

TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 23

25th Malaysian Society of Plant Physiology Conference

Environmental Conservation: Role of Plant Physiology

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**ENVIRONMENTAL CONSERVATION:
ROLE OF PLANT PHYSIOLOGY**

25th Malaysian Society of Plant Physiology Conference (MSPPC 2015)
held at Sunway Lost World Hotel, Tambun, Ipoh, Perak, Malaysia. 18-20 August 2015

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CHAPTER 1

PLANT GROWTH AND DEVELOPMENT

Preliminary Analyses: Effect of Different Irrigation Systems on the Growth and Plant Nutrient Content in Rubber (*Hevea brasiliensis*) Nursery Seedlings

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Introduction

Rubber has been planted widely in South East Asia for more than a century and has contributed significantly to the economy of the country. With rising increase of its (latex) demand, high quality seedlings need to be produced to ensure steady supply of the latex. This can only be achieved by producing high quality seedlings (Waizah et al., 2011). The growth of rubber seedlings is greatly influenced by the condition of their production including irrigation, soil or substrate quality, drainage and fertilization. BX-1 system (Rb 900 and BX-1 growing media) is a new nursery planting system introduced by Humibox Sdn. Bhd. with the purpose of replacing the traditional (polybag with soil) way of raising seedlings. Utilisation of container nurseries is rapidly developing in Malaysia and the world at large, because it gives a better productivity and better organisation of the production. Different water application methods affect the growth of nursery seedlings regardless of source and rate of nutrient solution (Argo and Biernbaum, 1994). The main objective of the study was to determine the best irrigation method namely overhead sprinkler, drip irrigation and capillary wick system to be used in BX-1 system for rubber nursery production. The irrigation systems were compared with one another in terms of their influence on seedling growth and nutrient content.

Materials and Methods

The experiment was conducted under a rain shelter at Field No. 15, Agrobio Complex, Universiti Putra Malaysia (2^o 59' 4.96'' N, 101^o 44' 0.70'' E). One month old RRIM 2000 was transplanted in 710 cm³ Rb 900 tube filled with 230g of BX-1 media. Each tube was planted with one seedling. The field experiment data shown was for 6 months from December 2014 to May 2015.

The experimental design was RCB (Randomised Complete Block), with four treatments and three replications per treatment. Each experimental plot consisted of a single tray or tube stand (1.5 m long 0.5 m wide and 1 m high) that accommodated 10 Rb 900 tubes. A total of 120 RRIM 2000 rubber seedlings were utilised in the experiment with 10 plants per experimental unit x 12 plots. The treatments were BX-1 system with overhead sprinkler as T1 (SPR), BX-1 system with drip irrigation as T2 (DRP), Bx-1 system with capillary wick system as T3 (WCK) and polybag-soil with capillary wick system as T4 (CTRL). Treatments were applied every day in the morning.

The water content in growing media was measured using moisture meter (FieldScout TDR 100-6440FS, Spectrum Technology, Inc., USA) every day before irrigation to monitor the moisture status of the media. In the first month of the experiment, 45 ml of water was supplied to SPR and DRP while WCK and CTRL received an average of 57 ml and 53 ml respectively. In the second, third and fourth month, SPR and DRP were supplied with 60 ml while WCK and CTRL received an average of 60, 60, 53 ml and 57, 58, 57 ml respectively. In the fifth and sixth months, SPR and DRP were supplied with 70 ml each while WCK and CTRL consumed an average of 57, 55 ml and 60, 57 ml respectively. A mini weather station was placed inside the rain shelter to monitor the microclimatic condition under which the rubber seedlings are grown.

Plant growth parameters were taken every 30 days after which a destructive sampling were taken for leaf nutrient analysis.

The soil and media were also analysed for their physical and chemical characteristics. All the physical and chemical analyses were conducted using the standard procedures (Jones, 2001).

All data collected were tested using Statistical Analyses System (SAS 9.4 SAS system for windows by SAS Institute Inc., Cary, NC, USA). GLM (General Linear Model) and ANOVA (Analysis of Variance) were used to determine the significant treatment effect on various measured properties with the significant different of $p < 0.05$. Test SNK (Student–Newman-Keuls) for mean separation was used to detect significant different between means.

Results and Discussion

The BX-1 media had higher nutrient contents than Munchong (Tropeptic Haplorthox) soil series (Table 1). The pH of the BX-1 was 6.4 (Table 1) which indicated the availability of nutrients while the soil pH was 4.6 which was rather acidic. It also had rather higher electrical conductivity (EC) of 1.2 dS m^{-1} , which did not affect the growth of the plant overall. The BX-1 media had a very low bulk density (0.135 Mg m^{-3}) which is a good characteristic of growing media as it allows easy handling unlike mineral soil (1.43 Mg m^{-3}) which makes handling and transportation difficult. About 58% of the media was water and its water retention (Figure 1A) showed saturation at 95% volumetric moisture content, field capacity at 31% and permanent wilting point at 20%. The available water content of this media was up to 11% while the soil (Munchong) has 3% available water content (Figure 1B). The hydraulic conductivity of the BX-1 media was very rapid (32 cm hr^{-1}) while the Munchong (Tropeptic Haplorthox) soil had rapid (17.5 cm hr^{-1}) hydraulic conductivity (Table 1).

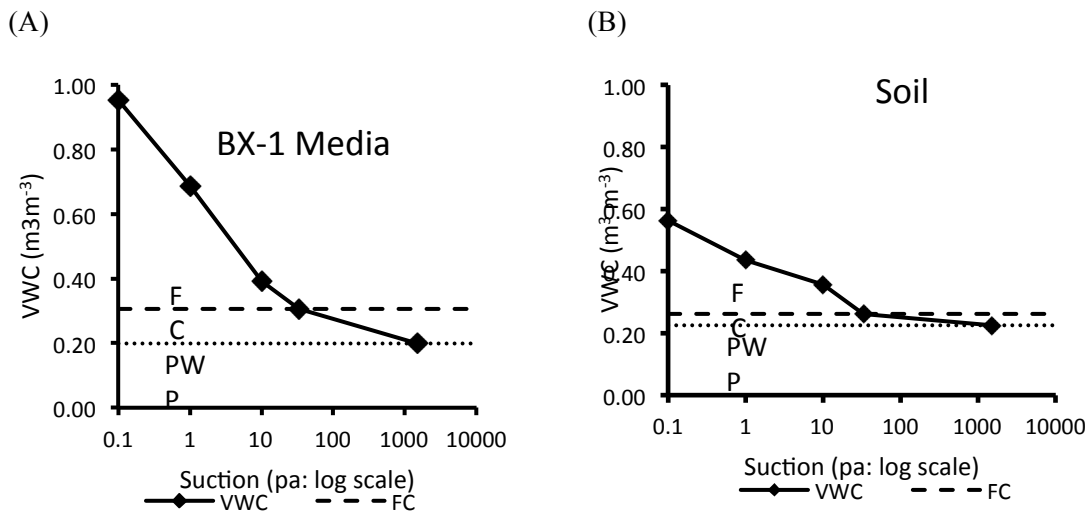


Figure 1. Water Retention Curve for BX-1 media (a) and Soil (b).
 VWC= Volumetric Water Content, FC= Field Capacity, PWP= Permanent Wilting Point

Table 1. Physical and chemical characteristics of BX-1 media and Munchong soil series

Physical properties			Chemical properties			
	Bx-1	Soil		BX-1	Soil	
Bulk density	0.135	1.43	mg m ⁻³	pH	6.4	4.67
Total porosity	57	46	%	EC	1.2	0.04 dS m ⁻¹
Moisture content	58	18.1	%	CEC	63.21	8.32 cmol.kg ⁻¹
HC	32	17.5	cm hr ⁻¹	C	34.25	1.37 %
SAT	0.95	0.56	m ³ m ⁻³	N	1.27	0.13 %
FC	0.31	0.26	m ³ m ⁻³	S	0.75	0.03 %
PWP	0.20	0.23	m ³ m ⁻³	P	680.57	8.34 ug g ⁻¹
				K	1779	41.27 ug g ⁻¹
				Ca	6223.67	459.33 ug g ⁻¹
				Mg	1709.33	85.47 ug g ⁻¹
				Na	17.93	5.43 ug g ⁻¹

HC= Hydraulic Conductivity, SAT= Saturation, FC= Field Capacity, PWP= Permanent Wilting Point

Analysis of variance (ANOVA) for the growth parameters showed that treatment and month interaction was significant at 5% level for plant height, specific leaf area, shoot: root ratio and total leaf area only. In plant height, the significant ($p < 0.05$) difference was observed in the second and last month. In the second month, the highest was observed in the WCK irrigation system and the lowest was recorded in the CTRL and in the last month, the highest was observed in DRP irrigation system which differed significantly with other treatments. The higher plant height observed in WCK and DRP could be attributed to the ability of the system to make the media nutrients available for plant uptake. Capillary wick irrigation compared with overhead irrigation reduced cumulative irrigation volume by 86% without reducing the yield (Bryant and Yeager, 2002). Overhead sprinklers for small containers cause high non-uniformity (Beeson and Yeager, 2003). In the specific leaf area (SLA), the significant difference was observed only in the second and sixth month of the experiment where all the treatments differed significantly with the control probably because of differences in nutrient content between the soil and BX-1 media. The total leaf area differed significantly between the treatments in all the months except in the sixth month in which no significant difference was observed in the month. From the first month to the fourth month of the experiment, WCK had the highest leaf area and the CTRL had the lowest. This was due to lower leaching and consequently lower nutrient loss in the WCK system. There was no significant difference between the treatments in terms of fresh and dry weight of the various parts of the seedlings except in the leaf dry weight, with the highest obtained in the WCK and the lowest in SPR (Figure 2). The lowest leaf dry weight obtained in SPR might be due to lower water use efficiency of the system.

The interaction between treatments and months was significant at 5% level for P, K, Ca and Mg. the results showed that there was no significant difference among the BX-1 media grown treatments but they differed significantly with the control (soil grown seedlings), because of the high media nutrient content (Table 1). The interaction of leaf nitrogen content and girth size was not significant at 5%, but the treatments main effect differed significantly with WCK and DRP recording the highest girth size and N leaf tissue content with the CTRL recording the lowest. Even though the CTRL had the lowest N nutrient content, the chemical analysis showed that the N nutrient content was within sufficient range.

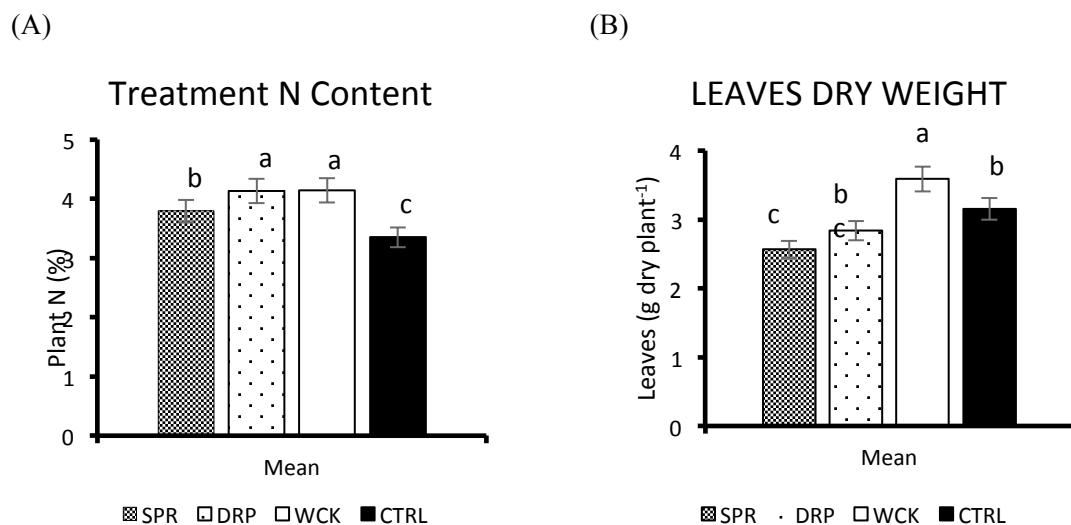


Figure 2. (a) Nitrogen content and (b) Leaves Dry weight of RRIM 2000 under different irrigation system. SPR= Sprinkler, DRP= Drip, WCK= Capillary Wick, CTRL= Control

Conclusions

WCK irrigation system gave the highest leaves dry weight, Girth size, total leaf area and Nitrogen leaves tissue content. This was because the WCK has higher nutrient use efficiency, lower leachate compared with SPR and DRP and consequently lower nutrient loss (Data not shown). The results also shows continues increase in virtually all the measured parameters as the months progressed except for the leaves Nutrient content (Data not shown). The research showed that BX-1 system can be adopted in a rubber seedlings nursery due to the good water retention and adequate available nutrients of the media (BX-1 media).

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Management of *Mucuna bracteata* with Plant Growth Regulator

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Introduction

Mucuna bracteata is one of the important leguminous cover crops adopted in inter rows in early establishment of oil palm and rubber plantations. It provides nitrogen to the main crops through symbiotic atmospheric nitrogen fixation (Mathews, 1998; Chee, 2007; Othman et al., 2012). It also suppresses growth of weeds as it is more vigorous and competitive in covering the ground as compared to many weed species. It, hence, is also effective in reducing soil erosion as the roots hold the soil particle and, at the same time, improve the water holding capacity in soil. As the plant parts die, it also enhances organic matter content of the soil.

Owing to the rapid growth behaviour, the fast extending vines of this cover crop have frequently been reported to climb and cover the young main crops when it is not maintained well with clipping and cutting off the climbing vines. The early growth of the juvenile main crops is then retarded following reduced photosynthetic capacity. Such exercise of trimming the undesired vine extension is generally carried out quarterly and as such, it implies additional management and labour costs. Herbicides are also applied by some planters to control this cover crop but such practice should be carried out carefully so as not to affect the young main crops. Application of plant growth retardant is sought after to reduce the labour intensive maintenance of this cover crop. This study aimed to determine the effectiveness of a few common plant growth regulators (PGRs) available in Malaysian market for management of the growth of *M. bracteata*.

