lopez-Gomez and Gomez Lim protocol (1992) with minor modification. RNA isolation from seed, leaves and flower was done according to Verwoerd et al. (1989). Total RNA from different ripening stages was converted into 5' RACE-Ready cDNA using SMART^{TM} RACE cDNA Amplification Kit (Clontech, USA) according to the manufacturer's instruction. 5' RACE were performed with gene-specific primers and PCR was carried out before cloning into pGEMT-Easy vector and sequenced at the Centre for Gene Analysis and Technology, Institute of Systems Biology, UKM.

Expression and purification of CpSUB1 in E. Coli

The region of proprotein CpSUB1 (pro-CpSUB1) cDNA without the signal peptide was amplified by *pfu* taq (Promega, USA) using forward and reverse primers. *Eco*RI and *Xho*I restriction sites were included in the sequence of forward and riverse primers, respectively. The resulting PCR product was digested with *Eco*RI and *Xho*I, and ligated into the *Eco*RI and *Xho*I sites of the expression vector, pET32b(+) (Novagen, USA) to give the desired in-frame product. The resulting pETSUB was transformed into *E. coli* Origami (DE3) for fusion expression.

Purification of the fusion protein was carried out using Ni^{2+}-resin (Novagen, USA) according to the manufacturer's instructions for inclusion body purification. The desired protein was eluted with imidazole and concentrated before separation in SDS-PAGE. Polyclonal antibody was generated by injecting the purified pETSUB fusion protein into rabbits obtained from Animal House, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Full length genomic DNA cloning

Extraction of genomic DNA from papaya leaves was performed according to Kaufman et al. (1995). The genomic nucleotide sequences were amplified by PCR approach using DNA genomic as template. Specific primers were designed based on the previously isolated papaya subtilisin cDNA sequence (Nuraziyan and Othman, 2005). The amplified genomic DNA fragments were then cloned into pGEMT-Easy vector and sequenced at the Centre for Gene Analysis and Technology, Institute of Systems Biology, UKM.

Results and Discussion

The size of the subtilisin gene sequence (*sub*) obtained using the PCR approach was 3.0 kb. The gene contained 9 exons and 8 introns of varying lengths and encoded a predicted protein of 772 amino acid residues (Figure 1).

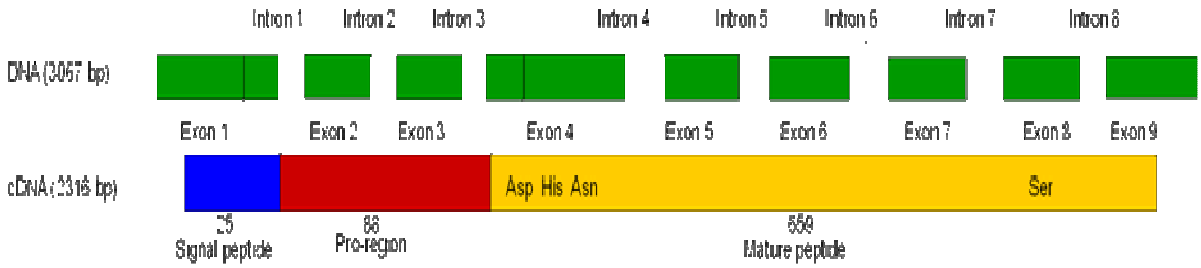

Figure 1. Organization of papaya subtilisin gene and mRNA. The amino acids forming the catalytic triad in the active site (Asp, His, Ser) and the conserved Asn are indicated.

RNA hybridization analysis showed that the expression of the *CpSUB1* gene increased from the mature green with maximum expression at 25% ripening stages and decreased thereafter. *CpSUB1* transcripts were only detected in mesocarp (Figure 2A and 2B).

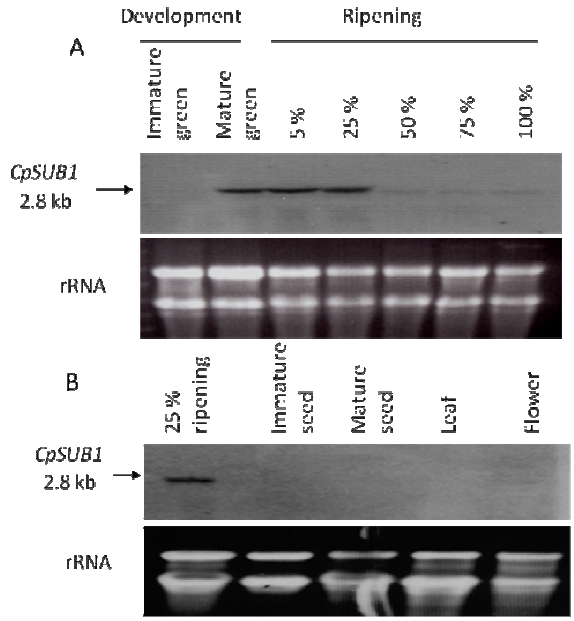

Figure 2. Northern blot analysis of *CpSUB1* gene. A. during papaya development and fruit ripening. B. in different organs

Protein gel blot analysis using anti-sub towards total protein extracted from all ripening stages revealed that a protein with a molecular mass of ~70 kDa was successfully expressed.

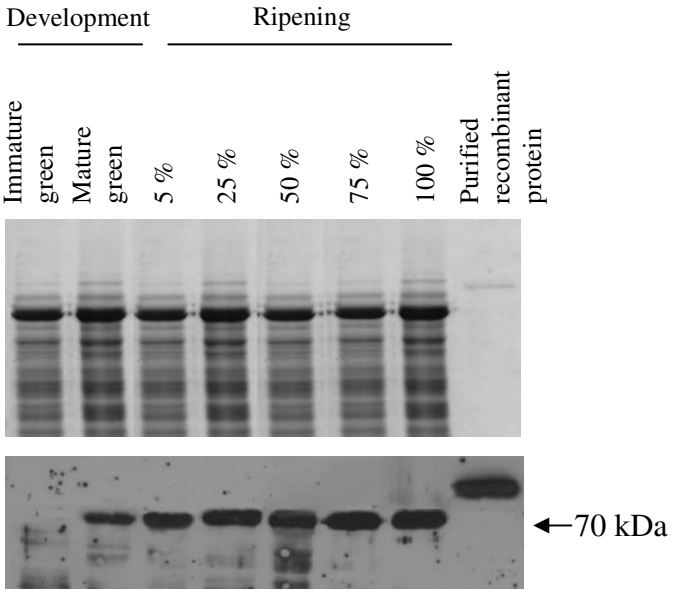

Figure 3. SDS-PAGE and Western blot analysis of total crude protein (30 µg per lane) from different development and ripening stages

Conclusion

RNA and protein gel blot analyses confirmed the presence of a mesocarp-specific subtilase during papaya fruit ripening.

Acknowledgements

This research was supported by the IRPA grant 01-02-02-0017-EA176 from the Ministry of Science, Technology and Innovation, Malaysia.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Protein Profiling for *Polygonum minus* Leaves

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Introduction

Polygonum minus or commonly known as kesum is an aromatic herb. It belongs to the family of Polygonaceae (Burkill, 1966) and the order of Caryophyllales. It flowers from August to September and can grow up to 0.5 m tall. The plant prefers sandy, loamy, clay soils and cool climate (15 °C - 24 °C). It is occasionally used as a flavouring ingredient in traditional foods in Asia, especially in Southeast Asia. The essential oil from kesum is thought to have high economic value as it is used in the food additive, flavour and fragrance industry (Karim, 1987). Traditionally, consumption of the kesum leaves was believed to increase blood body protection towards a variety of diseases (Balentine et al., 1997). Besides that, kesum is also known to have phytotherapeutic properties. The plant extract has been shown to display cytotoxic activity against human cervical carcinoma cell (Nomisah, 2005), anti-oxidant activity as well as anti-bacterial activity. Low-land kesum is found to have stronger aroma compared to high-land kesum.

Materials and Methods

Polygonum minus leaves were collected from high (Genting) and low (Lenggeng) land. Extraction of proteins from kesum leaves was best performed using phenol extraction method as describe by the manufacturer (TRI reagent, Molecular Research Center, Inc). One hundred µg and 450 µg of protein were loaded on 7 cm pH 3-10 and 18 cm pH 4-7 IPG strips (GE Healthcare), respectively. After silver staining, the gels were scanned with VersaDoc-imager (Biorad). The gel images were analyzed with the software package PDQuest 8.0 (Biorad) to calculate the number of spots and for comparison analysis. Protein samples were run on 2D-PAGE gel and the peptide mixtures were generated by an in-gel tryptic digest. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) experiments were performed on a MALDI micro MS™ MICROMASS mass spectrometer (Waters). For identification of proteins, the peptide mass finger printing data were used to search in NCBI databases for Viridiplantae using Mascott program (http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=PMF). The peptide mass fingerprinting of the proteins were scored with the Mowse score.

Results and Discussion

Protein extractions were performed from leaves collected using four different methods: Tris buffer method, direct lysis method, TCA-acetone method and phenol extraction method. Protein profile of *P. minus* was best produced by phenol extraction method (Figure 1). Two dimensional-PAGE (2D-PAGE) results showed that the phenol extraction method produced protein profiles with best resolved and separated protein spots. The phenol extraction method also produced the highest number of detectable protein spots.