Many PGRs in the family triazole are chemicals that are usually used to reduce internode elongation in plants. The commonly available triazoles in local market include paclobutrazol (PBZ) traded with the trade name Kaltaar®, Sumi-7® with active ingredient (a.i.) of uniconazole at 200 mg/l and Anvil® having 4.8% hexaconazole, while Folicur® with 26% tebuconazole and Score® having 23% difenoconazole are mainly used as fungicides, although they also have growth retardation properties when applied at higher dosages (MDAR, 2012). They inhibit the gibberellin biosynthesis pathway in plants by blocking cytochrome P-450 and synthesis of ergosterol (Rahman et al., 1989; Hassan, 1993; Chaney, 2005; Nouriyani et al., 2012; Runkle, 2012; Lolaei et al., 2012; Hua et al., 2014; Muslimin et al., 2014). These chemical compounds are usually applied as foliar spray and soil drenches (Deneke and Keever, 1992; Ruter, 1996; Jungklang and Saengnil, 2012). Triazoles are generally applied in low concentrations and they also have low toxicity (Prusakova et al., 2004). As vegetative growth of plants is retarded, flowering can be induced in some plant species with the more available energy, which otherwise, is usually spent on vegetative growth (Kulkarni et al., 2006; Abdel Rahim et al., 2011; Nouriyani et al., 2012). Triazoles treated plants have also frequently been reported to be more stress tolerant (Kucharska and Orlikowska, 2008; Chorbadijan et al., 2011).

Materials and Methods

Location of study

This study was conducted at Jasin campus, Universiti Teknologi MARA. It was done at an open area behind one of the rain shelters of Faculty of Plantation and Agrotechnology. The experimental site had average soil pH of 5.48 and the soil texture was clay loam. It is a flat terrain with no water body within radius of 1 km.

Test material

Mucuna bracteata at 11 months after field planting was used as test materials to determine its responses towards application of PGRs. Seedlings at six weeks after germination were planted at the study site in May 2014 followed by application of compost in June 2014 and organic fertilizer in July 2014 for enhancing the initial establishment of the seedlings after field planting. No further application of any fertilizer was carried out at the site thereafter. The cover crop was grown rain fed.

Experimental plot

Plots of 7x3 m were randomly established. Each plot was separated from other plots at a distance of at least 1 m. Each quadrat of 1x1 m marked within the plot was a sampling unit and represented a replicate of treatment. Plants in each quadrat were used for data collection following application of PGRs.

Plant growth regulator

A total of three experiments were carried out with three different PGRs. PGRs tested in this study were PBZ with the trade name Kaltaar®, having 25% a.i. of PBZ, Sumi-7® with a.i. of uniconazole at 200 mg/l and Anvil® having 4.8% hexaconazole.

Experimental procedure

Preliminary studies were carried out with some other plants of *M. bracteata* prior to experimentations on its growth inhibition using PBZ, uniconazole and hexaconazole. Then, experiments using these three PGRs were carried out concurrently in March 2015. The plants were 11 months old at the commencement of the experiments as mentioned. The plants were applied with different concentrations of PGRs. In the experiment with PBZ, PBZ treatments were 0 (control), 62.5, 125, 187.5 and 250 mg/l. Uniconazole was applied at 0 (control), 50, 100, 150 and 200 mg/l in another experiment. The experiment with hexaconazole, on the other hand, involved application with this PGR at 0 (control), 120, 240, 360 and 480 mg/l. As small plots were used in experimentations, application of PGRs was carried out respectively using a hand held sprayer after three calibrations. PGRs were applied at application volume of 400 l/ha. This application volume allowed all foliar plant part sprayed to run off with the respective PGR.

Data collection

Within each sampling unit (quadrat of 1x1 m), growth parameters measured to determine the rate of growth inhibition of *M. bracteata* after application of PGRs at different rates included the internode length and diameter of internode at first fully developed leaf position from apical shoot tip of the newly emerged branches, leaf area and relative chlorophyll content of the three first fully developed leaves as mentioned and the total leaf number within the sampling unit of 1x1 m. Data of five new branches within each sampling quadrat were recorded fortnightly while total leaf number was recorded monthly. Internode length was measured using a measuring tape. Calliper was used to measure the diameter of the internode. Leaf area was estimated after tracing the leaves on graph paper while relative chlorophyll content of leaves was determined using Minolta SPAD 502.

Experimental design and statistical analysis

Each experiment with any of the PGRs was arranged in randomized complete block design (RCBD) with five treatments and five replicates. Homogeneity test was first run with the sampling branches for each experiment. Subsequent data collected in each experiment were then subjected to analysis of variance (ANOVA) to determine the effects of the difference concentrations of PGR and treatment means were compared using Tukey's Honestly Significant Difference (HSD) test.

Results

Homogeneity test indicated that the experimental plots of *M. bracteata* were not significantly different in terms of internode length, diameter of internode, leaf area, relative chlorophyll content and leaf number per area. PBZ was effective in reducing internode length with application rate as low as 62.5 mg/l or with Kaltaar® of 0.25 ml/l, resulting in controlled vine extension and, hence, seemed to have great potential as a chemical means for management of the undesirable excessive growth of this cover crop (Table 1). This PGR did not affect the internode diameter at four weeks after application but by eight weeks after application of this PGR, treated plants had significantly bigger internode diameter and greener leaves as compared to the untreated controls. The leaves of the treated crops were, however, significantly smaller than the controls. PBZ did not affect the general appearance of this cover crop for its function as ground cover. It did not have any significant effect on the leaf number per m², indicating that PBZ could have also retarded new leaf formation.

Table 1: Mean comparison of growth parameters following treatment with paclobutrazol

Period (weeks) Rate (mg/l)	Internode length (cm)		Internode diameter (mm)		Leaf area (cm ²)		Relative chlorophyll content		Leaf number per m ²	
	4	8	4	8	4	8	4	8	4	8
0	9.16 ^a	20.86 ^a	0.24 ^a	0.33 ^b	59.13 ^a	52.21 ^a	35.61 ^a	39.22 ^c	252 ^a	354 ^a
62.5	1.86 ^b	3.74 ^b	0.29 ^a	0.49 ^a	36.11 ^b	19.72 ^b	35.40 ^a	40.67 ^{bc}	278 ^a	315 ^a
125	2.08 ^b	3.92 ^b	0.30 ^a	0.47 ^a	35.01 ^b	17.69 ^b	33.78 ^a	41.18 ^{ab}	308 ^a	349 ^a
187.5	2.26 ^b	3.33 ^b	0.26 ^a	0.47 ^a	30.97 ^b	16.40 ^b	37.28 ^a	42.92 ^{ab}	292 ^a	403 ^a
250	2.01 ^b	2.95 ^b	0.29 ^a	0.55 ^a	33.63 ^b	14.69 ^b	37.95 ^a	43.78 ^a	303 ^a	412 ^a

Note: Means having the same letter within the same column are not significantly different at 5% level of significance.

Uniconazole, on the other hand, should be applied at dosages up to only 100 mg/l on *M. bracteata* (Table 2). Higher dosages of 150 and 200 mg/l resulted in partial death in the experimental plots. This PGR was not effective in retarding the rapid vine extension of this cover crop. It also resulted in bigger internode diameter and enhanced chlorophyll pigmentation in the treated plants like that found with application of PBZ. In contrast, this PGR reduced leaf size. Application of uniconazole at rates up to 100 mg/l did not seem to retard new leaf development; there was significant increment in leaf number per m².

Table 2: Mean comparison of growth parameters following treatment with uniconazole

Period (weeks) Rate (mg/l)	Internode length (cm)		Internode diameter (mm)		Leaf area (cm ²)		Relative chlorophyll content		Leaf number per m ²	
	4	8	4	8	4	8	4	8	4	8
0	16.70 ^a	16.77 ^a	2.82 ^b	3.34 ^b	59.13 ^a	52.21 ^a	33.11 ^b	34.66 ^b	232 ^a	275 ^b
50	16.76 ^a	16.82 ^a	4.55 ^a	5.11 ^a	45.16 ^{ab}	44.04 ^a	36.55 ^a	39.22 ^a	207 ^b	314 ^{ab}
100	14.26 ^a	12.78 ^a	4.87 ^a	5.66 ^a	34.07 ^b	30.23 ^b	36.47 ^a	42.61 ^a	237 ^a	337 ^a

Note: Means having the same letter within the same column are not significantly different at 5% level of significance.

Hexaconazole was also generally found to have the rather similar effects as uniconazole in regulating the growth of *M. bracteata*. This PGR up to 480 mg/l neither effectively retarded the growth of the creeping vines nor internode diameter (Table 3). It also allowed normal new leaf development as that found with treatment with uniconazole. Nevertheless, it also resulted in smaller leaves and slightly greener leaves after treatment.

Table 3: Mean comparison of growth parameters following treatment with hexaconazole

Period (weeks) Rate (mg/l)	Internode length (cm)		Internode diameter (mm)		Leaf area (cm ²)		Relative chlorophyll content		Leaf number	
	4	8	4	8	4	8	4	8	4	8
0	7.80 ^a	11.95 ^a	2.75 ^b	3.37 ^a	60.41 ^a	52.47 ^a	38.96 ^b	37.38 ^b	353 ^d	371 ^c
120	4.77 ^a	9.04 ^a	3.97 ^a	3.57 ^a	40.35 ^b	60.53 ^a	37.60 ^b	40.20 ^b	398 ^c	431 ^b
240	3.56 ^a	10.74 ^a	4.05 ^a	3.71 ^a	33.51 ^{bc}	58.61 ^a	35.66 ^b	40.78 ^b	409 ^c	442 ^b
360	5.39 ^a	9.14 ^a	3.96 ^a	3.87 ^a	31.93 ^c	59.56 ^a	39.78 ^b	47.99 ^a	427 ^b	451 ^{ab}
480	8.14 ^a	13.90 ^a	4.17 ^a	3.86 ^a	33.26 ^{bc}	42.23 ^b	45.98 ^a	40.89 ^b	455 ^a	472 ^a

Means having the same letter within the same column are not significantly different at 5% level of significance.

Discussion and Conclusion

PBZ was the best among the three PGRs studied in effective growth retardation of *M. bracteata* in attempts to reduce physical trimming and cutting of the vigorous climbing vines. This has been widely reported to also retard growth of many other plant species (MDAR, 2012; Natarajan et al., 2012; Rosanna et al., 2014). Shorter internode is resulted from the inhibition of the gibberellin biosynthesis within cells in the treated plants. Cell division occurs but new shoot do not elongate during inhibition of gibberellin biosynthesis. In contrast, the internodes will be compressed into shorter length (Chaney, 2005). This will then greatly reduce the incidence of climbing vines in the context of application of PBZ for management of vigorous *M. bracteata*. It then can extend the labour intensive maintenance cycle of pruning the undesired climbing vines. PBZ at as low as 62.5 mg/l was sufficient to serve this purpose of suppressing the vine extension of *M. bracteata*. It has also exhibited persistent growth retardation effect in many previous studies. This has to be studied future on *M. bracteata* to determine the most effective application rates for maintaining this cover crop well while growth of the main crops remains unaffected or retarded by unnecessary high application rates of this PGR.

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Integrated of Nitrogen and Potassium: Effect on Coconut Seedling Growth at Different Sowing Methods

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Introduction

Coconuts (*Cocos nucifera*) are part of the daily food consumed by many people. Coconut contains a large quantity of water and when immature they are known as tender-nuts or jelly-nuts and harvested for drinking. When matured, they still contain some water and can be used as seed nuts or processed to give oil from the kernel, charcoal from the hard shell and coir from the fibrous husk. The endosperm is initially in its nuclear phase suspended within the coconut water. When dried, the coconut flesh is called copra. The oil and milk derived from copra are commonly used in cooking and frying. Coconut oil is also widely used in soaps and cosmetics. The husks and the leaves can be used as material to make varieties of products for furnishing and decoration. Coconuts is a large palm, growing up to 30 m (98 ft) tall, with pinnate leaves 4–6 m (13–20 ft) long, and pinnae 60–90 cm long; old leaves break away cleanly, leaving the trunk smooth. Coconuts are generally classified into two general types: tall and dwarf. On very fertile land, each plant of tall type coconut can yield up to 75 fruits per year compared to 30 fruits per year due to poor cultural practices. With proper cultural practices, coconut can produce their first fruit in 3-5 years after planting and it takes 12 – 17 years to reach the peak fruit production. The objectives of this study were to determine the optimum rate of nitrogen and potassium in the growth of coconut seedlings at different sowing methods.

Materials and Methods

The experiment was carried out in MARDI Hilir Perak located at the 3° 57' 28.9624" North and 100° 52' 3.9432 East at an above sea level height of 4 m. The experiment was carried out under rain-shelter condition to avoid major damage caused by pest and diseases. Accession of Matag was used. Four rates of nitrogen (N) at 0, 120, 240 and 360 g/plant/year and potassium (K) rate at 0, 144, 288 and 432 g/plant/year were applied. Fertilizer were divided into three equal portions and applied at 1st, 4th and 7th month after planting. Coconuts were germinated in the open nursery until they reach age of two months before application of different sowing methods and transferred into polybags. Two sowing methods were used in this experiment (open and close bottom) at 2 months aged coconut seedlings. The experiment site was marked out (or is it labeled) and split plot design was used. Transplanted coconuts seedlings were arranged under the rain-shelter with the distances of 2.5 x 2.5 feet in triangular system. Sprinkler system was used for uniform water distribution supply. Manual weeding with hand was done at 2, 5, and 8 month after transplanting. Weeding was carefully done and the weeds were separated. Data collections include stem girth, plants height, and number of roots, weight and length of roots.

Results and Discussion

Stem girth

It was observed that inorganic fertilizer (N & K) applications affect the growth of coconut seedlings. In open bottom sowing technique, combination of 360 N + 144 K gave the biggest girth of coconut seedlings stem and followed by 120 N + 144 K compared to other treatments (Figure 1). The control

plots that had fertilizer N and K only gave smaller stem girth. Stem girth was measured to the highest of 6 cm with the combination of N and K assisted by innovative sowing method.

Plant height

For the plants height, combination of 120 N + 144 K in open bottom sowing technique showed higher than other treatment and sowing method (Figure 2). The plant can reach up to 100 inches or 2.4 meters at 9 months old after germination. Result also shows that the increasing rate of N and K shows the reducing plants height. It is assumed that the inorganic soil matter does remain constant, and that N is supplied by the rain, crop residues and organic fertilizers, without including the released nutrients by mineralization of the inorganic matter.