A total of 929 and 940 protein spots were detected in the protein profiles of Genting and Lenggeng leaves, respectively (Figure 2). At least 93 protein spots were found to be unique in the protein profiles of Genting leaves and 104 protein spots in the Lenggeng leaves. At least 25 protein spots were found to be up-regulated in the protein profiles of Genting leaves and 26 protein spots down-regulated. The difference in protein profile of Genting and Lenggeng leaves are predicted to be caused by environmental stress.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

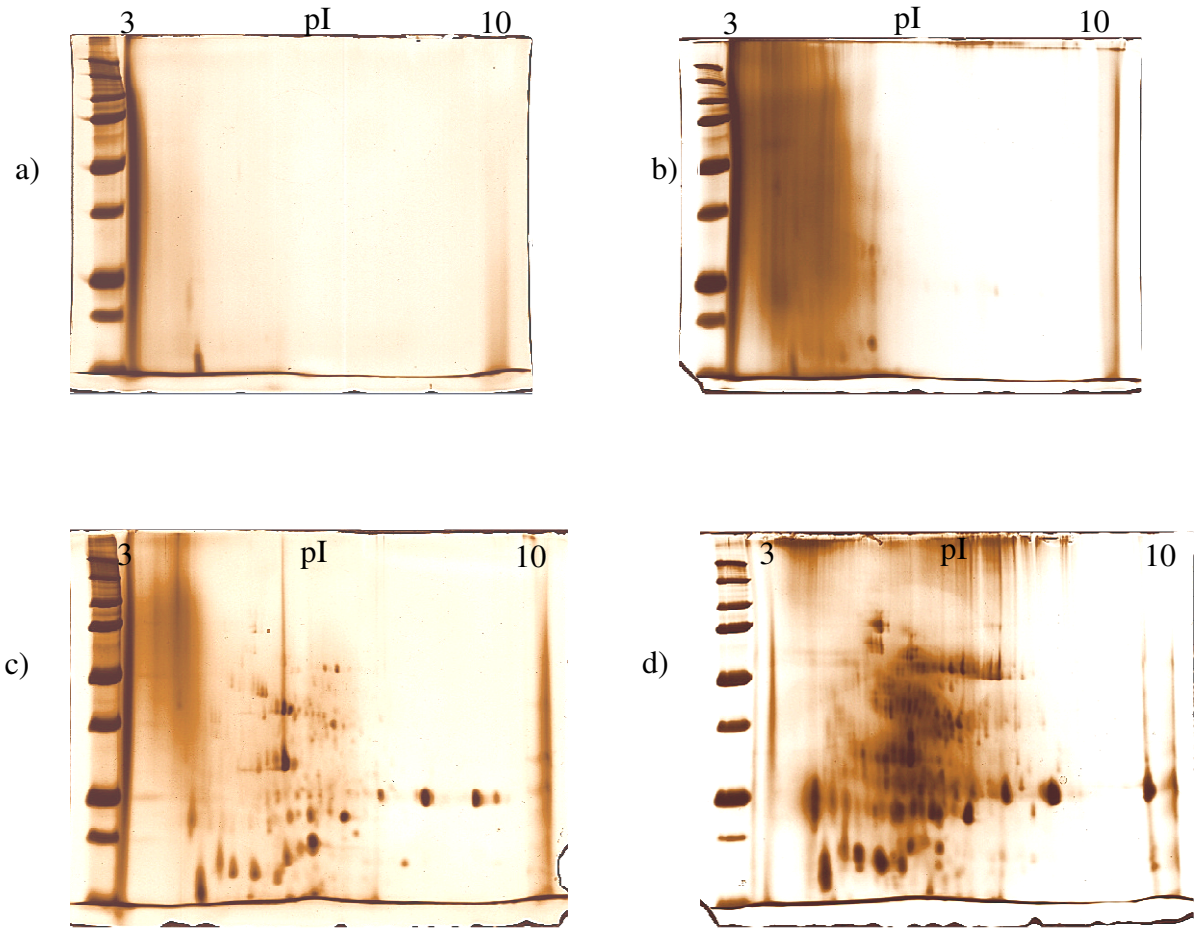

Figure 1. Two-dimensional gel electrophoresis of protein extraction optimization using (a) Tris base method (b) direct lysis method (c) TCA acetone method (d) Phenol method

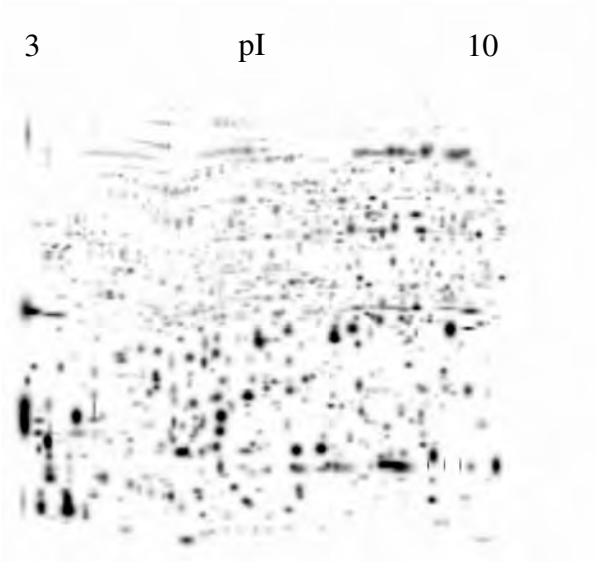

Figure 2. The master gel of 2D-PAGE of proteins expressed in Genting and Lenggeng

Three proteins have been identified using MASCOT search. Those proteins are ABB90028 from *Solanum tuberosum*, hypothetical protein B1136H02.5 from *Oryza sativa* and ATP synthase subunit

beta from *Nephrophyllidium crista-galli*. ABB90028 involves in ATP synthesis coupled electron transport while ATP synthase subunit beta produces ATP from ADP in the presence of a proton gradient across the membrane.

Conclusions

The protein profile of *P. minus* has been successfully produced using phenol extraction method. This method produced 929 and 940 protein spots for Genting and Lenggeng leaves respectively. Protein ABB90028, hypothetical protein B1136H02.5 and ATP synthase subunit beta might be expressed by *P. minus*.

Acknowledgements

This research was supported by Ministry of Science, Technology and Innovation: Research Grant UKM-MGI-NBD0008-2007

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 8

SEED TECHNOLOGY AND HIGH QUALITY PLANTING MATERIALS

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Desiccation Tolerance of *Euphoria malaiensis* Seeds

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Introduction

Propagation of many tropical fruit species is mainly by using seeds. Research on seed storage of these species is also carried out for germplasm conservation. Before seed storage can be implemented successfully, a full understanding of the requirements for seed germination and desiccation tolerance is essential (Tang and Long, 2008). Seeds sensitive to desiccation are common among species growing in permanently moist areas of tropical rainforest (Quedraogo et al., 1998; Tweddle et al., 2003; McDonald, 2004; Black et al., 2006). They are not amenable to long-term storage.

Euphoria malaiensis is a commercial tropical fruit species. It belongs to the same family as rambutan and pulasan, i.e. Sapindaceae. It is called Dragon's eye by the Chinese. The fruits are produced in bunches, globose with average diameter of 2 cm. Each fruit is covered by shell which is pale brown and rough to the touch. Within the shell is opalescent sweet aril covering the seed. There are substantial canning industries for the aril. The preserved dry aril is also a commercial product with medicinal values (Choo, 2000). The seeds within are extremely large relative to the thickness of aril (Sahadevan, 1987).

Euphoria malaiensis seeds are recalcitrant and therefore, short-lived and best sown fresh. As the seeds are killed rapidly when desiccated, it is important to know their lowest safe moisture content for proper handling prior to germination. However, very few documents on the seed handling and storage of this species were available. Systematic research is useful to better understand the behaviour of these seeds. This study was, hence, aimed to determine the lowest safe moisture content of *E. malaiensis* seeds.

Materials and Methods

The ripe fruits of *E. malaiensis* were obtained from the estate of Taman Kekal Pengeluaran Makanan, Rhu Tapai, Setiu, which is a 30 hectare-cultivation area. A pair of scateurs was used to harvest the fruits. The fruits were taken to the laboratory and the seeds were extracted carefully from the fruits by removing the aril that covered the seed. Then, the seeds were cleaned by running tap water. The cleaned seeds were then pat dried by using kitchen paper towel and subjected to ambient desiccation treatment.

Desiccation of seeds was carried out at Plantation Technology Laboratory 603 at Faculty of Applied Science, Universiti Teknologi MARA Shah Alam. A total of 960 seeds were used for experimentation. The seeds were randomly subjected to desiccation for 0, 1, 2, 3, 4, 5, 6 and 7 days. For each desiccation period, 60 seeds in six replicates, each with ten seeds, were drawn randomly for germination test. Seeds were germinated in moist sand sized 0.2 to 2.0 mm in enclosed plastic boxes on the racks outside the laboratory. The covers of boxes were opened for a few seconds daily to allow sufficient air (oxygen) to flow into the boxes for seed germination. A seed was considered as germinated seed when the emergence of radical was observed. The germination count was recorded for a period of two weeks.

Germination index was calculated as sum of ratios of the number of normal germinated seeds removed each day to the number of days since those seeds were sown.

Germination index = Σ [Number of normal germinated seeds/Number of days after sowing]

For each desiccation period, another 60 seeds in six replicates were also drawn randomly for the determination of seed moisture content. There were also ten seeds per replicate. The seeds were dried at 103°C for 16 hours in the oven and seed moisture content (MC) was calculated by using the following formula.

MC (%) = [(M1-M2)/(M1-M3)] X 100

where,
M1= Weight of seeds in moisture dish before oven drying (g)
M2= Weight of seeds in moisture dish after oven drying (g)
M3= Weight of moisture dish (g)

Results and Discussion

Euphoria malaiensis seeds were very sensitive to desiccation. The fresh seeds and those desiccated for only 1 day under ambient condition had 98% and 90% germination respectively (Table 1). Such seeds had above 30% moisture content (Table 1). However, seeds desiccated for 1 day under ambient condition were slightly less vigorous as compared to the fresh seeds (Table 1). The seed vigour was spelt out as germination index (Table 1). The fresh seeds started germination at 1 day-after sowing (DAS) but most of them germinated at 3 DAS (Figure 1). Some small number of fresh seeds germinated at 4, 5 and 6 DAS (Figure 1). Most of the seeds desiccated for 1 day germinated only at 3 or 4 DAS (Figure 1). Some of them germinated at 5 DAS and small number of them took even 9 or 11 days for germination (Figure 1).

When desiccation was prolonged to 2 days or longer under such condition, the seed moisture content dropped to below 30%, i.e. 27% with 2 day-desiccation, about 23% with 3 and 4 day-desiccation and below 20% with longer desiccation periods (Table 1). Most seeds were killed when moisture content was reduced to 27% (Table 1). There was only 8% germination with seed moisture content of approximately 27%. Almost all the seeds died with moisture content 23% or below. Such observations were also documented by Cruz (2007). When moisture content of *Theobroma grandiflorum* seeds was reduced from 88.6 to 30.8%, the seed germination was not affected. Germination, however, dropped significantly when seed moisture content was further reduced to below 30.8%.

Table 1. Moisture content and germination of *E. malaiensis* seeds after desiccation for varying periods

Desiccation (days)	Moisture Content (%)	Germination (%)	Germination Index
0	37.14	98.33	19.65
1	31.53	90.00	14.20
2	26.55	8.33	1.20
3	22.54	0.00	0.00
4	23.13	0.00	0.00
5	19.81	0.00	0.00
6	16.98	3.33	1.14
7	14.10	0.00	0.00

Euphoria malaiensis seeds had relatively high lowest safe moisture content in the range of 27-32%. Detail studies should be carried out to determine the more precise lowest safe moisture level for the seeds of this species. Seeds desiccated for 2 days or longer as studied showed wrinkled appearance. Hence, this change of appearance can probably be used as an indicator on the loss of viability of the seeds. Storage of *E. malaiensis* seeds may be a difficult task based on this study as seeds were killed at relatively high moisture content of 27%. Moist storage of the seeds as the alternative means of seed storage may also be difficult as the seeds are susceptible to fungal infestation when stored under any

conditions to maintain high seed moisture content of above 27%. In addition *E. malaiensis* seeds also germinated fast, i.e. within 3 to 4 days. Germinated seeds are difficult to handle during storage.

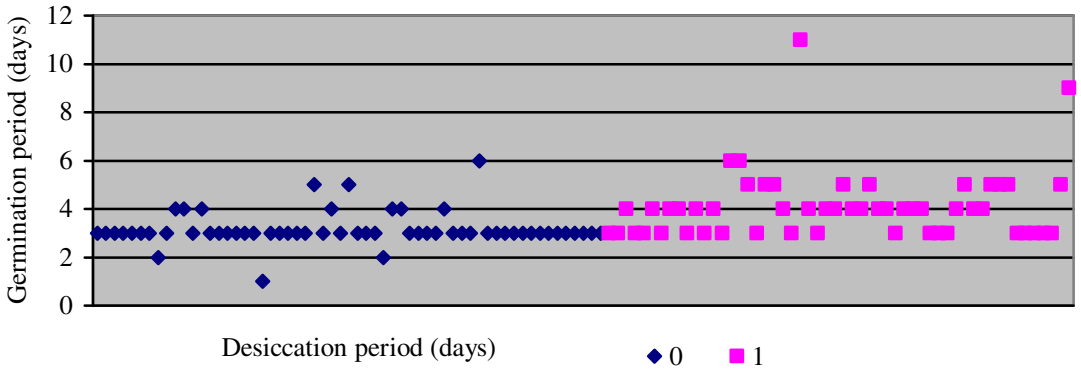

Figure 1. Germination period of *E. malaiensis* seeds after ambient desiccation for varying periods

Conclusions

Euphoria malaiensis seeds could only tolerate desiccation under ambient condition for up to 1 day with high germination of 90%, when the moisture contents were above 32%. Further desiccation for 2 days or longer under such condition was detrimental to the seeds even though the seed moisture content at 2 day-desiccation was still relatively high, i.e. approximately 27%. *Euphoria malaiensis* seeds can be concluded to have high lowest safe moisture content in the range of 27-32%.

Acknowledgements

The authors would like to thank the manager of the estate of Taman Kekal Pengeluaran Makanan, Rhu Tapai, Setiu, for supplying the test materials. We would also like to thank the Research Management Institute of Universiti Teknologi MARA for financial support of the project.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Shoot and Root Growth of Kelor Stem Cuttings (*Moringa oleifera)*

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Introduction

Moringa oleifera belongs to the family Moringaceae. Every part of this plant, including the pods and flowers, is valued especially for oil extraction. Kelor is known to be propagated either by seeds or cuttings. Cuttings root very easily and are usually preferred. Plants raised from seeds are highly variable. They take a long period to yield and produce fruits of inferior quality (Ramanchandran et al., 1990; Morton, 1991). In the Philippines, branches or cuttings may be set in the ground at distance of up to 1 m apart to form hedges or 'living fences'. While kelor grown by seed takes 2-3 years to start flowering, those propagated by cuttings only take around 15 months for the same event. However, there is insufficient documented information about using stem cuttings as planting materials. The objective of this experiment was, hence, aimed to examine the root and shoot initiation from six different types of stem cuttings of two varieties of kelor for propagation purposes.

Materials and Methods

Stem cuttings were collected from kelor trees around Sri Serdang and Universiti Putra Malaysia (UPM) campus. Two different accessions of kelor were identified: Accession A produced long and thin pods with smooth stem while Accession B had short and thick pods with rough stem. Cuttings of 30 – 35 cm long were taken from three different types of wood: hardwood, semi hardwood and soft wood for each accession. Before being propagated, all the leaves on each cutting were removed. The basal 10 cm part of each cutting was cut at a slant to give more surface area for root growth. The cuttings were propagated either fresh or after being dried for three days. Each cutting was planted to a depth of 10 cm in a raised propagation bed containing a moist medium of coarse sand under the rain shelter. Misting with water was done 2-3 times per day. The rooting bed was sprayed with 0.2% Thiram (Tetramethyldhiuram disulfide) two weeks before the rooting experiment and weekly subsequently to minimize fungal infection and contamination.

Two propagation experiments were carried out, each with cuttings of each accession as mentioned. Each treatment was replicated five times with 5 stem cuttings per replicate. Growth characteristics were measured at three week-intervals, i.e. from the third to the 12th week of propagation study.

Results and Discussion

The results showed that initiation of shoot occurred at the beginning of week two for both accessions. With both accessions, the collar of first leaf was visible about five days after propagation. At the early stage, very little shoot elongation occurred. In Accession B, the initiation and elongation of leaf and shoot were nearly completed on the 6th week. From week six, the shoot elongated rapidly. Then a continuous rapid shoot elongation occurred. The time between the appearances of new leaf stages started to shorten. This could be because of the large difference among individual plots resulting in very low coefficients of determination. Correlations between root formation and axillary bud growth had been reported earlier for *Schefflera* and *Stephanotis* (Hansen and Kristensen, 2000). The bud showed rapid internodes elongation until the last stage (beginning bloom). Dried hardwood cuttings produced the first shoot because of the presence of natural hormone, gibberellins, at the shoot tip. At the same time, when cuttings started to develop, a steady and continuous nutrient is needed for shoot elongation. Shoot height was not significantly affected by type of cuttings and propagation duration in this study.

The results showed that root elongation started on week three. An exponential response between root length and propagation duration occurred from week three to week six. However, from week six to week nine, the increase in root length was consistent. There was a rapid increase in root length again from week nine to twelve. A significant and similar increase in root length occurred with both accessions with the dried hardwood and dried semi-hardwood (Figures 1 and 2). Uniform performances were noticed from week three to week twelve. Root length of dried softwood cuttings was not significantly different with Accession A. The maximum root length of cuttings of both accessions was almost identical. Each root produced 3-4 secondary roots. According to the observation during the final duration of the study, the roots grew extensively and were distributed in the propagation bed. The nodal root system began to elongate from week six onwards. A set of nodal roots began development on each progressively higher node of the cutting (Yamashita and Immamura, 2007).

For both accessions, root length and root number were negligible on week three with fresh softwood cuttings (Figures 2C and 2D). There was growth of shoot in these cuttings except for fresh hardwood, dried hardwood and fresh semihardwood. Evidently, shoot growth occurred due to available reserves in the cuttings even when nutrient was not supplied. In this growth phase, the water uptake by the cuttings was by passive movement from the soil to the surface of cutting and then to the bud. Shoot production in this plant occurred on week nine of propagation in the absence of roots. Roots are of cause needed for longer growth period.

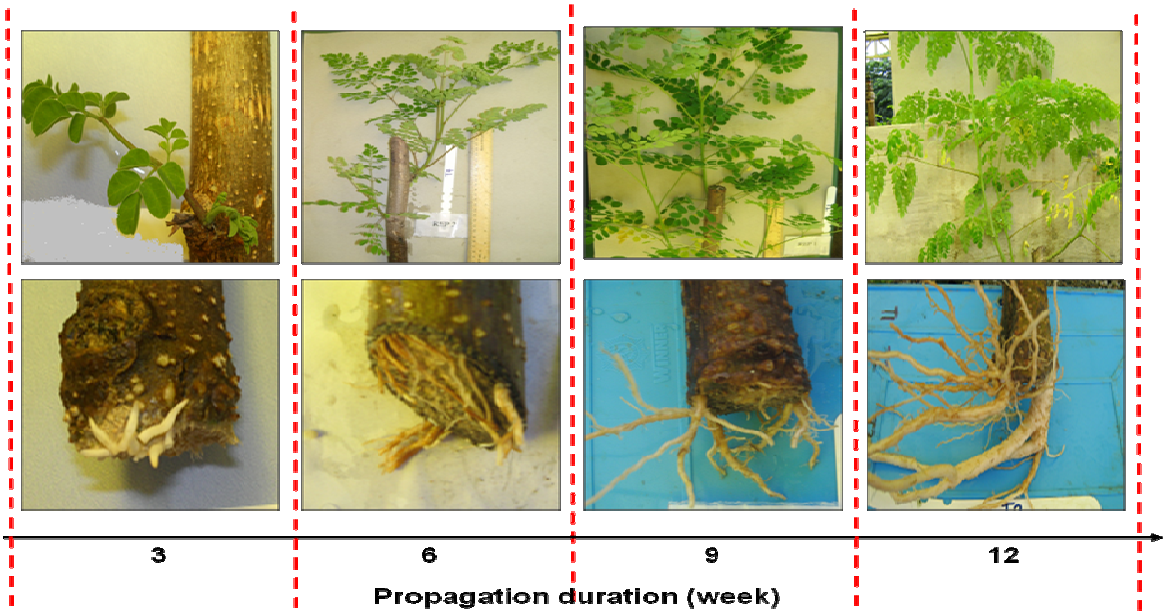

Figure 1. Growth and development of fresh semi-hardwood shoots and roots of kelor cuttings of Accession A