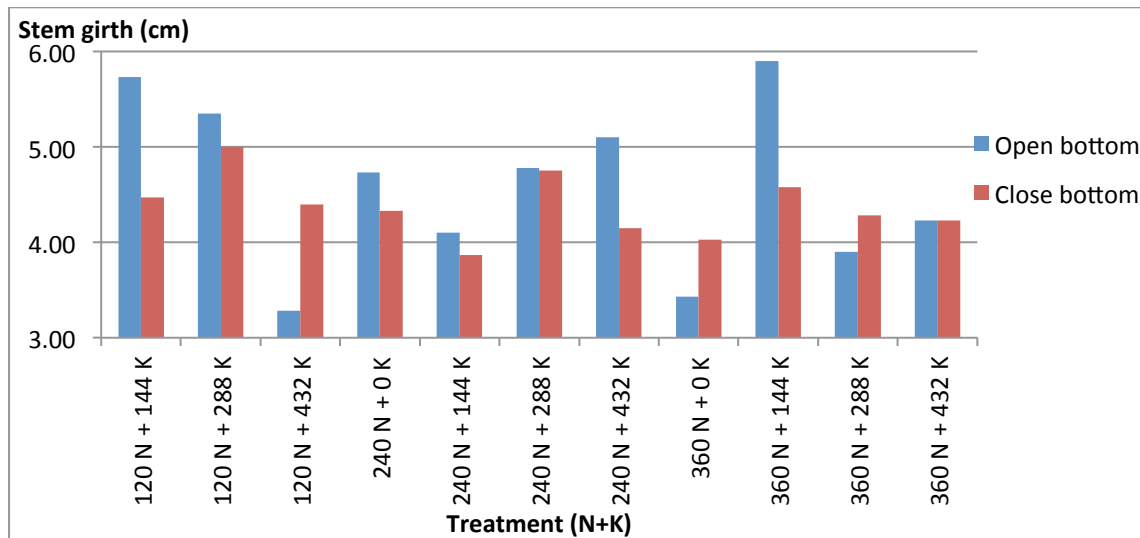


Figure 1. Stem girth of coconuts seedling affected by different rate of N and K

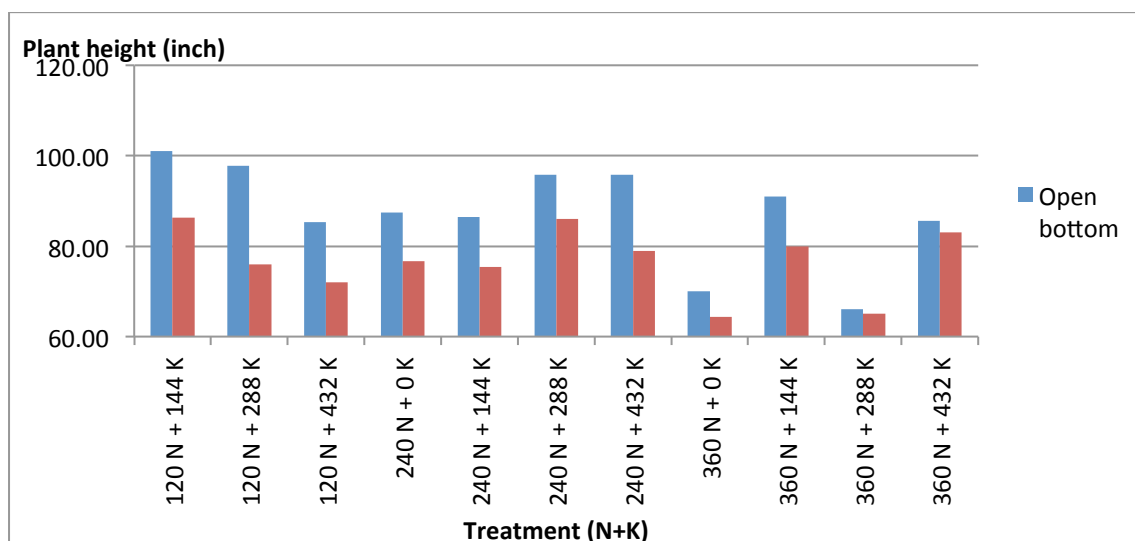


Figure 2. Plant height of coconuts seedling affected by different rate of N and K

Weight of roots

Figure 3 shows the result of roots weight for coconuts seedling under different sowing type and fertilizer combination. Combinations of 120 N + 144 K in open bottom sowing technique have higher than other treatments and sowing methods (Figure 2). The root weights are able to reach up till 700 g/plant compare to other combinations of fertilizer and sowing method.

Length of roots

Figure 4 shows the result of roots length for each coconut seedling. Result shows that with open bottom sowing technique, combination of 240 N + 288 K gave longest roots of coconut seedlings and followed by 120 N + 144 K compared to other treatments. The length of roots can reach up to 80 cm with best combination of N and K assisted with open bottom sowing technique. However, if the nutrient recycling rate from the soil by the extractor is known, that is, the relation between the recycled nutrient quantity by the extractor and the nutrient quantity applied to the soil, it is possible to estimate the available quantity for plants, according to the soil volume the roots explore.

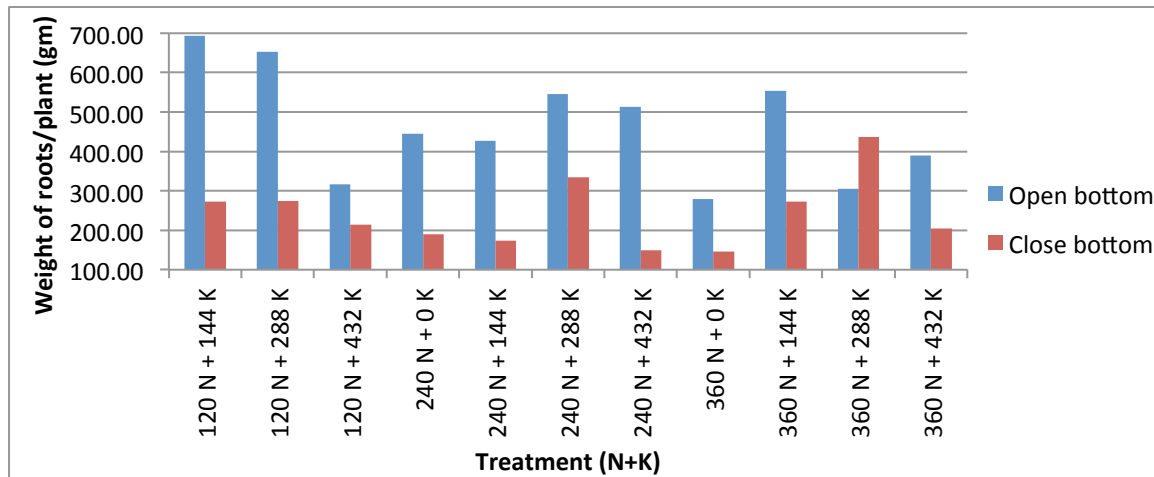


Figure 3. Weight of roots per plant of coconuts seedling affected by different rate of N and K

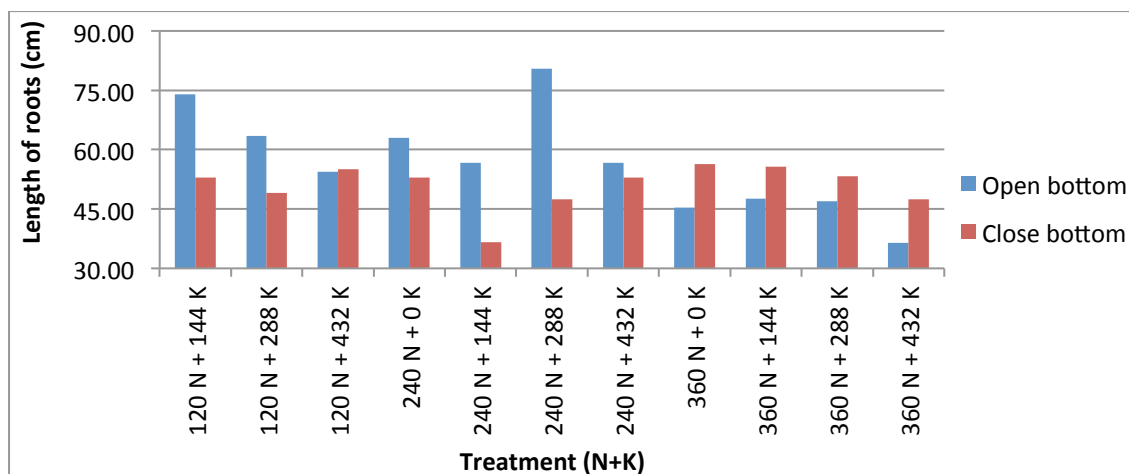


Figure 4. Averages length of roots per plant of coconuts seedling affected by different rate of N and K

Conclusions

From this study, results obtained from all the parameters indicate that there were significant growth of coconut seedlings between the treated seedlings and control. The results showed excellent effect of combination N and K with new technique of sowing method. It assisted in roots growth performance and plants physical changes. Therefore it is advisable to the farmers to have combination of N and K in a good rate. With open bottom sowing method, using 120 g N and 144 g K per plants/year gave the best growth in terms of performance in coconut seedlings under controlled condition.

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New Culm Productivity Assessment of *Gigantochloa scortechinii* in Response to Clump Density Applied

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Introduction

Gigantochloa scortechinii is one of the most commonly used bamboo species endemic to the Peninsular Malaysia. It occurs extensively in logged-over forests, particularly in the state of Kedah, Perak, Kelantan, Selangor and Pahang. The species thrives best in sites with well-drained sandy to clay loam soils with slight acidic condition (soil pH of 5.0–6.5).

Like other commercial species of bamboo, *G. scortechinii* stands have been very much depleted and the supply continues to decline due to unregulated exploitation. Furthermore, these bamboos grow wild, scattered and are practically unmanaged. At present, there are no proper management measures being practised to sustain the production of this raw material from natural forests. There is no information on maintaining proper number of culms per clump suitable for harvesting practices to support management of bamboo stands in Malaysia. Most of the harvesting activities of the resource are unsystematic and haphazard in nature (Azmy et al., 1997).

Thus, this study is an integral part in the management regime of natural bamboo stands for improving production and sustainability of this species. In view of current problems, a study was conducted to determine the best clump density for optimum productivity of *G. scortechinii*.

Materials and Methods

Description of study sites

The study was conducted in logged over areas in Betau, Kuala Lipis in Pahang. The study area has a flat and undulating topography. The mean annual rainfall, temperature and humidity of the study site is 1500 mm, 30°C and 89% respectively. Natural stand of *G. scortechinii* dominated the areas with a scattered distribution. The clump density was between 204–250 bamboo clumps per hectare.

Clump density

Three clump densities of *G. scortechinii* natural stands were classified, consisting of 10–25, 26–40 and >40 culms per clump respectively. Initial harvesting was employed to extract the older bamboo culms and then setting up the clump density treatments. The harvesting techniques used in this study was based on the various harvesting techniques and clump management that have been practiced in India (Lakshmana, 1988).

The trial was conducted in a one hectare trial plot. A total of 15 clumps for each clump density were selected randomly and observed in this trial. The parameters observed are number of new culms produced, its height and diameter at breast height (DBH). The assessment was carried out at 18 months after harvesting had been applied. Every new culm emerged will be marked with coloured paint. Data were subjected to Analysis of Variance (ANOVA).

Results and Discussion

The results from this study showed that clump density gave an effect on the emergence of new culms within the duration of 18 months after harvesting had been carried out. Table 1 reveals that clump

type had high significant influence on the emergence of the new culms after felling. However, no significant difference was observed on DBH and height of the bamboo culms.

Table 1. Analysis of variances on growth of *G. scortechinii* at 18 months after felling

Source	DF	Total no. new culms	Height	DBH
Clump Density	2	4214.0**	6.4 ^{ns}	0.7 ^{ns}

Note: ns – not significant at $P < 0.05$; *- significant at $P < 0.05$, **- highly significant at $P < 0.001$

The mean yield revealed that culm density of more than 40 culms per clump resulted in higher culm production (Table 2). Felling of culm groups with above 40 culms per clump gave significantly higher number of culm production with average of 10.5 culms and 7.2 and 3.8 culms were recorded for categories of 26-40 and 10-25 culms per clump, respectively.

Table 2. Effects of clump density on number of new culms produced, height and DBH of *G. scortechinii* at 18 months after harvesting treatments

Clump Density (no. of culms per clump)	No. of new culms	Height (m)	DBH (cm)
10–25	3.8c	14.8b	6.9a
26–40	7.2b	16.0ab	6.8a
>40	10.5a	15.1ab	7.2a

Note: Values with the same letter(s) are not significantly different at $P < 0.05$.

From this study, it indicated that harvesting intensity should be looked into and this is closely related to culms removable and density of a bamboo clump. The clump size also gave significant effect on culm production. From this study, it showed that bamboo clumps having more than 26 culms per clump can be used as a basis of minimum number of clump density that produced 7 new culms which is economic for bamboo production. Bamboo clumps having less than 26 culms per clump can be harvested selectively to attain better culm production. Thus, harvesting should be done selectively to the smaller clumps until it reaches the limit, i.e. until the clump size reaches more than 26 culms per clump. It is important to have a systematic management of bamboo resources to ensure adequate and continuous supply of bamboo culms over a long period of time. Similar results on *Dendrocalamus strictus* were reported by Varmah and Bahadur (1980).

Conclusions

Based on the results of the study conducted, *G. scortechinii* natural stands can be managed systematically. The results indicated that clump density gave highly significant effects on the emergence of new culms, culm quality and above-ground biomass at 18 months after felling. Furthermore, it is recommended that only bamboo clumps with the density of 26 culms per clump and below should be harvested selectively. This proper clump density and harvesting practice can be used to manage the natural stands of bamboo sustainably.

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Sweet Potato Growth and Yield as Affected by Application of Inorganic Fertilizer and Biofertilizer

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Introduction

Sweet potato (*Ipomea batatas*) belongs to Convolvulaceae family and is a short term food crop that offers many economic opportunities in downstream processing of food products. Its high nutrient content can be observed in the exceedingly rich beta-carotene and phytonutrients, including polysaccharide-related molecules such as batatins and batatosides (Tumuhimbise et al., 2009). Sweet potatoes also contain storage proteins called sporamins which have unique antioxidant properties (Failla et al., 2009). They are also a very good source of vitamin C, manganese, copper, pantothenic acid, and vitamin B6. Additionally, they are a good source of potassium, dietary fiber, niacin, vitamin B1, vitamin B2, and phosphate. Globally, it is among the important food crops in the world, after wheat, rice, maize, potato, and barley. The tubers are used in the production of industrial starch, alcohol, pectin and others. Considerable interest is being shown in the cultivation of sweet potato as it gives high yield and sweet potato is relatively an easy crop to grow. Based on statistics, the production of sweet potato in Malaysia was 26,582 tonne in 2011 and increased to 26,688 tonne in 2013 with cultivated area of 2,229 ha in 2011 to 2,505 ha in 2013 (Jabatan Pertanian, 2013). Many growers use solely inorganic fertilizer in the cultivation of sweet potato. The combination of inorganic fertilizer with organic matter can create a beneficial interaction in maintaining high soil fertility. According Yeng et al., (2012), both inorganic and organic inputs are needed to increase crop production in Guinea Savanna zone. The importance of organic fertilizer is seen in its little or no soluble salt content and its application in large quantity without at risk of damaging crop roots and soil microorganisms. It helps to break the organic materials into inorganic water soluble forms for plant use increase water retention and encourages the biological activity of the soil (Kareem, 2013). Thus, the present study was conducted to determine the effects of biofertilizer applied with different rates of inorganic fertilizer on growth and yield of orange sweet potato variety.

Materials and Methods

The study was conducted on mineral soil at Field 15, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. Previously, the experimental plot was grown with pasture grass. The experiment started in early May until August 2015. Stem cuttings of 30 cm from plants aged 2 months were used as planting materials. The planting materials were obtained from Ulu Chuchuh, Sepang, Selangor. The cuttings were planted at a distance of 25 cm between plant and 30 cm between the ridges. There were four treatments (NPK 12:12:17:2 fertilizer at 280 kg ha⁻¹ (control), NPK 12:12:17:2 fertilizer at 280 kg ha⁻¹ + Biofertilizer, NPK 12:12:17:2 at 400 kg ha⁻¹ and NPK 12:12:17:2 at 400 kg ha⁻¹ + Biofertilizer) arranged in Randomized Complete Block Design of 16 experimental plots with four replications. Biofertilizer was applied as organic fertilizer at rate of 4 t ha⁻¹. Chicken manure were applied to experimental plots at 5 t ha⁻¹ prior planting. NPK fertilizer were applied in three split applications at 21, 35 and 56 days after planting. Weeds were controlled manually every week starting from the planting date.

Measurements and Data Analysis

Tuber yield was recorded once for every two weeks starting from three weeks after planting by successive harvesting using 0.2 m² quadrat. Maximum tuber yield was calculated as the highest fresh yield of sweet potato over the whole experimental period for each treatment. Fresh yield was weighed using a digital balance and tuber samples were oven dried at 70 °C to constant weight. Radiation interception (PAR) and leaf area index (LAI) were measured using a Decagon AccuPAR model LP-80 PAR/LAI Ceptometer (Decagon Devices, Inc., Washington, USA). Measurements were taken weekly once the canopy had formed. The fraction of radiation intercepted (Fi) was determined using the techniques of Gallagher and Biscoe (1978): $F_i = (1 - T_i)$ where T_i was the transmitted radiation. The radiation use efficiency (RUE) was calculated as the slope of the linear relationship between accumulated crop biomass and accumulated intercepted PAR. The regression line was forced through the origin based on assumption that when accumulated intercepted PAR was zero, no dry matter was produced.

Data analysis

Data were subject to analysis of variance and mean values were compared using Least Significant Difference at the 0.05 level.

Results

Maximum Tuber Yield

Maximum tuber yields showed no significant differences between treatments. The highest yield was 15,370 kg ha⁻¹ from the combination of 280 kg ha⁻¹ NPK fertilizer and biofertilizer and the lowest was 10,560 kg ha⁻¹ from the combination of 400 kg ha⁻¹ NPK fertilizer and biofertilizer (Figure 1).

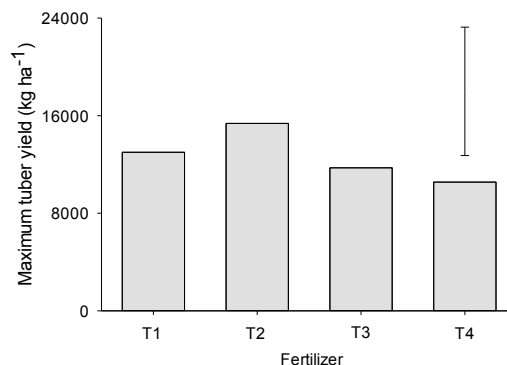


Figure 1: Maximum tuber yield of (T1: 280 kg ha⁻¹ NPK fertilizer (control), T2: 280 kg ha⁻¹ NPK fertilizer + Biofertilizer, T3: 400 kg ha⁻¹ NPK fertilizer and T4: 400 kg ha⁻¹ NPK fertilizer + Biofertilizer) of orange sweet potato.

Intercepted PAR and LAI

From the exponential curve the critical LAI (LAI_{crit}) at 90% of intercepted PAR was calculated to be 4.21 for (a) T1, 3.96 for (b) T2, 4.12 and 4.18 for (c) T3 and (d) T4, respectively (Figure 2). Figures 2a-d show all treatments intercepted radiation up to 90%, which meant that they did reach the LAI_{crit}. The LAI_{crit} for all treatments was achieved at 62 to 77 days after sowing (Figure 2.1a-d).

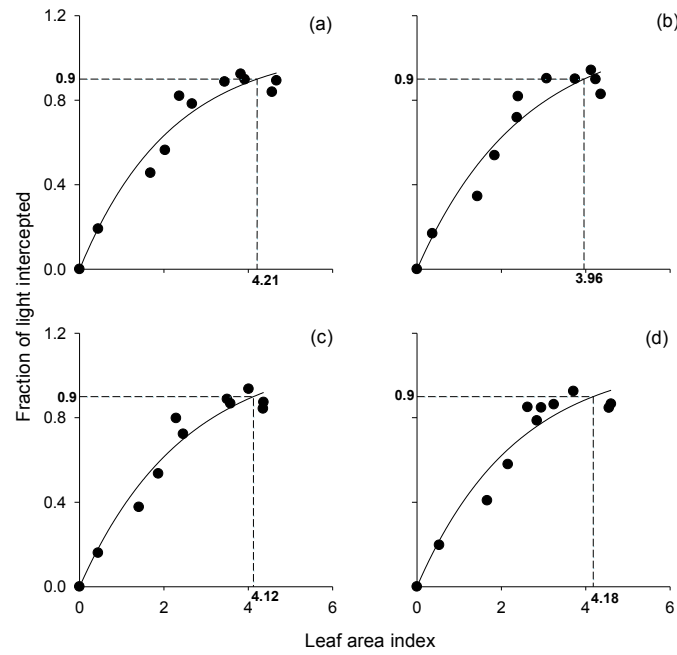


Figure 2: The relationship between the fraction of radiation intercepted against leaf area index of (a) (T1) 280 kg ha⁻¹ NPK fertilizer (control), (b) (T2) 280 kg ha⁻¹ NPK fertilizer + Biofertilizer, (c) (T3) 400 kg ha⁻¹ NPK fertilizer and (d) (T4) 400 kg ha⁻¹ NPK fertilizer + Biofertilizer of orange sweet potato.

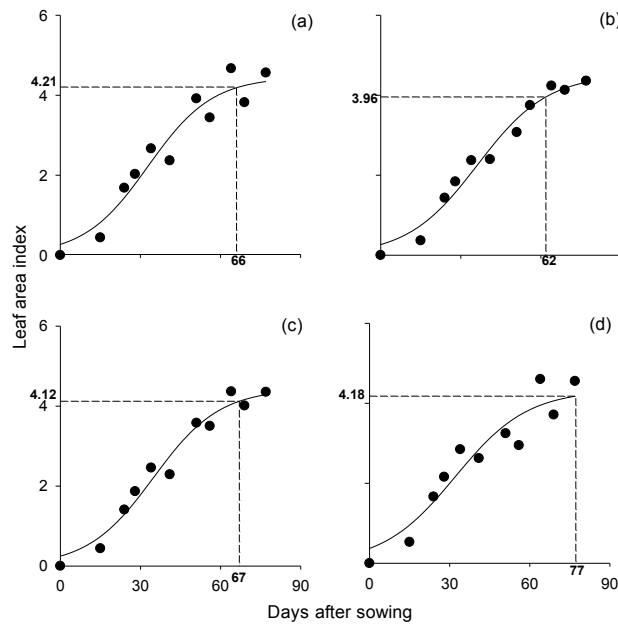


Figure 2.1: The relationship between the leaf area index against days after sowing of (a) (T1) 280 kg ha⁻¹ NPK fertilizer (control), (b) (T2) 280 kg ha⁻¹ NPK fertilizer + Biofertilizer, (c) (T3) 400 kg ha⁻¹ NPK fertilizer and (d) (T4) 400 kg ha⁻¹ NPK fertilizer + Biofertilizer of orange sweet potato.

Total intercepted photosynthetically active radiation

No significant difference for the total intercepted photosynthetically active radiation (PAR) was detected for all treatments (Table 1).

Table 1: Total intercepted photosynthetically radiation radiation (PAR) of orange sweet potato receiving different inorganic fertilizer and biofertilizer treatments.

Fertilizer	Total intercepted PAR
(a) 280 kg ha ⁻¹ NPK fertilizer	444 ^a
(b) 280 kg ha ⁻¹ NPK fertilizer + Biofertilizer	432 ^a
(c) 400 kg ha ⁻¹ NPK fertilizer	426 ^a
(d) 400 kg ha ⁻¹ NPK fertilizer + Biofertilizer	438 ^a

Means with the same letter are not significantly different ($P>0.05$) using LSD

Radiation use efficiency (RUE)

There was no significant difference among fertilizer treatments in RUE similar with other measurements (Table 2). Radiation use efficiency ranged between 1.75 (400 kg ha⁻¹ NPK fertilizer) to 2.36 (280 kg ha⁻¹ NPK fertilizer + Biofertilizer).

Table 2: Radiation use efficiency of orange sweet potato receiving different inorganic fertilizer and biofertilizer treatments.

Fertilizer	Radiation use efficiency
(a) 280 kg ha ⁻¹ NPK fertilizer	2.02 ^a
(b) 280 kg ha ⁻¹ NPK fertilizer + Biofertilizer	2.36 ^a
(c) 400 kg ha ⁻¹ NPK fertilizer	1.75 ^a
(d) 400 kg ha ⁻¹ NPK fertilizer + Biofertilizer	1.89 ^a

Means with the same letter are not significantly different ($P>0.05$) using LSD

Discussion

In the present study, the objectives of the study to determine the effects of biofertilizer applied with different percentage of inorganic fertilizer in combination with biofertilizer on growth and yield of orange sweet potato did not show positive results. Maximum tuber yield, total intercepted PAR and RUE were not significantly different among treatments. The results were in contrast with the finding of Agbede, (2010) which indicated that application of NPK fertilizer and poultry manure significantly increased the tuber yield of sweet potato, however, the application of NPK fertilizer or poultry manure alone did not show any significant difference. No significant difference in amount of intercepted PAR and RUE indicated equal efficiency of PAR conversion into biomass for all treatments applied. Generally if there is any positive yield response, it will be associated with increment in interception of PAR and high RUE by understanding the interaction of PAR and RUE in response with biomass. Shangakkara et al., (2004) stated that the replenishment of nutrient and enhanced quality of tropical soils could be achieved through the addition of fertilizers, organic matter or a combination of both. The lack of significant response exhibited by the orange sweet potato could be attributed to the lack of survival rate of microbes in the soil applied with biofertilizer and it could be related to the crop itself. Sweet potato has the ability to produce long roots and vines which can extract nutrients and enable it to survive even under poor soils with high aluminium content (Janssens, 2010). The other factor that could explain for the absence of significant effects on tuber yields and other measurements could be the widespread incidence of sweet potato weevil which started in late June.

Conclusion

The application of NPK fertilizer and biofertilizer did not show any significant difference for all treatments, however, it was observed that the application of NPK fertilizer at 400 kg ha⁻¹ and biofertilizer at 4 t ha⁻¹ led to production of moderate yield of orange sweet potato in comparison with standard practice.

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Response of Five *Citrus Hystrix* Provenances to Different Fertilizer Applications

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Introduction

Citrus hystrix, very well known in Malaysia as 'limau purut' and in Thailand as 'som makrut' is a species of family Rutaceae that have a great economic potential (Muhammad Nor,1999). The Kaffir Lime Tree is a thorny tree with aromatic and distinctively shaped "double" leaves. The kaffir lime is a rough and has green fruit. In Malaysia, this plant was planted in the house garden together with other plants such as lemon grass, ginger, and pandan (Muhammad Nor,1999). Essential oils obtained from the leaves and fruits are widely used in medicinal preparations, perfumery and flavor ingredients. The chemical compounds from these extractive contents were found to be potential sources as raw materials for the development of our local herbal industries. In order to establish products based on an essential oil industry in the country, it requires selection of planting materials, which contain high quantity and quality of essential oils. Realizing the importance of this plant, the Plant Improvement Programme of Forest Research Institute Malaysia (FRIM) has come out with a breeding strategy to produce good planting materials of *C. hystrix*. Thus, one of the aspects that breeder needs to investigate is fertilization aspects at the nursery stages. This study was conducted with the objectives of i) to investigate the response of *C. hystrix* seedlings to two types of fertilizer applications (granule and liquid) and, ii) to determine which provenances responded better to the two fertilizer applications.

Materials and Methods

A total of 450 seedlings at the age of one month from five provenances (Mata Ayer, Perlis; Yan, Kedah; Banting, Selangor; Teluk Intan; Perak and Raub, Pahang) were used in this study. These provenances were selected based on a previous study on a selection high yielding mother trees for high citronellal content (ranging from 40-85%). Experiment were handled in 6' x 8' polybags size and was conducted at FRIM's Nursery, Kepong. The experimental layout is using the randomized complete block design, arranged in a factorial experiment of five provenances and two types of fertilizers with 30 seedlings for each provenancesper block. The treatments consisted of two fertilizer types: NPK Green (8:8:8), Flora Mas (NPKMgSCa, 20:15:10:1:3:2 + 1% trace elements) and control (no fertilizer). NPK Green was applied by ring method at the rate of 100 mg per seedlings. Whereas, for Floral Mas, 6 ml of liquids Floral Mas mixed into 432 ml of water was applied by sprayed directly to the leaves. The data of plant height and diameter were taken monthly in a period of 6 months. The data collected were subjected to analysis of variance (ANOVA) and treatment means were separated by Duncan Multiple Range Test (DMRT).

Results and Discussion

Based on Analysis of Variance (ANOVA) shows in Table 1 and 2, main effects of provenance on plant height (0.003) and diameter (0.004) of *C. hystrix* seedlings were highly significant. It was indicated that seedlings in each provenances are varied from each other due to genetic itself. However there is no significant different (0.175 and 0.233) on the main effect of fertilizing treatment. Interaction of fertilizer treatment and provenances also indicated a highly significant difference on plant height and diameter (0.000) growth at significance level, $P \leq 0.05$. The findings are similar with study did by Nnabude et al. (2015). The results of the study indicated non-significant differences

among the tomato varieties and rates of treatment applied while the interaction between fertilizer and tomato varieties significantly affected the plant height relative to other growth parameters. Therefore, further analysis of means using DMRT were conducted to determine the effects of fertilizer types and provenance on plant height and diameter of *C. hystrix* seedlings.

Table 1: ANOVA of height response of *Citrus hystrix* seedlings from five provenances applied with two types of fertilizers

Source of variation	df	Mean squares	P<0.05
Provenance	4	207.19	0.003**
Treatment	2	43.37	0.175 ^{ns}
Provenance x Treatment	8	19.86	0.000**
Error	435	4.05	
Total	449		

ns is insignificant and ** significant at $P \leq 0.05$

Table 2: ANOVA of diameter response of *Citrus hystrix* seedlings from five provenances treated with two types of fertilizers

Source of variation	df	Mean squares	P<0.05
Provenance	4	15.28	0.004**
Treatment	2	2.89	0.233 ^{ns}
Provenance x Treatment	8	1.64	0.000**
Error	435	0.27	
Total	449		

ns is insignificant and ** significant at $P \leq 0.05$.