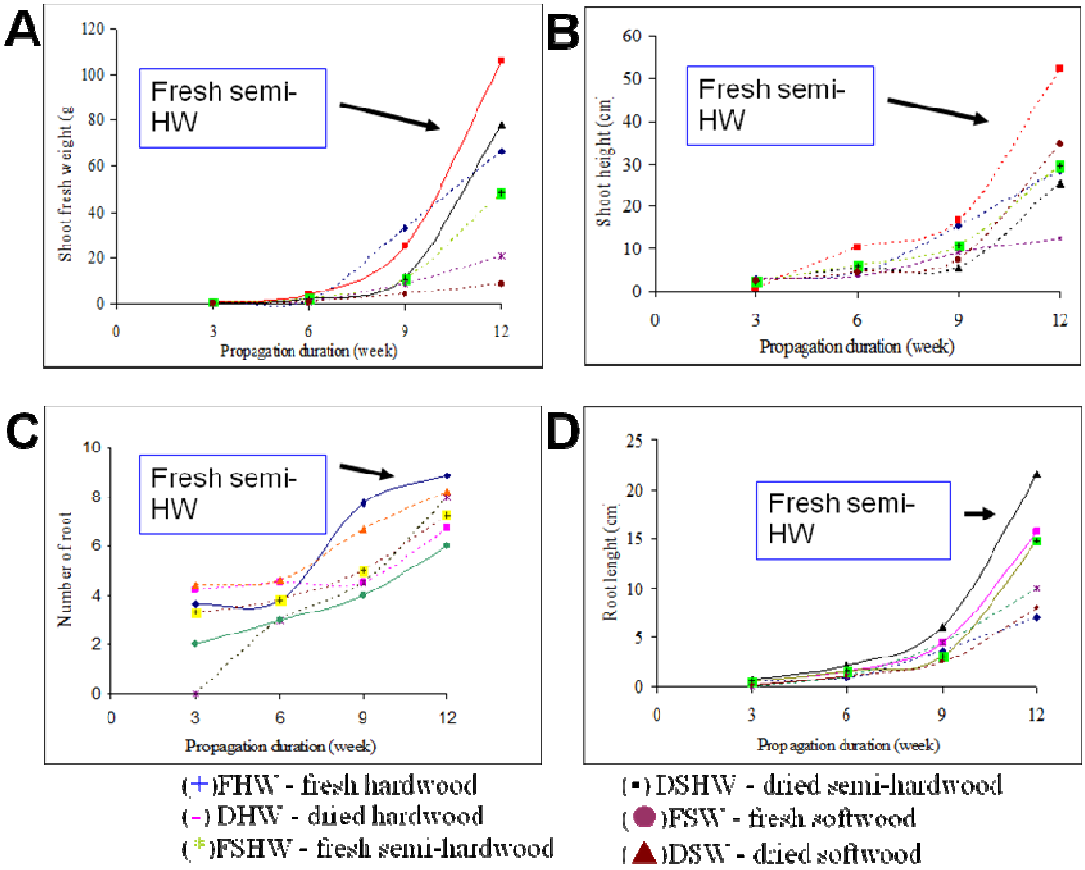

Figure 2. Shoot fresh weight (A), shoot height (B), number of root (C) and root length (D) of cuttings from Accessions A and B cuttings of kelor during 12 weeks of propagation (x), $y = Axe^{bx}$, where A and b are constants.

Conclusions

In conclusion, cuttings of dried hardwood are recommended as planting material based on the results presented here by both accessions, especially for the purpose of shoot production while fresh semi-hardwood cuttings could be recommended as planting materials having the best root growth. Good watering should be practiced to induce the formation of shoot, adventitious root and overall growth of *Moringa oleifera* cuttings.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

CHAPTER 9

MODELING AND SIMULATION

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Allometric Relationship of Trees Based on Ecological Grouping in Hill Dipterocarp Forest, Peninsular Malaysia

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Introduction

Tropical rainforests owned a diverse and complex tree structure than other forest types (Claussen and Maycock, 1995). Competition with light and different growth rate presented a multilayer of crown in tropical rain forest. Kohyama (1996) wrote the existence of multiple species in the tropical forest create the multiple of crown layers of a forest stand. Modelling the tree allometry based on the tree height and dbh allometry, helps to understand the characteristic of this complex forest. Osunkuya et al. (2007) found that crown position is one of the keys for better understanding of the forest structure and dynamics.

Sposito and Santos (2001) wrote that it is important to know the relationship between size and shape in trees when to understand the differences among species, competitive interactions, and the structure and dynamics of the forests. The developed of allometric function in each forest was vary because of tree density, tree size, taller and shorter species, both within and between species, demography and forest types (King, 1990; Aiba and Kohyama, 1996, 1997; Davies et al., 1998). There is a need to develop an allometric function in each study site for better understanding of the forest structure and function. In this study, we examine the allometric function of total height and dbh of trees from various ecological groups of a hill dipterocarp forest.

Materials and Methods

The study area is located in the Semangkok Forest Reserve (FR), Selangor, Peninsular Malaysia (3.0N, 103.0E). Semangkok FR is a hill dipterocarp forest of the sub-type Seraya Ridge forest due to the predominance of large *Shorea curtisii* Dyer *ex* King on the ridges. The plot dimension is 200 x 300 m across the topography. The Semangkok FR plot has been monitored for long-term changes in the population dynamics of trees. The demography of the plot was narrow ridge and steep slope ranging 340 - 450 m above sea level (Niiyama et al., 1999).

All species with tree abundance of more than 20 individuals were measured. A total of 2951 trees were measured and 64 species were identified. All trees which ≥ 5 cm DBH were measured from December 2007 until July 2008. Measurement of tree height was taken using a rangefinder. Tree height was considered as the length of tree from base ground until the highest foliage. Tree less than 5 m tall were measured directly with measuring pole. DBH were measured on non-buttressed trees at a height of 1.3 m above ground level and 20 cm above buttress on buttressed trees.

Data Analysis

Data summary: All trees were categorized to ecological functional groups based on Manokaran and Swaine (1994):

(a) Emergent - mature tree, highly light demanding, long lived species, mostly with spreading crowns and growth above the canopy level for primary forest and reach more than 30 m height.
(b) Canopy - mature tree, highly light demanding, long lived species, crown of each individuals form the forest canopy and trees height range between 20-30 m.

(c) Understorey - shade-tolerant species, dominant at the lower strata below canopy and tree heights usually below 20 m.
(d) Treelet - tolerant of shade, light-demanding and form as a part of the mature forest.

Summary statistics of collected data on Ht and DBH measurements are showed in Table 1. Individuals with any evident crown damage, dead, fallen tree, stem with top broken, main stem dead and sprout were discarded from the analysis. The tree mean diameter indicated the differences in the size of the trees in the size of the trees in relation to its functional groups.

Table 1. Descriptive statistics for the Height-DBH data set

Ecological group	Number of trees	Tree diameter (cm)		Tree height (m)	
		Mean	Range	Mean	Range
Emergent	433	37.2	5.0 - 181.5	19.8	3.1 - 59.3
Canopy	1326	18.4	5.0 - 120.5	13.0	3.0 - 48.2
Understorey	1139	12.2	5.0 - 82.5	9.7	1.8 - 49.8
Treelet	53	8.0	5.0 - 24.8	6.7	2.2 - 13.0

Regression analysis: Three regression models were tested to determine the best fitted model for the height~dbh allometry. The regression model tested with linear, logistic and non-linear model (Table 2).

Table 2. General form of the regression model

Types	Model	
Linear:	$Ht = b_0 + b_1DBH + \varepsilon$	[1]
Logistic:	$Ht = b_0 / (1 + \exp (b_1 + b_2DBH) + \varepsilon$	[2]
Exponential:	$Ht = a + \exp (b_0 + b_1DBH^{b2}) + \varepsilon$	[3]

Where:
Ht = tree height (m)
DBH = diameter at breast height (cm)
b_i = regression coefficients
a = 4.5 foot ≈ 1.3 meters above ground; Ht = 4.5ft when DBH = 0
ε = error terms

Height is treated as dependent variable while DBH as independent variable. The exponential model was adapted from Wang and Hann (1988) research in the R statistical package program (version 2.9.2).

Each fitted model will be evaluated on its goodness-of-fit by using root of mean square error (RMSE), Akaike Information Criterion (AIC), Bayesian Information Criterion (BIC), coefficient of determination (R^2) and Furnival Index (FI). The best fitted model that explained the relationship between Ht-DBH for these ecological group will be chosen if; i) scatter plot and residual plot of fitted model produced a normal residual distribution and constant variance of error terms, ii) get the lowest value of RMSE, AIC, BIC and FI, iii) and the highest value of R^2.

Results

Based on the criteria mentioned above, the exponential model was selected for emergent, canopy and understorey, while the logistic model for treelet. Mentioned below we present a more detail explanations on the chosen fitted model for each ecological group. The detail of the goodness of fit results for all models is given in Table 3.