The variability of *C. hystrix* provenances plant growth in response to applied fertilizer types are presented in Table 3. Plant height and diameter were all significantly ($P < 0.05$) affected by *C. hystrix* provenances and fertilizer types. *C. hystrix* provenance from Banting, Selangor and Yan, Kedah had the highest plant height and diameter with 7.47 cm and 7.22 cm, respectively. This is followed by provenances from Raub, Pahang (6.34 cm) and Mata Ayer, Perlis (6.22 cm) and Teluk Intan, Perak (3.65 cm). In terms of diameter, provenances Banting, Selangor recorded the highest diameter (2.07 mm), whereas Teluk Intan, Perak recorded the lowest (1.03 mm). It was observed the application of Flora Mas fertilizer gave the higher value in plant height (6.75 cm) and diameter (1.88 mm) even though it was not significantly different from NPK Green fertilizer. The results indicated the application either one of that fertilizers used gave significantly different from control and the growth response are positive with some increment.

Table 3: Effects of fertilizer types and provenances on plant height and diameter of *C. hystrix*. Values with the same letter are not significantly different at $P \leq 0.05$

Fertilizer type	Provenances					Mean
Plant height (cm)	Banting, Selangor	Teluk Intan, perak	Mata Ayer, Perlis	Raub, Pahang	Yan, Kedah	
Control	6.20	3.81	5.34	6.10	6.96	5.68 ^b
NPK Green	6.71	3.27	6.79	6.19	7.57	6.11 ^{ab}
Floral Mas	9.51	3.86	6.52	6.73	7.13	6.75 ^a
Mean	7.47 ^a	3.65 ^c	6.22 ^b	6.34 ^b	7.22 ^a	
Diameter (mm)						
Control	1.83	1.01	1.78	1.65	1.76	1.60 ^b
NPK Green	1.78	0.90	1.75	1.88	2.25	1.71 ^{ab}
Floral Mas	2.60	1.16	1.87	1.87	1.89	1.88 ^a
Mean	2.07 ^a	1.03 ^c	1.79 ^b	1.79 ^b	1.97 ^{ab}	

Conclusion

As a conclusion, application two types of fertilizer whether Floral Mas (liquid) or NPK Green (granule) can improved the seedlings growth in plant height and diameter of *C. hystrix* from all provenances. Interaction effect between fertilizer and provenances showed seedlings from provenances Banting, Selangor gave higher increment in plant height and diameter compared to other provenances. In order to produce high quality and large quantity in planting materials in future, it is recommended to use Floral Mas to improve the growth of *C. hystrix* due to its better response.

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CHAPTER 2

PLANT PHYSIOLOGICAL ECOLOGY

Leaf Temperature and Leaf-to-Air Vapour Pressure Deficit of Black Pepper (*Piper nigrum* L.) Grown on Kenaf (*Hibiscus cannabinus* L.) Based Composite Post

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Introduction

Black pepper (*Piper nigrum* L.) is generally grown from stem cuttings. The root system is developed from adventitious roots formed at nodes that are buried in the soil at planting. As the vegetative (orthotropic) shoot climbs upward, a bunch of short adventitious roots also develops to help the shoot cling to the support. It is well known that black pepper vines require a reliable support for proper growth, development and yield. Currently in Sarawak, the preferred support material is the wooden pole of the Belian tree (*Eusideroxylon zwageri* Teijsm. & Binn.), which is a high density, heavy, construction timber resistant to termites (Paulus, 2011). The ironwood poles are very durable and can remain undamaged for many years. However, the belian post acquisition is the most expensive item in the establishment of a pepper garden apart from being limited in supply.

Kenaf (*Hibiscus cannabinus* L.) being a cheap, fast growing plant and its fibers possess good mechanical properties is abundant in the north eastern part of Peninsular Malaysia particularly in the state of Kedah and Kelantan. Due to its favourable strength to weight ratio, ease in handling, sustainability and local availability, kenaf fibers have been used as reinforcement in many types of materials including concrete, building blocks and other structural and non-structural applications (Bhutta et al., 2013). Suitable as natural fiber bio-composite, kenaf fibers are emerging as a promising alternative post material that will provide a much needed boost to the pepper industry in Malaysia.

Providing an ideal supporting post plays a pivotal role in the successful growth and development of pepper vines. It is important to note that materials used as support post do not absorb or emits excessive heat that can increase surrounding temperature when exposed to daily sunlight radiation. Wahid and Sitepu (1987) observed that support post material were believed to be the key element affecting pepper physiological response which have close correlation with its growth and yield development. Like other crops, Mathai (1983) and Vijayakumar et al. (1984) found that excessive exposure to heat from sunlight radiation decreased black pepper carbon fixation and some physiological disorders were developed even under favourable soil moisture conditions. Although there is no studies so far associating pepper physiological response to increasing temperature and leaf-to-air vapour pressure deficit (VPD) however the negative responses of leaf stomatal conductance and photosynthetic rate to increasing VPD and leaf temperature have been discovered on crops such as corn and wheat (Warkentin et al., 1992; Darlington et al., 1997).

There is no documentation so far on the growth performance of black pepper using kenaf based composite post. In this study, black pepper gas exchange rates were used as growth indicator in response to microclimatic effect under which several posts system was applied. Therefore, this study was conducted with the following objectives: (i) to determine the leaf temperature and leaf-to-air vapour pressure deficit (VPD) under kenaf based composite posts system, (ii) to investigate the gas exchange rates of black pepper under kenaf based composite posts system and (iii) to examine possible interactive effects between leaf-to-air VPD and leaf stomatal conductance of black pepper.

Materials and Methods

The experiment was carried out in the field and conducted in an 8 m x 8 m plot located in Bau, Sarawak. The crop involved in this study was *Piper nigrum* L. var. Semongok Aman. Pepper cuttings were first rooted in a sand bed. After 4 weeks, the rooted cuttings were selected and transplanted to the planting site.

In the current study, three (3) types of kenaf based composite posts were used and provided by the National Kenaf and Tobacco Board. The kenaf based composite posts are shown in Table 1. Belian wooden poles were used as a standard. The dimension of supports used in this study was measured at about 5 cm x 5 cm x 2 meters long. The study was conducted from the month of September 2014 to May 2015.

Table 1: Type of kenaf based composite post and its composition

Type of kenaf based composite post	Composition
Kenaf extrusion	60% kenaf fiber yarn, 40% polyvinyl chloride (PVC)
Kenaf pultrusion	80% glass fiber yarn, 10% glass fiber mat, 10% kenaf fiber yarn
Kenafcrete	10% kenaf fiber yarn, 20% piling rod, 70% cement

Experimental design and treatments

The experiment was a completely randomized design (CRD) with four (4) treatments and replicated five (5) times. The treatments based on support post materials were: (i) control– belian wooden poles as standard support post, (ii) Kenaf extrusion, (iii) Kenaf pultrusion and (iv) Kenafcrete.

Leaf temperature and leaf-to-air vapour pressure deficit (VPD) measurement

Following the method by Day (2000), leaf temperature and VPD were computed based on air temperature, measured by a fine wire thermocouple using a LICOR LI-6400 XT (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA).

Piper nigrum gas exchange rate measurement

Gas exchange measurement was determined according to the method by DiCristina and Germino (2006), carried out on young fully expanded leaves with the same orientation and the same layer in the crown (middle bottom). Measurements of net photosynthesis on an area basis (A) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and leaf stomatal conductance (g_s) ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of twenty five (25) different leaves per treatment were monitored using a LICOR LI-6400 XT (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set to PAR at $900 \mu\text{mol m}^{-2} \text{ s}^{-1}$ which was presumed to be the intensity where photosynthetic rates for black pepper would be maximal (Mathai, 1983; Vijayakumar et al., 1984). The CO_2 flow into the chamber was maintained at a concentration of $400 \mu\text{mol mol}^{-1}$. The humidity flow into the chamber was fixed at $500 \mu\text{mol s}^{-1}$. Measurement was done on gas exchange parameters at between 1100 to 1200 h.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) with the SPSS software (version 15, SPSS Inc., Chicago, USA). The Tukey's Honest Significance Difference (HSD) Test, at $\alpha = 0.05$ level of significance was done to compare the means. The relationship between leaf temperature and leaf-to-air VPD were correlated using regression of best fit.

Results and Discussion

Leaf temperature and leaf-to-air vapour pressure deficit (VPD)

The influence of kenaf based composite posts on black pepper leaf temperature is presented in Table 2. The black pepper supported by kenafcrete post demonstrated a significantly higher leaf temperature when compared to the belian, kenaf extrusion and kenaf pultrusion posts. Since kenafcrete consisted mainly of concrete material, this might contribute to the increase of leaf temperature. The finding was almost similar to a study done by Sivaraman et al. (1999) which reported that concrete poles tend to heat up during summer under exposed conditions. Leaf temperature of vines supported by belian, kenaf extrusion and kenaf pultrusion posts showed no significant difference indicating that in terms of temperature microclimatic effect on the leaves, both kenaf extrusion and pultrusion were on par with the belian.

Table 2 shows the result of leaf-to-air vapour pressure deficit (VPD) subjected to different treatments. Leaf-to-air VPD under kenafcrete support post recorded the highest value at 1.90 kPa while that of the kenaf pultrusion support post exhibited the lowest value at 1.71 kPa. This result might be due to the surface characteristics of the support posts that affected heat transmission into the environment. Agarwal and Gupta (2011) studied that a coarse and dull coloured surface such as concrete is more likely to absorb and emits heat and therefore, increase surrounding temperature when compared to a lighter coloured and smooth surface. In other crops such as corn and wheat, a small increase in temperature can increase leaf-to-air VPD during exposure to extreme heat radiation (Day, 2000; Will et al., 2013).

Table 2: Effect of kenaf based composite posts on leaf temperature and leaf-to-air VPD of pepper

Support Post Material	Leaf temperature (°C)	Leaf-to-air VPD (kPa)
Belian	35.66 ± 1.13 ^b	1.81 ± 0.28 ^b
Kenaf extrusion	35.74 ± 1.51 ^b	1.76 ± 0.21 ^{bc}
Kenaf pultrusion	35.68 ± 1.41 ^b	1.71 ± 0.31 ^c
Kenafcrete	36.27 ± 0.98 ^a	1.90 ± 0.23 ^a

Note: Means with different alphabets within column indicate significant difference between treatments using LSD at 0.05 probability level (Means ± S.D., $n = 25$).

Piper nigrum photosynthetic and leaf stomatal conductance rate

The photosynthetic rates (A) of plants supported by kenaf pultrusion posts recorded the highest value which indicated better growth performance for pepper vines supported by this type of post (Table 3). The favourable outcome from the result of net photosynthetic rate was that kenaf pultrusion posts

were able to support the growth of black pepper vines fairly well and in a similar manner as the belian hardwood. The *A* rate was reduced by 35% in black pepper vines subjected to kenafcrete when compared to that of kenaf pultrusion. The result for net photosynthetic rate of black pepper might well be associated with changes in leaf temperature and leaf-to-air VPD (Table 2). Day (2000) reported that increased in leaf-to-air VPD and temperature, acting singly or interactively, reduced photosynthetic carbon gain of a plant. The negative response of *A* rate to increasing VPD in plants have been described in several studies as well (Warkentin et al., 1992; Darlington et al., 1997).

The results in Table 3 shows that the leaf stomatal conductance (*g_s*) of black pepper supported by kenafcrete declined by 53% of the kenaf pultrusion value. With regard to external factors, stomata respond to many environmental factors including leaf temperature and leaf-to-air VPD (Jones, 1992). Vann et al. (1994) discovered significant inhibition of both *A* and *g_s* rates in most plants at air temperatures >34°C and related the response to current range limits and changes that might be linked to a warming climate. Table 3 also shows that there was no significant difference (*p* < 0.05) between the treatment belian and kenaf pultrusion suggesting that leaf stomatal conductance of black pepper responded positively when supported by kenaf pultrusion and belian hardwood posts.

Table 3: Effect of kenaf based composite posts on net photosynthetic rate and leaf stomatal conductance of *Piper nigrum* L.

Treatment	Net photosynthetic rate (<i>A</i>) ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	Leaf stomatal conductance (<i>g_s</i>) ($\text{mol m}^{-2}\text{s}^{-1}$)
Belian	9.82±2.50 ^a	0.20± 0.07 ^{ab}
Kenaf extrusion	7.33±4.37 ^b	0.18± 0.13 ^b
Kenaf pultrusion	10.89± 1.63 ^a	0.30± 0.12 ^a
Kenafcrete	7.10±4.84 ^b	0.14± 0.09 ^b

Note: Means with different alphabets within column indicate significant difference between treatments using LSD at 0.05 probability level (Means ± S.D., *n* = 25).

Figure 1 shows the relationship between *P. nigrum* L. leaf temperature and leaf-to-air VPD. The strong relationship between *P. nigrum* L. leaf temperature and leaf-to-air VPD regardless of treatments showed a polynomial cubic regression line of zero intercept with $r^2 = 0.89$ indicating that higher leaf-to-air VPD increased the leaf temperature of black pepper. This finding concurs with a report by Will et al. (2013) on several crop species which revealed that a 40% increase in leaf-to-air VPD was linked to a rise in leaf temperature ranging from 33°C to 37°C.

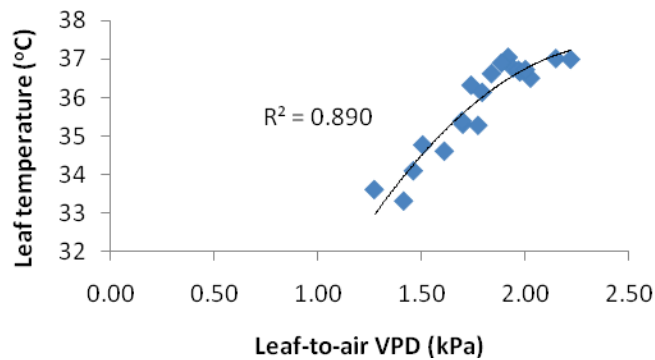


Figure 1: Relationship between leaf temperature ($^{\circ}\text{C}$) and leaf-to-air vapour pressure deficit (kPa) subjected to different pepper support systems. Values are means \pm s.e. of twenty leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determined coefficient are included.

Similarly, the relationship between leaf stomatal conductance (g_s) rate of *P. nigrum* L. and leaf-to-air vapour pressure deficit (VPD) subjected to different treatments were fairly correlated, $r^2 = 0.61$ (Figure 2). The relationship between the two regardless of treatments was best described by a polynomial cubic regression line of zero intercept which explained a value of around 61% of the variation in leaf temperature. The outcome depicted close relations between the two in which g_s decreased with increasing leaf-to-air VPD. In response to period of high vapour pressure deficit, most plants have the ability to acclimatize, combining morphological and physiological modifications, which will improve their capacity to survive heat (Liang et al., 1997). For example, stomata imposed a critical control over water loss and exchange of gases between the atmosphere and leaf cells (Lecoeur et al., 1995) and limits transpiration of plants exposed to severe heat radiation and avoids leaf water potential becoming too negative (Kramer, 1987; Ryan et al., 1994; Kessler, 2008).

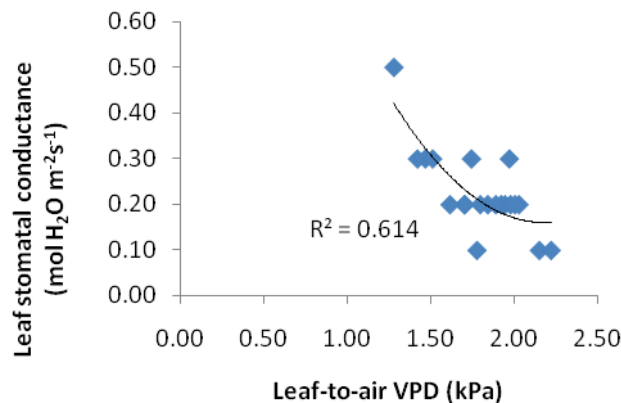


Figure 2: Relationship between leaf stomatal conductance and leaf-to-air vapour pressure deficit (kPa) subjected to different pepper support systems. Values are means \pm s.e. of twenty leaves taken from different plants per treatment. The regression line (continuous) is shown. The values of the determined coefficient are included.

Conclusions

This preliminary eight months study has shown that the kenaf based pultrusion black pepper support posts can performed as well as the belian hardwood of Sarawak. Kenafcrete and kenaf extrusion however performed not as favourably as the kenaf pultrusion and belian. It was revealed that

kenafcrete treatment contributed to an adverse microclimate environment for the growth of black pepper by showing significantly higher leaf temperature and leaf-to-air VPD. Net photosynthesis rates (A) and leaf stomatal conductance (g_s) of black pepper supported by kenaf pultrusion were comparatively higher than that of treatment belian, kenaf extrusion and kenafcrete. The leaf temperature and leaf stomatal conductance of *P. nigrum* L. were found significantly correlated to leaf-to-air VPD. Although longer period of time is needed to assess the ability of kenaf based composite posts particularly when supporting huge mature pepper vines, this study has provided an early indication of the promising potential of kenaf pultrusion as a feasible replacement for the fast extinct belian hardwood.

Acknowledgments

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Assessment of Hydraulic Conductivity on Different Sizes of Air-Layered *Azadirachta excelsa* (Jack) M. Jacobs

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Introduction

Water is important to all living things, including trees. It acts as a medium to transport water and nutrients inside the tree through xylem and phloem, facilitate photosynthesis and store to support cells turgidity. When photosynthesis and respiration reach a compensation point, their rates should be equal, so water plays an important role in the process. In hydraulic limitation hypothesis, it suggests that as length of tree increases, water transport system will extend later, delay the photosynthesis mechanism and thus retards the plant growth. Tree size can influence the process of height growth and the proportion of trunks, branches and crowns are important in enabling trees to withstand mechanical forces, light intercept and water transport to their foliage (King, 2011).

For tropical trees, it has been shown that total daily use of stored water increases with tree size (Goldstein et al., 1998). Meanwhile, tall trees often exhibit morphological and physiological changes to face hydraulic resistance limitations, include greater sapwood hydraulic conductivity and embolism resistance (Ishii, 2011). To further justify this hypothesis, hydraulic conductivity (K) parameter is chosen as it gives an important indicator on woody plant water relations in adaptation on new environment. The main objective of the study is to examine whether the changes in hydraulic conductivity affect the different sizes of air-layered *Azadirachta excelsa* with similar age range.

Materials and Methods

The study was conducted in the nursery of Faculty of Forestry, UPM. This study was continued from the previous study on biochemical processes with thirty air-layered plants from different branches length (30, 40, 50, and 60 cm) and three levels of donor height (3, 4 and 5 m height above ground level), that labelled as L1H1, L1H3, L2H1 and L2H2 mixed in treatments (Yap et al., 2014). All air-layered branches were measured by removing bark about 5 cm long early in the morning and quickly attached to the pressure coupling set filled with boiling distilled water and finally, tested with High Pressure Flow Meter (HPFM Gen3) in three categories: branches with leaves, branches without leaves and root branches. The correct flow range was determined after Zero Flow Volts was set to zero transducer, and then the function of the transient measurement of conductance was set as this was the quickest method for gathering hydraulic conductance data and also minimizing any plugging effects caused by the plants' natural healing process (Anonymous, 2009). The K values for each replicate in every category were recorded at two seconds intervals up to forty measurements and all data were subjected to the repeated measures analysis.

Results and Discussion

The ANOVA showed that there was no significant difference for hydraulic conductivity and respiration among different parts of tested replicates, so no post hoc test is executed. Since the results showed there were no significant differences in level (donor height) and length (plant height) for the parameter, thus no size-related trends were observed.

Table 1. Summary of ANOVA for hydraulic conductance parameters

Source of Variation	Parameter ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Df	Mean Square	F-value
Level	K	1	5.29e-9	0.04 ^{ns}
	Resp	1	1.97e-5	0.37 ^{ns}
Length	K	2	2.44e-8	0.16 ^{ns}
	Resp	2	4.46e-5	0.84 ^{ns}

ns: not significant

On the other hand, in regression relationship graphs at figure below, all replicates in the categories of branch with leaves and branch without leaves, showed slight positive correlations between 0.01 to 0.15 with increasing parameters, except for leaf area parameter, showed least positive correlation with $R^2 = 0.0023$, merely zero. There were bigger correlations with increasing coupling lengths and average diameters of branches in categories of branch without leaves, than in the categories of branch with leaves, where the R^2 values are 0.1404 and 0.0217, respectively.

A slight strong relationship in hydraulic conductivity with increasing branch length in both categories: with leaves and without leaves, as where the branches without leaves had tighter relationship than that found for the branches with leaves, as water supply rates with increment of tree size depend on the hydraulic resistance of water flow pathway and the steepness of water potential gradient between leaf and soil (Zach et al., 2010). Different hydraulic conductivities in air-layered plants were due to difference in branch length on flow path from soil to leaves and growth of branches. A less loose relationship is showed in hydraulic conductivity with increasing average branch diameter in both categories too, while hydraulic conductivity showed most loose relation with increasing leaf areas, that do not affected the water transportation inside the branches.

However, all replicates in the categories of root branches, showed slight negative correlations. The increment of lengths had higher correlation than the increment of average diameter, where the R^2 values are 0.0326 and 0.0092, respectively. The root showed the decline trend with increasing length and diameter, but length showed a closer relationship than diameter due to the disadvantage of root measurement with HPFM that the direction of flow is opposite to the normal direction of transpiration (Tyree et al., 1995).

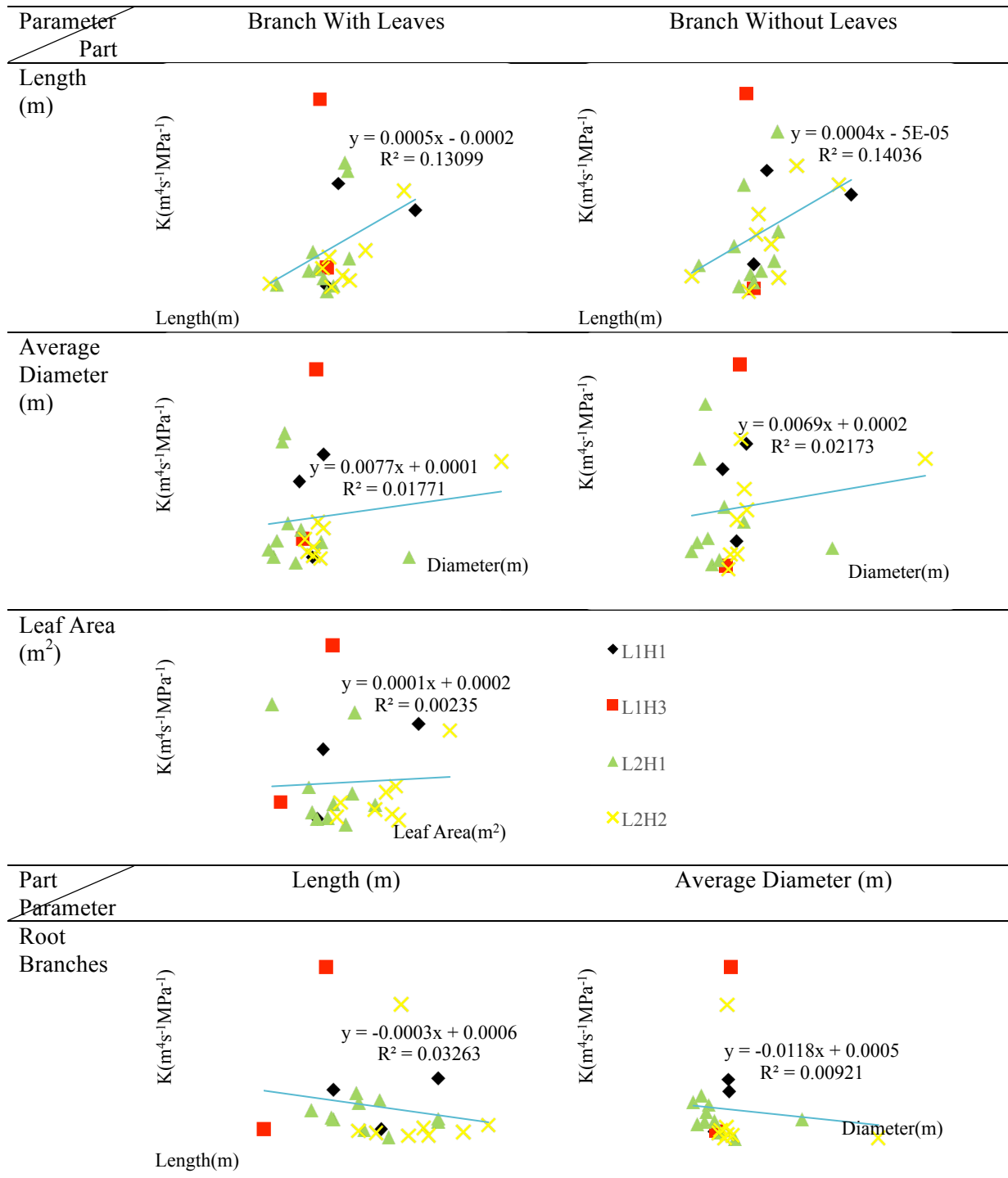


Figure 1. Hydraulic Conductivity of Different Level and Length of Branches

Conclusions

The little differences on selected size of air-layered trees were hard to prove that hydraulic constraint affects tree height through photosynthesis. Although the length of branches gave more effects on water transport than average diameter of branches at all categories, but length alone may not be fully responsible to changes of hydraulic conductivity. More researches have to be done to further investigate the effects of water transport system to overall tree growth.

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CHAPTER 3

BIOTECHNOLOGY

Genetic Engineering of Eksotika Papaya for Resistance to Papaya Dieback Disease

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Introduction

Papaya is an important fruit in the world. The export value of papaya in Malaysia is estimated RM100 - 120 million per year (Rabu et al., 2005). One of the major diseases that affect Malaysian papaya industry is papaya dieback disease. This disease is capable to destroy the whole papaya plantation and resulting total production loss. The pathogen that caused the disease have been identified and confirmed as *Erwinia mallotivora* bacteria belong to *Enterobacteriaceae* family (Noriha et al., 2011).

The common agricultural practices such as spraying of pesticides or antibiotics are not effective in controlling the disease. Early detection of the disease symptoms and destroying the affected plants seems to be the best control strategy at the moment. An alternative prevention method should be developed, and one of possible ways is by applying modern biotechnology approach. Development of this transgenic papaya will help revive the papaya industry in Malaysia.

Strategically, the development of transgenic papaya variety with enhanced resistance to dieback disease can be achieved by equipping the plants with the ability to disrupt the essential element for bacterial growth known as quorum sensing via genetic engineering approach. Two potential genes against papaya dieback were successfully isolated, characterized and cloned: two *Acyl-homoserine lactonase* (*Ahl lactonase* CHB37 and *Ahl lactonase* C151). These genes, which the antimicrobial activity have been validated *in vitro*, were transformed into Malaysian Eksotika papaya by using *Agrobacterium*-mediated transformation method. The contained evaluation of T₀ plants resistance degree towards *Erwinia mallotivora* is now in progress.

Materials and Methods

Embryogenesis Callus Induction of Eksotika Papaya

Immature zygotic embryos were obtained from immature fruit 90 days after pollination and were used as explants for callus induction. The fruits were kindly provided by MARDI Pontian, Johor. Embryogenic callus were induced by culturing the zygotic embryo on half-strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), 50 mg/L myo-inositol, full strength MS vitamin (thiamine-HCl, pyridoxine, glycine and nicotinic acid), 60 g/L sucrose, 45.2 µM 2,4-D, 0.14 g/L adenine hemisulfate, 400 mg/L glutamine, 250 mg/L carbenicillin and 3.2 g/L gelrite.

Agrobacterium-mediated Transformation and Regeneration of Transformants

The plasmid pCAMBIA2301 harboring the *Ahl lactonase genes* (*Acyl-homoserine lactonase* CHB37 and *Acyl-homoserine lactonase* C151) were introduced into *Agrobacterium tumefaciens* strain LBA 4404. One-month-old embryogenic calli were transformed with *pCAMBIA2301:Ahl lactonase* using an established method for *Agrobacterium*-mediated transformation of Eksotika papaya (Vilasini et al., 2000). The transformed calli were selected on half-strength Murashige and Skoog (MS) basal salts medium supplemented with kanamycin. The first selection was carried out on MS medium supplemented with 75 mg/L kanamycin followed by another three months selection on 150 mg/L

kanamycin. The calli that survived on the selection medium were then transferred onto hormonal-free maturation medium (De Fossard, 1974) for a month. Proliferating calli were then cultured on the De Fossard regeneration medium supplemented with 0.89 μM 6-benzyladenine (BA), 1.1 μM α -naphthaleneacetic acid (NAA), and 150 ml/L coconut water for shoot regeneration.

PCR Analysis of Putative Transgenic Papaya Lines

The presence of transgenes in the putative transgenic plants was verified using PCR analysis. The genomic DNA of the papaya leaves were extracted for about 100 mg for each sample by using the Qiagen kit (Qiagen, Hilden, Germany). During PCR analysis, ~50 ng of extracted genomic DNA samples were used.