Table 3. Parameter estimates and regression coefficients and associated statistics for these ecological groups

Ecological group	Parameter estimates								
	Constant 'a'	b_0	b_1	b_2	RMSE	R^2	AIC	BIC	FI
EMERGENT									
Linear	-	9.3130 ***	0.2825 ***	-	2.5610	0.7642	2788.03	2800.24	41.15
Logistic	-	39.9454 ***	1.5773 ***	0.0518 ***	4.7630	0.8617	2585.53	2601.81	76.53
Exponential	4.5	4.238 ***	-8.7805 ***	-0.5404 ***	4.6200	0.8617	2559.13	2575.42	74.24
CANOPY									
Linear	-	6.5562 ***	0.3821 ***	-	3.851	0.6673	7343.02	7358.68	46.55
Logistic	-	28.604 ***	1.3429 ***	0.0713 ***	3.655	0.7006	7205.12	7225.98	44.18
Exponential	4.5	4.2641 ***	-7.1249 ***	-0.4474 ***	3.578	0.7131	7169.52	7169.52	43.25
UNDERSTOREY									
Linear	-	5.1240 ***	0.4113 ***	-	3.078	0.5743	5797.40	5812.51	28.41
Logistic	-	27.8657 ***	1.3883 ***	0.0678 ***	3.124	0.5620	5832.06	5852.21	28.83
Exponential	4.5	6.1547 ***	-7.7379 ***	-0.2280 ***	3.045	0.5838	5773.76	5793.91	28.10
TREELET									
Linear	-	4.3587 ***	0.3598 ***	-	2.086	0.2581	232.30	238.215	14.25
Logistic	-	9.5586 ***	2.9409 *	0.5874 *	1.807	0.4597	213.99	221.79	13.25
Exponential	-	2.3199 ***	-54.9440 ns	-2.6378 ns	1.824	0.4495	214.96	222.77	13.37

*Significant at $P < 0.05$ = ***; ns = not significant; b_i = regression coefficients;*
RMSE = square root of mean square error; R^2 = coefficient of determination;
AIC = Akaike Information Criterion; BIC =Bayesian Information Criterion; FI = index of Furnival

Emergent – Regression model [4] or the exponential model represented the best fitted model for this functional group. The estimated goodness-of-fit for this fitted model were RMSE = 4.62, R^2 = 0.8617, AIC = 2559.13, BIC = 2575.42 and FI = 74.24, respectively. The regression coefficients of the fitted model were statistically significant at $P < 0.001$.

$$Ht = 4.5 + \exp (4.2389 + 8.7806*DBH^{0.5404}) \quad [4]$$

Canopy – Regression model [5] or the exponential model represented the best fitted model for this functional group with the estimated RMSE = 3.578, R^2 = 0.7131, AIC = 7148.76, BIC = 7169.52 and FI = 43.25, respectively. The regression coefficients of canopy fitted model were statistically significant at $P<0.001$. The coefficient of determination, R^2, showed that the error terms of fitted model explained 71% of the variation.

$$Ht = 4.5 + \exp (4.2641 + 7.1249*DBH^{0.4474}) \quad [5]$$

Understorey – Scatter plot of understorey's Ht-DBH relationships showed that the relationship was best explained by exponential model [6]. The residual plot of the fitted model present the variance of the error terms is constant between the zero lines. The estimated regression coefficients were statistically significant at $P < 0.001$ and also the values of estimated goodness-of-fit; RMSE = 3.045, R^2 = 0.5838, AIC = 5773.76, BIC = 793.91 and FI = 28.10, respectively. The variability on Ht is shared with DBH at 58%. Thus, the estimated values and the scatter plot with residual plot showed that the exponential model [6] was fit the Ht-DBH relationships in understorey group.

$$Ht = 4.5 + \exp (6.1547 + 7.7379*DBH^{0.2280}) \quad [6]$$

Treelet – Unlike other functional group, the treelet height~dbh allometry function was best explained by the logistic model [7]. This model was chosen followed from the values of RMSE = 1.81, R^2 = 0.4597, AIC = 213.99, BIC = 221.79 and FI = 13.25, respectively. The regression coefficient of b_1 is statistically significant at $P < 0.0001$ meanwhile b_2 and b_3 are statistically significant at $P < 0.05$.

$$Ht = 9.5586 / (1 + \exp (2.9409 + 0.5874*DBH) \quad [7]$$

The scatter plots in Figure 1 showed nonlinear regression function on Ht-DBH relationships for all ecological groups. Figure 2 showed the residual plot against fitted values of each fitted model in Figure 1. These residual plots displayed the residuals fall within a horizontal band centered around 0. The relationship between Ht-DBH parameters was found significantly nonlinear ($P<0.05$) for all ecological groups.

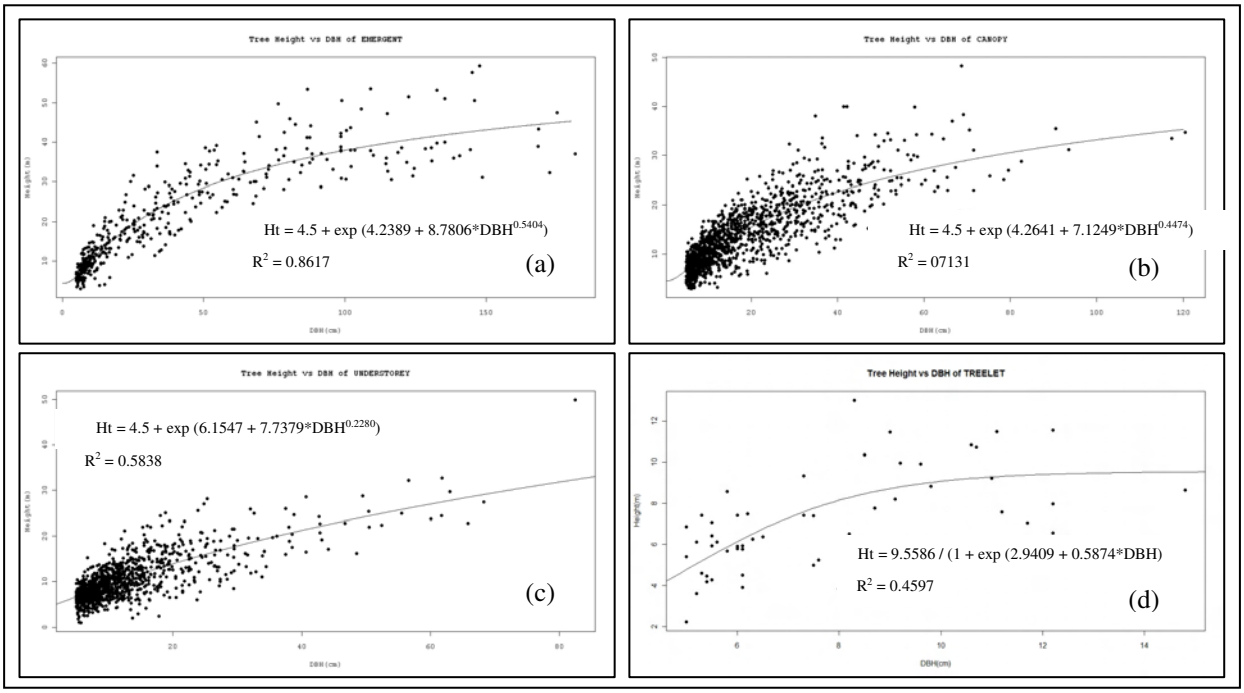

Figure 1. Scatter plot of Ht-DBH at Semangkok FR; (a) emergent, (b) canopy (c) understorey (d) treelet

Discussion

Each ecological group presented a consistent pattern of non-linear height-diameter equations in natural tropical forest tree populations. This pattern generally describe that as a tree grows in the trunk diameter, tree height becomes slowly decrease and stabilized.

Height-DBH relationship of emergent, canopy and understorey layers (Figure 1 and 2) in Semangkok Forest Reserve was best fitted by the exponential model [4], [5] and [6]. Treelet relationship [7] behaves differently as compare to the other ecological groups. Based on the criteria for model selection (as mentioned in data analysis section), logistic was the preferred model that can best explained the relationships on this tree group. However, number of measured treelet trees in this study site was only 51 trees, and these trees were limited in ranges of DBH and tree height. Therefore, the specific equations developed may not apply to trees outside this size range, and should be used with caution.

The understorey showed a shallower allometric slope than emergent and canopy trees, likely affected by low light level and shading by above crown. Meanwhile small tree species in treelet group show an asymptotic relationship that explained trees in this group couldn't grow to bigger size tree with limited tree height and diameter and also heavily shaded by larger trees.

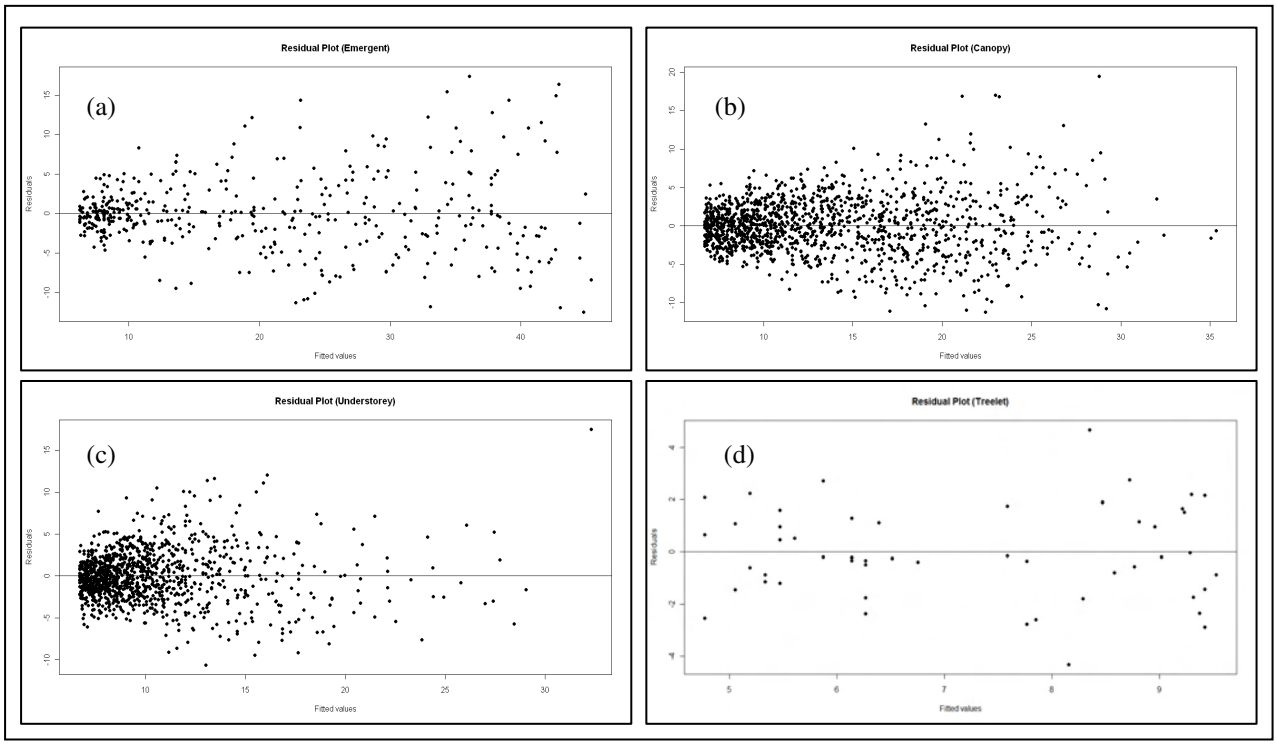

Figure 2. Residual plot of 4 types of ecological group: (a) emergent, (b) canopy (c) understorey (d) treelet

Conclusions

The output of this study was parallel with the finding of Aiba and Kohyama (1996; 1997) and Manokaran (1988) that allometric relationships between Ht and DBH show a nonlinear relationship and average of tree height falls within the range of each ecological group. The height-diameter equations of these ecological groups can be used to estimate tree height based on tree diameter for Seraya-Ridge Forest. These models were recommended for the development of total aboveground biomass estimation model and site volume prediction

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Preliminary Validation of a New and Simple Equation to Estimate Net Rainfall under Various Canopies

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Introduction

During a rainfall event, water either penetrates the canopy, falling directly to the field floor, or it is intercepted by the canopy. Tree canopies modify raindrop trajectories by partitioning the incident rainfall into throughfall and stemflow. A proportion of the incident rainfall is intercepted by and retained temporarily on leaf surface, branches and stems.