Evaluation of Dieback Resistance in Transgenic Papaya Lines T₀

Twenty transgenic Eksotika papaya lines were grown in a control environment under Transgenic Glasshouse condition for evaluation of *Erwinia mallotivora* resistance. Three-months-old plants were used for inoculation. The plants were inoculated with *Erwinia mallotivora* by injection method. Non-transformed papaya plants were used as a positive control to validate symptoms development after inoculation. Symptoms appeared were observed and recorded every day.

Results and Discussion

Two potential genes against papaya dieback disease, *Acyl-homoserine lactonase* CHB37 and *Acyl-homoserine lactonase* C151 from antagonist bacteria (*Bacillus cereus* and *Bacillus thuringiensis*), were successfully isolated, characterized and cloned. Both genes showed about 88% nucleic acid sequence similarity. Generally, *Ahl lactonase* is capable to inactivate acyl-homoserine lactones (AHLs) activity that is crucial for bacterial quorum sensing. The inactivation mechanism is executed by hydrolysing the lactone bond of AHLs. Thus, transgenic papaya plants expressing *Ahl lactonase* are capable to quench the pathogen quorum-sensing signalling and may enhance the defence against dieback disease. For development of gene cassettes, the sense orientations of *Ahl lactonase* genes were cloned into the plant transformation vector, pCAMBIA2301. This vector contained selectable marker gene, *neomycin phosphotransferase (nptII)* which is conferring resistance to antibiotic kanamycin. The inserted genes were driven by a cauliflower mosaic virus 35S promoter and nos terminator.

Generation of transgenic plants have been successfully carried out via *Agrobacterium*-mediated method. *Agrobacterium* was used for transformation method as there is evidence that a low or single copy number can be found in most *Agrobacterium*-transformed plants. A total of 3000 embryogenic calli of Eksotika papaya were transformed with the two different *Ahl lactonase* constructs. Hundred putative transgenic papaya lines were recovered after selection on kanamycin medium and these putative transgenic lines were successfully regenerated on De Fossard salts medium supplemented with 0.89 μM 6-benzyladenine (BA) and 1.1 μM α -naphthaleneacetic acid (NAA). Genomic DNA from young shoot of these T₀ plants were extracted and used for PCR analysis to confirm the integration of inserted transgenes. The PCR results showed that out of 100 putative transgenic papaya lines analysed, 80 were positive for the presence of *Ahl lactonase* and *nptII* genes. The positive transgenic lines obtained were transferred to rooting medium, acclimatized and hardened at Transgenic Glasshouse.

The positive transgenic plants were screened for plants resistance toward *Erwinia mallotivora* bacteria by using injection method. Currently, twenty positive transgenic lines have been screened with bacteria *Erwinia mallotivora* under Transgenic Glasshouse Condition. Symptoms appeared were observed and recorded every day. Symptoms development appeared within 3 days after inoculation in

non-transformed control plants but was delayed for 10 days in the transgenic lines. After 3 days of inoculation, non-transformed plants showed yellow mottling, distortion on young leaves, water-soaked and subsequently died after 7 days of inoculation. Different results were observed for transgenic plants, whereby after 30 days of inoculation, transgenic plants still able to survive and formed new shoot (Figure 1). These results suggested that it should be possible to engineer transgenic Eksotika resistant to papaya dieback by using *AHL lactonase* gene.

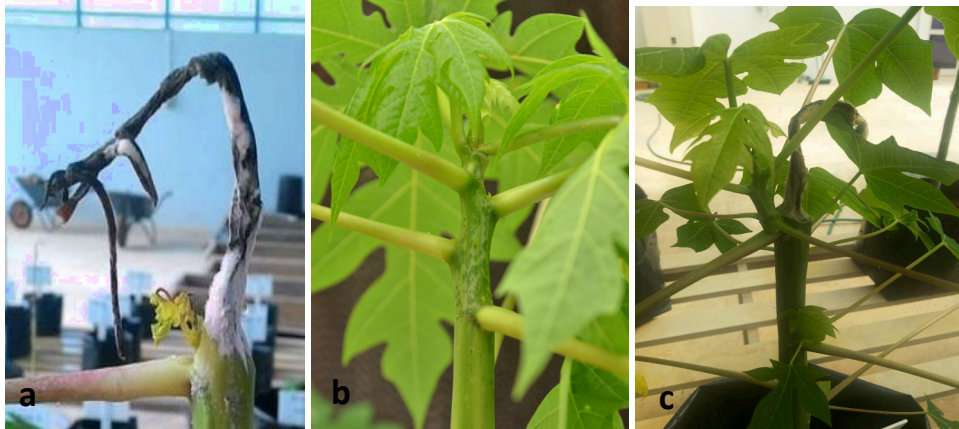


Figure 1: Screening of transgenic Eksotika papaya plants against *Erwinia mallotivora*. a; Non-transformed papaya died after 7 days inoculation b: Symptom observations of the inoculated transgenic papaya lines containing *Acyl-homoserine lactonase* CHB37-derived transgenes after 10 days of inoculation and c: Transgenic lines still survived after 30 days inoculation and formed new shoot.

Conclusion

Preliminary experiments indicated that Eksotika papaya showed substantial delayed of infection and showed mild symptom after inoculation with *Erwinia mallotivora*.

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Glucomannan Content of *Amorphophallus* spp. in Peninsular Malaysia

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Introduction

Plants of the *Amorphophallus* spp., belonging to the Araceae family, consist of more than 200 species distributed across the tropical and subtropical regions, including Malaysia (Sedayu et al., 2010). Locally, the plant is called loki, lekir, sarek and mayak (Burkil, 1996). *Amorphophallus* plant thrives well on fertile and well-drained soil. Recently, there is a high demand for the *Amorphophallus* plant from the international markets. The plant is highly sought for its glucomannan (GM) content, a compound suitable for various food, beverage, pharmaceutical and textile industries. GM is a water-soluble polysaccharide, comprising β -1,4-linked D-glycopyranose and β -D mannopyranose, which has an acetyl group randomly spread in every 10-19 units derived from the corm of *Amorphophallus* spp. Currently, many efforts have been put, particularly, in Malaysia, to find a plant-based medicine capsule, such as GM, which seems to be the most promising source of a plant-based gelatin for the halal industry. This is because of its unique gelling properties. Also, as a natural biopolymer, GM is good for packaging purpose due to its excellent film-forming ability, good biocompatibility and biodegradability (Wu et al., 2012). Since the GM is found in the corm, there is a need to collect accessions from around Malaysia and determine the amount of GM in the different size corms. Therefore, the study was carried out to determine the effect of accession and corm size on GM content of the collected *Amorphophallus* spp. in Peninsular Malaysia.

Materials and Methods

Planting materials collection

Sixty accessions of the *Amorphophallus* spp. were collected in six locations in Peninsular Malaysia [i) KKP: Kubur Panjang, Kedah; ii) PBJ: Bukit Jambul, Penang; iii) PUK: Ulu Kenas, Perak; iv) PT: Taiping, Perak; v) SHL: Hulu Langat, Selangor; vi) KKB: Kota Bahru, Kelantan]. The collected samples were established and cultivated under Brazil nut trees (*Bertholletia excels*) which provided 50% shade at the Taman Pertanian, UPM. The plants were maintained with basic cultural practices (watering, compost application and weeding). The *Amorphophallus* spp. corms were harvested at 12 months old for GM content determination.

Determination of GM content

Corms weighing about 200 ± 50 , 500 ± 50 and 1500 ± 100 g were selected. The crude flour (CF) was prepared by peeling off the corm epidermis. Then, the corms were washed and sliced into pieces of 2-3 mm thickness. The corm slices were oven-dried at 120 °C for 40 min followed by further drying at 60 °C for 3-4 days. The dried corm slices were ground and sieved using a 425 μ m sieve aperture

(Fisher Scientific Ltd, U.K.) to obtain the CF (Wu et al., 2002). The GM content was determined based on reducing sugar hydrolysis extraction using 3,5-dinitrosalicylic acid (DNS) colorimetric method (Chua et al., 2012). The glucose contents of GM solution extract and GM hydrolysate were determined from a linear regression of glucose standard curve and the GM content (%) was calculated based on the equation below:

$$KGM \text{ content } (\%) = \frac{5000 f (5T - T_0)}{m}$$

where, f = correction factor, T = glucose content of GM hydrolysate (mg), T_0 = glucose content of GM solution extract (mg), m = mass of CF.

Data analysis

Experiment 1 was conducted using a completely randomized design (CRD) with three replications of 12 treatment combinations of accessions (comprised KKP, PBJ, PUK, PT, SHL and KKB) based on corm sizes (200±50, 500±50 and 1500±100 g/corm). In experiment 2, a CRD was conducted in a factorial arrangement of treatments (four accessions × two corm weights), with three replications. The four accessions, KKP, PBJ, PUK, and KKB, had complete sizes of 200±50 and 500±50 g/corm. The data were analyzed using the ANOVA and mean separation was carried out by least significant difference (LSD) test (SAS ver. 9.3, Institute Inc., Cary, NC, USA).

Results and Discussion

The *Amorphophallus* spp. corms sprouted one month after planting and continued to develop and grow leaves for 4-5 months. Then, they went into dormancy for two months before they sprouted again and grew for another 4-5 months before they went into a second dormancy period. At this second dormant stage, the corms were harvested at about 12 months old for GM determination. At every dormancy stage, the leaves turned yellow, became wilted and dried, then dropped and detached from the corm. The corm sizes varied between accessions. Thus, not all accessions had the complete corm sizes, except the KKB accessions. Three available corm sizes, in the range of 200±50, 500±50 and 1500±100 g/corm, were used to determine the GM content (Table 1). It was not possible to obtain uniform and desired corm size from the first-generation corms. This was because the mother corm that had been used as the planting materials varied in sizes.

The CF was brownish in colour with a slight fishy smell. The GM content of experiment 1, as determined by the 3,5-dinitrosalicylic acid (3,5-DNS) colorimetric assay, were significantly different depending on accessions based on corm sizes (Figure 1). The results showed that the SHL200 had 4.87% and 5.74% higher GM content compared to KKB1500 and PT1500, respectively. All the accessions had relatively low GM contents. Similar studies on *A. paeoniifolius* by An et al. (2010) and Mekkerdchoo et al. (2013) reported GM content of 8-9% in the corms. On the contrary, Shahbudin (2012) reported GM content of 14.9% in young corms and 50.21% in mature corms. It was previously reported that GM content varies with the *Amorphophallus* spp. GM content of more than 50% had been reported in *A. konjac*, *A. muelleri* and *A. albus* (Liu, 2004).

Table 1. Accessions of *Amorphophallus* spp. and their respective corm sizes, 200, 500 and 1500 g, used for glucomannan (GM) content determination.

Accession	Corm size (g/corm)		
	200±50	500±50	1500±100
KKP	√	√	na
PBJ	√	√	na
PUK	√	√	na
PT	na	√	√
SHL	√	na	na
KKB	√	√	√

na = not available.

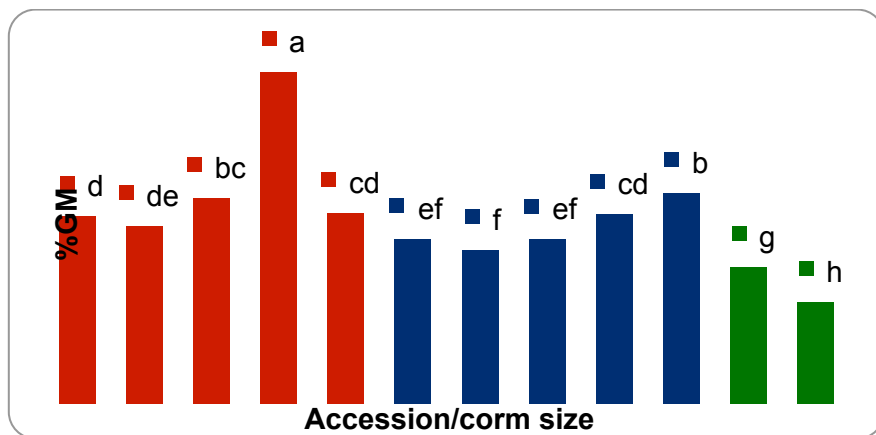


Figure 1. Comparison of the glucomannan content of accessions based on corm sizes (200, 500 and 1500 g) of Kota Bahru, Kelantan (KKB); Bukit Jambul, Penang (PBJ); Ulu Kenas, Perak (PUK); Hulu Langat, Selangor (SHL); Kubur Panjang, Kedah (KKP); Taiping, Perak (PT). Means with the same letter are not significantly different at $P \leq 0.05$ by LSD.

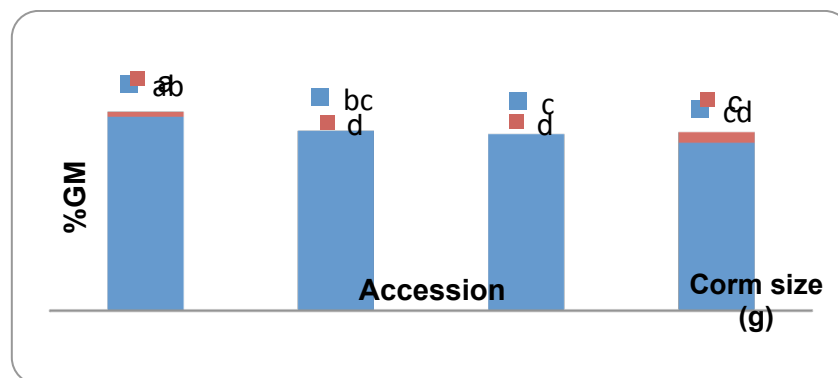


Figure 2. Interaction between accession and corm size on the glucomannan content of Ulu Kenas, Perak (PUK); Kubur Panjang, Kedah (KKP); Kota Bahru, Kelantan (KKB); Bukit Jambul, Penang (PBJ) and corm sizes (200 and 500 g). Means with the same letter are not significantly different at $P \leq 0.05$ by pooled LSD.

In experiment 2, the analysis was conducted for the accessions with a complete data of corm sizes (Table 1) to determine the interaction of accessions \times corm sizes. The results showed that there was a significant interaction effect between the accessions \times corm sizes on the corm GM content (Figure 2). For accessions KKP and KKB, the larger corms (500 g/corm) had significantly lower GM content compared to the smaller corms (200 g/corm). However, the large and small corms of PUK were not significantly different. A similar result was shown by PBJ. The physiological stress during the corm transplanting to a new environment could also affect the GM content in the corm of the accessions. The physiological stress has been reported to enhance the aging of young potato seed. The aging potato seed was found to influence the internal biochemistry of the tuber (Pavlista, 2004).