Many predictive models have been developed to predict rainfall interception according to the characteristics of rainfall and canopy. For this research, we want to determine the exact values of (T_f + S_f) that reach the ground after rain. Therefore, to find the actual value, we will compare it by using the experimental equations and the new model.

The main objective of this study was to validate a new and simple net rainfall model.

Materials and Methods

To improve the description of rainfall, we have a new model for the prediction of rainfall interception by plant canopies. As we know, not all of the rainfall will reach the ground because of the interception by the plant canopy. Interception can be as high as 50% of rainfall in some areas. From the actual value of gross precipitation, only 60% to 90% throughfall (T_f) and only 0% to 10% stemflow (S_f) will reach the ground.

T_f = 60% to 90% of P_g
S_f = 0% to 10% of P_g

In this study, the estimation from the model was compared against field measurement and data reported from literature. From the literature, four types of trees data were calculated: oil palm, rubber tree, tropical rain forest and pine forest. The test of model accuracy was done by comparing the estimated values against measured values. To estimate the values of the throughfall (T_f) and stemflow (S_f), the equation was:

$$T_f + S_f = P_g \bullet exp\ (-G \times LAI)$$

$$G = G_{max} - \left[\frac{P_g}{(C+P_g)} \bullet (G_{max} \times G_{min}) \right]$$

Where,C = 1 and LAI = total one-sided leaf area above a unit of ground area (m^2/m^2)

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

From this model, we can find the error value that accure in daily rainfall by comparing the result.

Mean absolute error (MAE) was calculated as:

$$MAE = \frac{\sum_{i=1}^{N} | P_i - O_i |}{N}$$

Where P_i and O_i are predicted and observed values, respectively, and N is the number of observations.

Results and Discussion

By using the mean absolute error between estimated and measured values for oil palm trees (Zulkifli et al., 2006) was 1.039 mm (LAI = 4), rubber trees (Zulkifli et al., 2003) was 1.4796 mm (LAI = 5), tropical rain forest (Germer et al., 2005) was 1.2952 mm (LAI = 7) and pine forest (Shachnovich et al., 2006) was 1.006 mm (LAI = 6).

The accuracy of the estimated values is shown by the graph of estimated vs. measured (Figure 1).

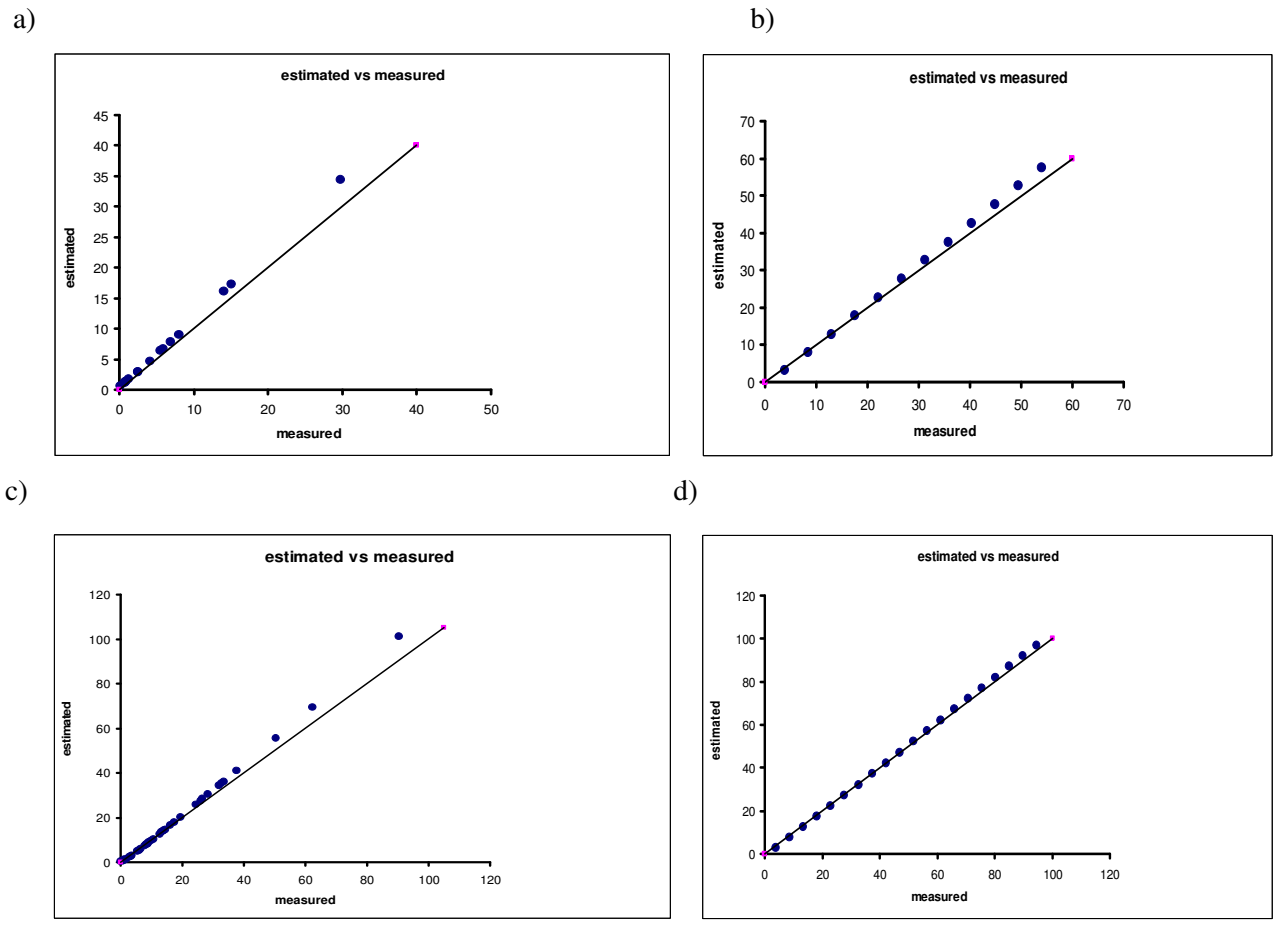

Figure 1. Comparison between measured and estimated net rainfall under canopies of: (a) Oil palm trees, (b) rubber trees, (c) tropical rain forest, and (d) pine forest

Conclusion

The graph of the estimated value and measured value showed that almost all of the points of the estimated values crossed the measured value. The accuracy was high.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Estimating Accuracy of Soil Water Characteristics using Saxton-Rawls Model for Several Malaysian Soils

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Introduction

Soil water characteristics give the relationship between the amount of water in the soil (i.e. gravimetric or volumetric water content) and soil suction (i.e. matric suction at low suction and total suction at high suction). Soil water characteristic is an important soil physical property because it describes how strongly a soil holds water. It reveals the maximum amount of water the soil could store (i.e water content at saturation point), as well as the maximum amount of the water potentially available to the plants (i.e the difference between water content between field capacity point and permanent wilting point). However, direct determination of soil water characteristic can be expensive and time consuming (Minansny et al., 1999). Consequently, estimating soil water characteristic from readily available parameters has been developed by many soil physicist and engineers (Rawls, 1982). Many methods and equations exist just to estimate the soil water characteristic from multiple soil properties, typically bulk density, texture and organic matter (Saxton and Rawls, 2006).

The accuracy of eight modern estimation methods against field data across many regions in USA has been compared by Gijsman et al. (2002). They concluded that texture based method by Saxton et al was the most accurate method. The root mean square error (RMSE) for the Saxton et al. (1986) method was lowered by 64% as compared to the average RMSE for other methods. Furthermore, the main advantage of Saxton et al. (1986) methods is that it requires only information on the soil texture (sand and clay fractions) and organic matter (in per cent) to predict the soil water characteristic. This method was updated and improved recently by Saxton and Rawls (2006) who calibrated this method again over 1700 different soil types stored in the USDA/NRCS National Soil Characterization database (Soil Survey Staff, 2004).

Therefore, the purpose of this study was to evaluate the accuracy of the Saxton-Rawls model (Saxton and Rawls, 2006) for predicting the soil water characteristics, namely the soil water content at saturation point, field capacity, and wilting point, for several Malaysian soil series.

Materials and Methods

Nine soil types (order Entisol, Ultisol, and Oxisol) were used: Munchong (72.65% clay, 17.98% sand), Melaka (52.8% clay, 20.63% sand), Rengam (41.28% clay, 51.86% sand), two Bungor soils (26.14 % clay, 57.65% sand; 30.43% clay, 65.11% sand), two Serdang soils (30.22% clay, 45.39% sand; 13.59% clay, 82.70% sand), Holyrood (11.35% clay, 87.12% sand), and Sungai Buloh (9.97% clay, 86.67% sand). These data were provided by Teh (1996) with a wide range of texture classes that were collected from soil depth 0-150 mm. These soils were collected from various locations in UPM campus as well as from Sg. Buloh. The percentages of sand, clay, and organic matter for each soil type were used in the Saxton-Rawls model to determine the water content at saturation, field capacity, and wilting point. The predicted values were then compared against the measured data to determine the model accuracy. For Malaysian soils, their organic matter content typically ranges between 0.5 to 1.5%.

Results and Discussion

Provided that the sand contents in soils were less than 80%, the Saxton-Rawls model was insensitive to the organic matter content between this range of 0.5 to 1.5%. For example, the predicted soil water

characteristics, when soil organic matter level was set at 0.5% differed, on average, between 3 to 6% than that when organic matter was set at 1.5%. Most importantly, results (Table 1) also showed that the mean absolute error (MAE) between Saxton-Rawls' prediction and measured values for saturation point, field capacity, and wilting point ranged between 18 to 26%, with a mean of 21.13%. For saturation point, the MAE was 17.84%, field capacity was 19.16%, and wilting point was 25.83%. These levels of error were deemed rather large. The model also showed bias: the higher the clay contents in the soil, the larger the prediction error. Consequently, the Saxton-Rawls model was calibrated for the nine Malaysian soils. The following quadratic equation was used to calibrate the Saxton-Rawls model: $Y = -aX^2 + aX$, where Y and X are the calibrated and uncalibrated predicted value, respectively; and a is a parameter. For predicting the soil water content at saturation, field capacity, and wilting point, the parameter a is 2.109, 1.558, and 1.490, respectively.

Table 1. Data for calculated, measured and error values for saturation point, field capacity and permanent wilting point on nine soil types.

		saturation point			**field capacity**			**permanent wilting point**		
	BD	calculated	measured	error	calculated	measured	error	calculated	measured	error
Munchong	0.99	0.563410867	0.53	6.303937	0.521253835	0.31	68.1464	0.420983	0.27	55.91967
Melaka	1.18	0.505701015	0.67	24.52224	0.436384588	0.4	9.096147	0.311466	0.32	2.667001
Rengam	1.29	0.429910492	0.63	31.76024	0.359830946	0.36	0.046959	0.250603	0.32	21.68654
Bungor1	1.34	0.40984063	0.51	19.63909	0.261927163	0.37	29.20887	0.162625	0.26	37.45186
Bungor2	1.34	0.403610149	0.55	26.61634	0.276028008	0.27	2.232596	0.187868	0.21	10.53917
Serdang1	1.19	0.425632272	0.51	16.54269	0.304583027	0.33	7.702113	0.186279	0.25	25.48855
Serdang2	1.36	0.405686601	0.43	5.654279	0.140355989	0.19	26.12843	0.086415	0.15	42.39032
Holyrood	1.35	0.410828432	0.46	10.68947	0.11833487	0.14	15.47509	0.072306	0.1	27.69406
Sg. Buloh	1.26	0.414059294	0.51	18.8119	0.111286096	0.13	14.39531	0.06398	0.07	8.600468
				17.8378			19.1591			25.8264

Conclusions

The study found that it was necessary to calibrate the Saxton-Rawls method to increase its accuracy to estimate soil water characteristic for Malaysian mineral soils. Since the Saxton-Rawls method was tested only for nine different soil types, so it would be better if this model could be tested on a wide range of particle distribution in Malaysian mineral soils. With calibration, the Saxton-Rawls model gave a prediction error of between 9 and 15%, with a mean of 11.61%. This error is lower by 45% than the mean prediction error from the uncalibrated model.

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ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

Predictive Models for the Estimation of Mass of *Artocarpus integer* (Chempedak) Seeds

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Introduction

Many tropical fruits are cultivated in South-East Asia. Some of them, such as durian, mangosteen and rambutan, are well known as commercial fruit species. Some other tropical fruits receive less attention despite having great potential for commercial use. *Artocarpus integer* is among the most typical fruits in this category. It belongs to the family Moraceae (Kanzaki et al., 1997).

Artocarpus integer is a fruit native to Malaysia, Indonesia, Thailand, Brunei, Myanmar and Philippines. It is commonly known as Chempedak. It is similar to *Artocarpus heterophyllus* (Jackfruit) in appearance and in the way the fruit is used, but smaller in size, with less pith, more seeds, and softer pulp. The fruits have many local varieties that come from different parts of Indonesia, eastern Thailand and Malaysia. This fruit requires moist condition, deep fertile soil, and full sunlight for optimum growth.

Seeds of *A. integer* are recalcitrant (Roberts, 1973; Wendell, 1999). They are sensitive to drying. Oven drying is commonly used to determine the seed moisture content (MC). Alternative means to determine the seed MC may be useful especially in the determination of lowest safe moisture content (LSMC) (Masetto et al., 2008). This study was aimed at determining the relationships of the morphological parameters and fresh weight to the mass of the seeds of *A. integer* after ambient air drying. The models can then be used to estimate the seed MC without the normal destructive procedure of oven drying.

Materials and Methods

Seeds of *A. integer* were used as test materials. A total of 250 mature but unripe seeds were used in this study. The seeds were subjected to 10 desiccation treatments, i.e. 0, 1, 2, 3, 4, 5, 6, 7, 8 and 10 days to bring the seed MC from initial MC to MC of about 20%. For each desiccation period, 25 seeds were drawn randomly. The seeds were each measured for fresh weight (FW) and morphological parameters followed by oven drying to determine the mass and MC of each seed. Each seed was placed in an aluminum boat for the determination of FW by using an analytical balance. Then, a caliper was used to measure the length (l), width (w), and thickness (t) of the seed. The seed in the aluminum boat was then dried in the oven at 103^{o}C. Drying took place for 16 hours (ISTA, 1995). After oven drying, each seed in aluminum boat was cooled down to room temperature in a desiccator. Then, the oven-dried seed in the aluminum boat was weighed again and lastly, the aluminum boat was emptied and weighed. MC was calculated as:

$$MC(\%) = \frac{\text{[Fresh weight of seed in aluminum boat before oven drying(g)-weight of oven dried seed in aluminum boat(g)]}}{\text{[Fresh weight of seed in aluminum boat before oven drying(g)-weight of aluminum boat(g)]}} \times 100$$

The area of the seed (A) was computed as l x w, and the volume of seed (v) was computed as l x w x t. A total of two third of the data was used to build suitable models to estimate the mass of *A. integer* seeds. The estimated mass was then used to estimate the seed MC. The remaining one third collected data were used to validate the models developed in the model building procedure.

Model building

In the model building procedure, the relationships between the seed mass and its morphology and FW were determined (Suratman et al., 2004). Predictor and response variables were used in this model development. Predictor variables (X) in this study were l(x_1), w(x_2), t(x_3), FW(x_4), A(x_5), v(x_6), FW/A(x_7) and FW/V(x_8), while response variable ($\hat{Y}$) was the mass. Relationships between mass ($\hat{Y}$) and predictor variables (X) were studied by using scatter plots. The Pearson's correlation coefficients (r) were calculated. A few models were suggested based on R^2, R_a^2, SE_E and significance at 0.05 levels. The residuals of the models were plotted and examined to avoid lack of fit.

Model validation

The models developed as mentioned were validated by using the validation data set. The predictive accuracy of the models was interpreted and concluded.

Results and Discussion

Model building

With model building data set, mass was significantly and strongly correlated to A and w was also significantly and strongly correlated to A with correlation coefficients greater than 0.800 respectively (Table 1). Other pairs of parameters had relatively weaker relationships.

Table 1. Correlation matrix

	mass	L	w	T	A	v	FW	FW/A	FW/v
Mass	1.00								
l	0.599***	1.00							
w	0.303***	0.280***	1.00						
t	0.528***	0.365***	0.341***	1.00					
A	0.557***	0.776***	0.820***	0.439***	1.00				
v	0.637***	0.691***	0.712***	0.808***	0.878***	1.00			
FW	0.830***	0.767***	0.508***	0.691***	0.786***	0.871***	1.00		
FW/A	0.594***	0.230**	-0.235**	0.526***	-0.026 ns	0.256**	0.583***	1.00	
FW/v	0.078 ns	-0.136 ns	-0.580***	-0.437***	-0.469***	-0.532***	-0.088 ns	0.520***	1.00

*Notes: *,**,*** are significant at the 0.05, 0.01 and 0.001 probability levels, ns = not significant*

For the estimation of mass of *A. integer* seeds, a total of 6 different models with different predictor variables were selected based on R^2, R_a^2, Mallows-Cp and SE_E (Tables 2 and 3). All models were highly significant with P value < 0.001.

Table 2. Comparison of models fitted to mass for model building (n = 250)

Model no	Predictor variable	p	R^2 (%)	R_a^2 (%)	Mallows C-p	SE_E (g)
1	v, FW	2	71.8	71.5	6.3	0.6195
2	A, FW	2	71.2	70.9	9.8	0.6257
3	t, A, FW	3	72.3	71.8	5.3	0.6161
4	A, v, FW	3	72.1	71.6	6.1	0.6175
5	t, A, FW, FW /v	4	72.6	72.0	5.0	0.6138
6	t, A, FW, FW/A	4	72.6	71.9	5.4	0.6144

Notes: p = number of predictor variables in the model

Table 3. Model equation to estimate the mass of *A. integer* seeds (n = 250)

Model no	Equation
1	$\hat{Y} = 0.494 - 0.108\ v + 0.631\ FW$
2	$\hat{Y} = 1.19 - 0.227\ A + 0.571\ FW$
3	$\hat{Y} = 1.96 - 0.631\ t - 0.262\ A + 0.644\ FW$
4	$\hat{Y} = 0.814 - 0.111\ A - 0.0778\ v + 0.637\ FW$
5	$\hat{Y} = 5.19 - 1.46\ t - 0.493\ A + 0.819\ FW - 2.37\ FW/v$
6	$\hat{Y} = 4.07 - 0.652\ t - 0.560\ A + 0.874\ FW - 1.60\ FW/A$

Predicted MCs calculated based on the mass of seeds estimated by using the models were significantly correlated to observe MC (Table 4). Model 2 was found to give lower Pearson's correlation coefficient (r) in this context and, hence, was less suitable for the estimation of MC of *A. integer* seeds.

Table 4. Pearson's correlation coefficient of Observed moisture content (MC) and Predicted moisture content (MC) based on models developed

		Observed MC
Predicted MC	Model 1	0.399^{***}
	Model 2	0.187^{*}
	Model 3	0.428^{***}
	Model 4	0.426^{***}
	Model 5	0.427^{***}
	Model 6	0.421^{***}

Notes: *,**,*** are significant at the 0.05, 0.01 and 0.001 probability levels

Model validation

In validation procedure, the fitted line plots of predicted mass versus observed mass and plots of estimation of residuals gave fairly good indication for acceptable estimation of mass of these seeds. Generally, there was not much difference in the predictive performance among these models (Figures 1 and 2). Validation procedure indicated that the models did not show a lot of biases (Tables 5).

Table 5. Comparison of models fitted to mass in model validation (n = 250)

Model no	Predicted variables	P	I^2 (%)	I_a^2 (%)	RMSE (g)
1	v, FW	2	62.1	61.3	0.5515
2	A, FW	2	62.7	61.8	0.5476
3	t, A, FW	3	63.2	62.0	0.5467
4	A, v, FW	3	63.2	62.0	0.5466
5	t, A, FW, FW/v	4	63.3	61.5	0.5497
6	t, A, FW, FW/A	4	63.2	61.5	0.5498

Notes: p = number of predictor variables in the model

Model 2 was eliminated based on correlation analysis of observed MC and predicted MC in validation of models (Table 6). Model 3 was found the best for estimation of mass of *A. integer* seeds $\hat{Y} = 1.96 - 0.631\ t - 0.262\ A + 0.644\ FW$.

The estimated mass was also the best for estimation of MC of *A. integer* seeds among all these models.

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

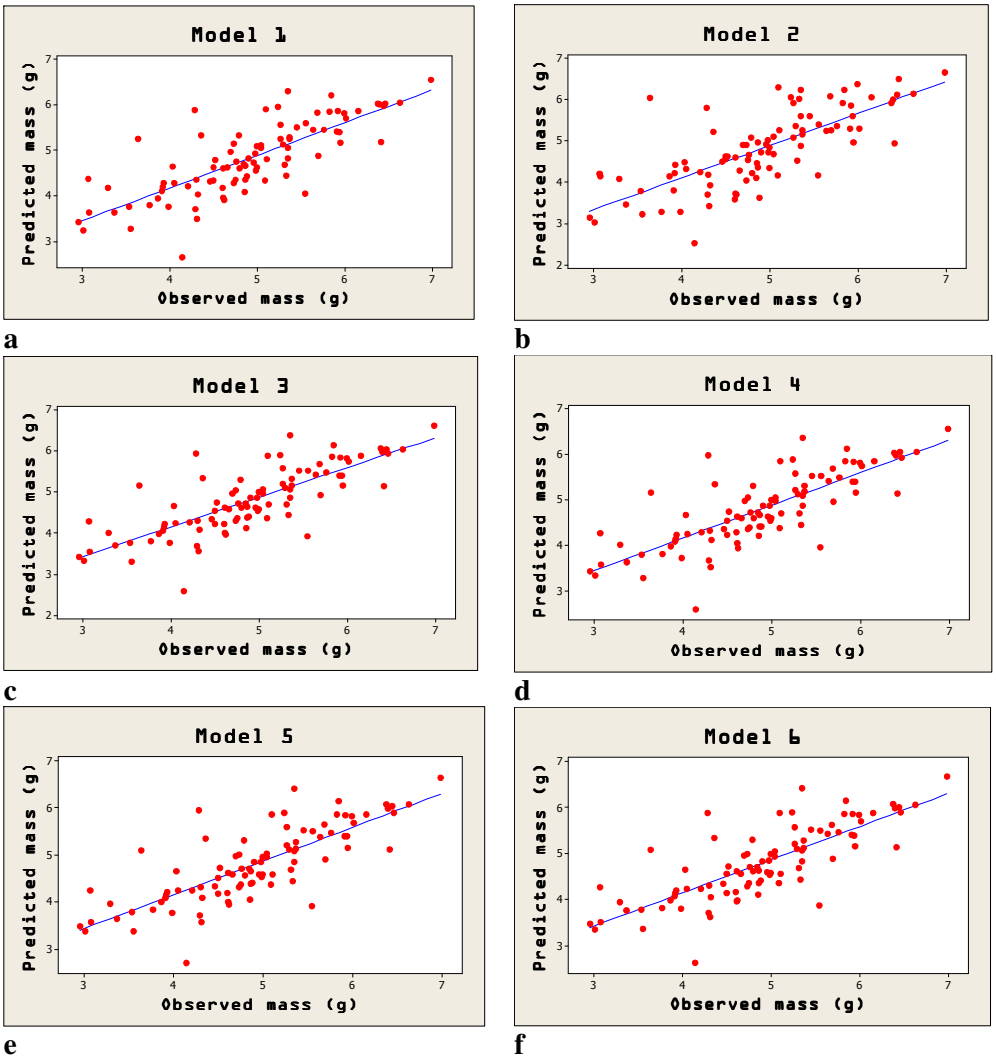

Figure 1. Predicted mass versus observed mass with validation data set based on (a) Model 1 (S=0.5019; R^2=61.9%; R_{adj}^2=61.5%), (b) Model 2 (S=0.6145; R^2=56.0%; R_{adj}^2=55.5%), (c) Model 3 (S=0.4946; R^2=62.8%; R_{adj}^2=62.4%), (d) Model 4 (S=0.4929; R^2=62.9%; R_{adj}^2=62.5%), (e) Model 5 (S=0.4909; R^2=62.9%; R_{adj}^2=62.5%), (f) Model 6 (S=0.4934; R^2=62.9%; R_{adj}^2=62.4%)

ISBN: 978-967-344-188-4
Trans. Malaysian Soc. Plant Physiol. 18

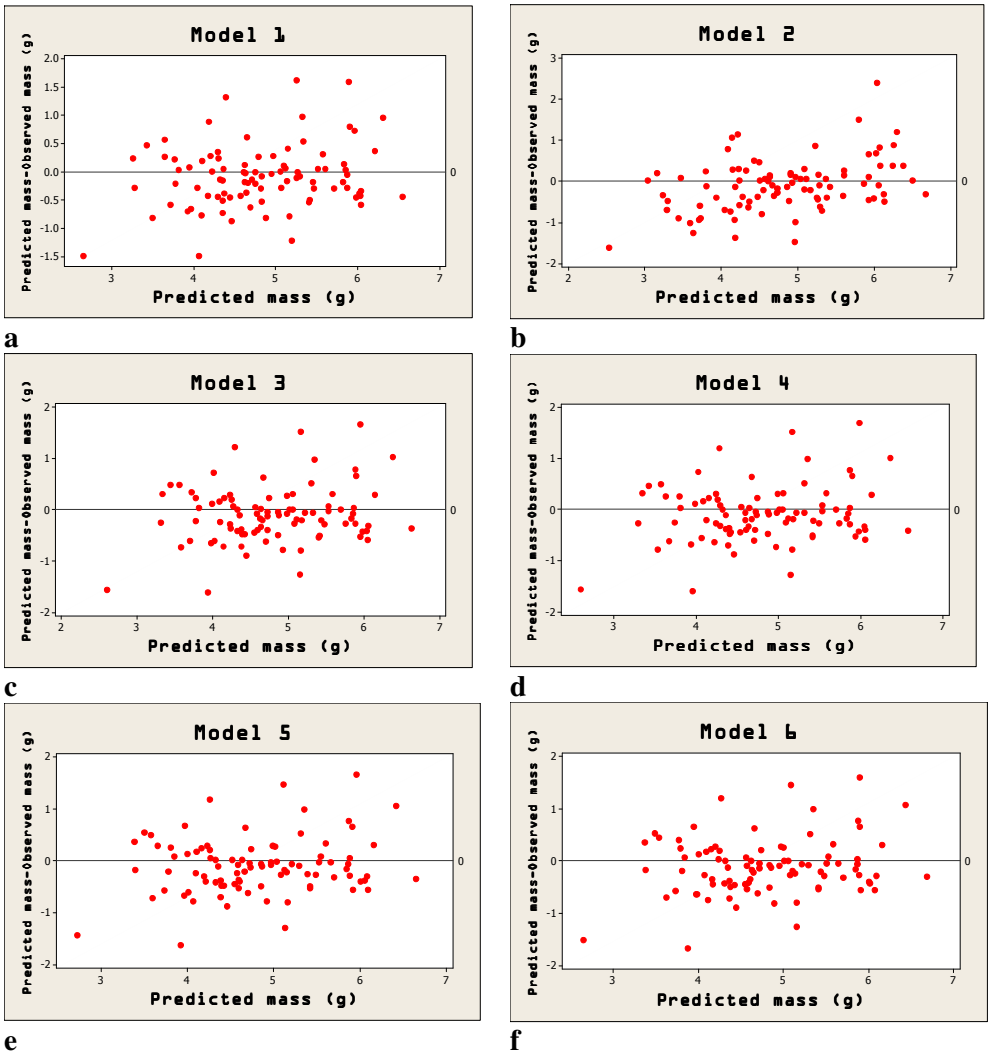

Figure 2. Predicted mass-Observed mass versus predicted mass with validation data set based on (a) Model 1, (b) Model 2, (c) Model 3, (d) Model 4, (e) Model 5, (f) Model 6

Table 6. Pearson correlation coefficient of Observed moisture content (MC) and Predicted moisture content (MC) based on validation data

		Observed MC
Predicted MC	Model 1	0.347**
	Model 2	0.065 ns
	Model 3	0.369***
	Model 4	0.376***
	Model 5	0.403***
	Model 6	0.375***

*Notes: *,**,*** are significant at the 0.05, 0.01 and 0.001 probability levels*

Conclusions

Mass of *A. integer* seeds was significantly and highly correlated to morphological parameters and fresh weight. Model building and validation procedures suggested that mass of *A. integer* seeds could be estimated by using Model 5:

$Y = 1.96 – 0.631\ t - 0.262\ A + 0.644\ FW$

The R^2, R_a^2, SE_E and I^2, I_a^2 and RMSE were 0.723, 0.718, 0.6161 g and 0.632, 0.620, 0.5467 g. Seed MC calculated based on the mass of seed estimated by using this model was also significantly correlated to the observed seed MC. Estimation of seed MC of *A. integer* based on the under study procedure may be beneficial for the determination of lowest safe moisture content of seeds of this species.

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