Conclusions

The GM contents of the accessions were in the range of 2.55-8.29%. The selection of GM cannot be based on accession or corm size as the GM content obtained was not consistent due to the interaction effect between the accessions and corm sizes. The content of GM in the corm provided a basic knowledge and understanding of the use of the plant for future plant improvement studies.

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Establishment of Salt Tolerant Rice via Overexpression of *Omega-3-Desaturase*

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Introduction

Salinity stress associated with plant physiological water deficit that drive plant control water lost by restricting stomata open which is directly inhibit photosynthesis resulted in slow growth rate and delayed maturity (Yadav et al., 2010). Exploration of *Omega-3-Desaturase Gene (O3D)* prove that the high omega-3 polyunsaturated fatty acids (PUFAs) enrichment in membrane phospholipid regulates cell turgidity in saline environment (Allen & Bartlett, 2002; Sakuradani et al., 2005). It also is a precursor of jasmonate that reduce sodium ion uptakes in plant. Enhanced genomic annotation can be utilized in molecular breeding for production of new rice variety with additional *O3D*, coupled with the advances in tissue culture protocol of rice. *Agrobacterium*-mediated transformation of plant is the most popular transformation protocol due to low cost and simpler DNA insertion compare to biolistic and microinjection (Gelvin, 2003). However early studies have shown that monocot crop was outside from the range of *Agrobacterium* infection and only works on 'competent cell' (Dong et al., 1997; Hiei et al., 1997; Rashid et al., 1996). Thus, embryogenic calli and freshly derived shoot during primary seed germination was used as a recipient in order to elucidate the transformability of embryogenic and non embryogenic rice sectors.

Materials and Methods

The rice seed, *Oryza sativa* L. ssp. *indica* variety MR219, MR263 and SS1-42 were used as a model. The *Agrobacterium tumefaciens* strain LBA 4404 harboring pCAMBIA 1304 with *omega-3-desaturase gene* (GenBank accession number EU100100) constructed with cauliflower mosaic virus (CAMV) 35S promoter was kindly supplied by Assoc. Prof. Dr Cha Thye San, UMT.

Transformation was conducted as Cha et al., (2011). *Agrobacterium tumefaciens* was streaked on solid LB media without the addition of phenolic for 2 nights at 26.8 °C in the dark. Then, *A. tumefaciens* was cultured overnight in liquid LB medium containing 0 (control), 100, 200, and 300 µM phenolic compound at 26.8 °C with a vigorous agitation at 200 rpm, in the dark. After 16 h, the bacteria culture was transferred into 50 mL falcon tube and centrifuged at 10000 rpm for 15 min at 4 °C. The supernatant was discarded and the bacterial cell pellet was resuspended using liquid co-cultivation media, a modified B5 media containing phenolic compound to adjust the concentration of bacteria cells nearer to 0.4 using spectrophotometer at 600 nm absorbance (A_{600} 1.0 corresponds to 1×10^8 cells/ mL). Little modification based on Toki's work in 1997, the plant was transferred into empty falcon tube followed by mixing the bacterium suspension and gently shaken for 3 minutes to allow the bacterial infection toward explants. Then, the plant was poured and blotted dry using sterile tissue paper in a petri dish and eventually cultured in solid B5 medium containing phenolic compound with a pH of 5.5. The culture was incubated for 5 days in the dark at 25 °C. For heat shock treatments, the mixture of *Agrobacterium* and plant in liquid B5 medium was subjected to 42 °C in a water bath also for 3 min. Transformation efficiencies was accessed by antibiotic screening and GUS assay. Putative transformant then was analysed by PCR as the plant regenerated.

Results and Discussion

A. tumefaciens exhibit different level of virulence towards type of explant for all tested rice cultivars (Figure 1). The result shows same escalation pattern as the increment of acetosyringone concentration for all tested rice type. 300 μ M of acetosyringone with 3 min heat-shock treatment at 42°C slightly increased the transformation rate for MR219, MR263 and SS1-42 varieties. Based on antibiotic screening, the survival rate calli were 80.8 ± 9.2 %, 88.9 ± 5.1 % and 87.3 ± 6.4 %, respectively. ANOVA justified that there is no significant difference between none and heat shock treatments in MR variety while yes in SS1-42. Meanwhile, 78.73 ± 3.94 % of SS1-42 shoot survived in transformant selection when treated with 300 μ M acetosyringone with heat shock treatment during co-cultivation. The low transformation rate in phenolic free media validates that plant are capable to release phenolic compound itself but at low level and insufficient to trigger the *Agrobacterium* infection (Raineri et al., 1990; Cha et al., 2011).

Previous studies have shown that the presence of at least one ortho-methoxy group as in benzalacetone and chalcone derivatives is required for vir gene induction and could lead to higher transformation rates (Joubert et al., 2002). The proposed mechanism for phenolic induction of vir expression by Hess et al. (1991) stated that an acidic residue at the binding site of *Vir A* protein protonates the carbonyl oxygen and activates the phenol. According to this mechanism, it was suggested that the ability to protonate the basic residue and the transfer of an electron from phenol to the acidic residue should correlate with the vir induction power of phenolic molecules (Joubert et al., 2002). Acetosyringone induced heat shock protein (Hsp), an alpha-crystalline-type small heat shock protein (α -Hsp) in proteomic study (Lai et al., 2006). Further study of Hsp regulation discover AS-induced Hsp protein accumulation is regulated in VirB-dependent pathway and required for VirB protein accumulation, which is translated for efficient bacterium-target receiver complex (VirB/D4)-mediated DNA transfer and virulence (Tsai et al., 2009).

In addition, both plant and bacterium release heat shock protein (Hsp) in response toward heat shock treatment during transformation (Patel et al., 2013). Hsp secreted by plants are capable to regulate cell cycle to rest and prevent programmed cell death (Iordanskjy et al., 2004; Khanna et al., 2004). Thus, the combination of both phenolic compound and heat shock treatment conducted in this study is intriguing to induce Hsp in order to minimize detrimental effect of plant cell apoptosis triggered by *Agrobacterium* (Hansen, 2000).

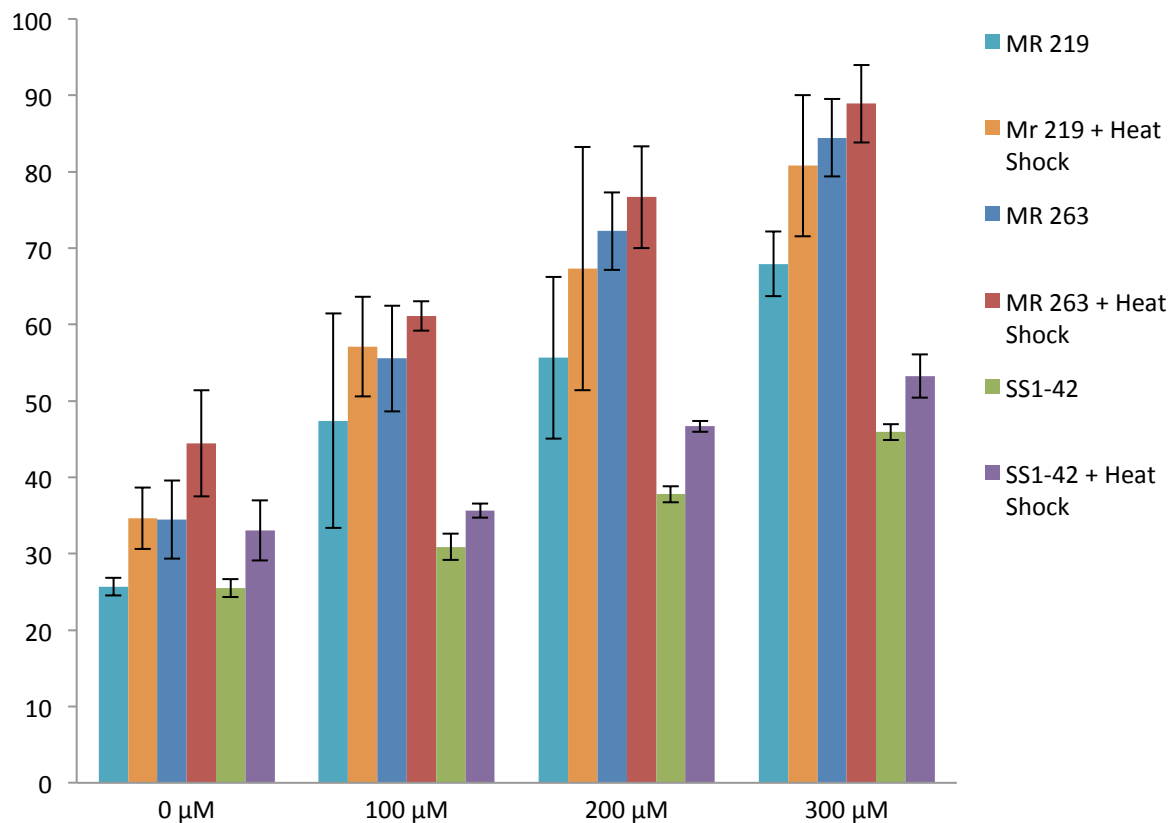


Figure 1. Acetosyringone concentration and heat shock efficiency on transformation rates

The first successful *Agrobacterium*-mediated transformation of *indica* rice only achieved using calli derived from scutellar as reported by Rashid et al., (1996) while direct transformation of rice root shows no transformant at all (Dong et al., 1997). This finding indicates that the ability *Agrobacterium* in transferring DNA is only limited to ‘competent’ cells because rice plant was outside from the host range of *Agrobacterium* (Hiei et al., 1997; Toki et al., 2006). Thus, the embryogenic part of rice must be selected as a target recipient in *Agrobacterium*-mediated transformation such as embryogenic calli and germinating tissue developed during primary stage of seedling.

The uses of rice embryogenic calli also useful for the production of somaclonal variant in *Agrobacterium*-mediated transformation which is crucial to ensure transgenic line that inherit extra genetic material (Hiei & Komari, 2008; Zuraida et al., 2010). However, rice calli was considered as hard and recalcitrant toward tissue culture and culture protocol optimized in one lab are often not reproducible (Rashid et al., 1996; Hiei, 1997; Zaidi et al., 2006; Zuraida et al., 2010). In this study, some of the transformed shoot loses the desired gene as the absence of target band in PCR analysis. This gene escapism phenomenon was caused by chimeric distribution of inserted gene during regeneration of transgenic line (Hiei & Komari, 2008).

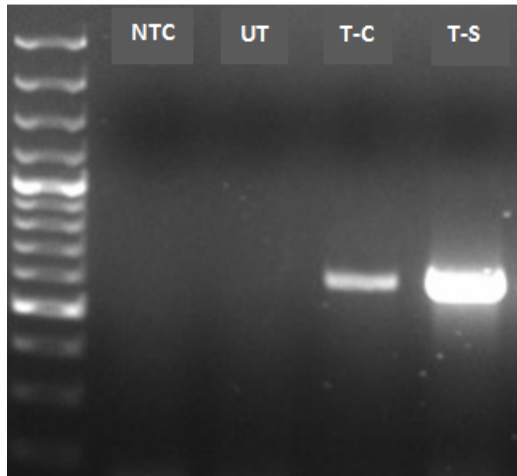


Figure 2. PCR Analysis of putative Transformed Calli (T-C) and Shoot (T-S)

Conclusion

Successful rice culture protocol is the main prerequisite in the production of genetically improved *indica* rice. A drawback of the most suitable target receiver of rice organ and tissue was important in order to produce stable transgenic rice. The heat shock treatment slightly enhance acetosyringone activity display the brighter hope in promoting bacterial infection towards targeted candidate instead of lowering the cost of future *Agrobacterium*-mediated transformation

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Expression of *N*-acylhomoserine Lactone Lactonase Genes in *Arabidopsis thaliana*

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Introduction

In Gram-negative bacteria, N-acyl homoserine lactones (AHLs) are the key signalling molecules to sense the population density in a phenomenon coined as quorum sensing (QS), or simply known as cell-to-cell communication (Williams *et al.*, 2000). The QS is responsible to regulate biofilm formation, virulence gene expression, and other important biochemical processes at certain population density threshold. Phytopathogens such as *Pseudomonas syringae* (potato, tomato, and *Arabidopsis* bacterial rot), *Erwinia mallotivora* (papaya dieback), *E. chrysanthemi* (pineapple bacterial rot) and *Ralstonia solanacearum* (banana bacterial disease) are all in this category of bacteria that use lactones to communicate. On the other hand, AHL-lactonases are a group of enzyme naturally produced by certain bacteria (i.e. *Bacillus spp.*) that target the lactones and capable of silencing communication in competitor bacteria (Wang *et al.*, 2004). Knowing this work of nature, lactonases can be utilized to curb wide plant diseases when produced in transgenic plants. Several studies that involve incorporation of lactonase gene into foreign organisms, either for recombinant protein production or resistant transgenic plant development, have presented promising results. A couple of studies discussed transformation of lactonase gene conferred tobacco resistance against *Erwinia carotovora* infection (Dong *et al.*, 2001) and recombinant lactonase could attenuate *Aeromonas hydrophila* infection in Zebra fish (Cao *et al.*, 2012).

In this study, however, we are attempting to genetically *Arabidopsis thaliana* to increase its resistance against a bacterial infection using lactonase genes isolated from local bacterial isolates. *Arabidopsis thaliana* was chosen to be the target organism for genetic modification because the transformation approach of this model plant is relatively simple and does not require callus generation. Being a Gram-negative bacterium and utilizes AHL in QS, *Pseudomonas syringae* was selected as the virulent pathogen to test the effectiveness of lactonase when recombinantly produced in *Arabidopsis*. This effort will serve as a study platform to validate the *in-planta* transgene function which then will be applied in combating bacterial infections in other economically important crops.

Materials and Methods

Preparation of Arabidopsis thaliana for transformation

Wild type (Columbia ecotype, Col-0) *Arabidopsis* seeds were cold-stratified at 4°C for three days and sown in 10-cm diameter pots (about 5-6 seeds in a pot) containing compost soil, perlite and vermiculite in 4:1:1 ratio. After a week, seedlings were thinned to a single plant per pot and continuously grown under neutral light condition (12/12 hr day/night, 20 °C, ~70% r.h.) to promote robust rosette growth. Watering was done bottom-up and each plant was fertilized biweekly using diluted organic fertilizer (goat's manure mixture) according to manufacturer's instruction. Flowering took place on the 5th week post germination and the plants were ready for gene delivery.

Preparation of gene constructs

Two bacillus-origin lactonase genes (aiia-CHB18 and aiiA-SP24) were cloned in a plant expression vector pCAMBIA2301 replacing the GUS gene and two constructs, pCAMB-CHB18 and pCAMB-SP24, were developed. The pCAMBIA2301 vector contains kanamycin resistance gene for selection and both gene inserts are driven by cauliflower mosaic virus (CaMV) 35S promoter (35S-aiiA-NOST).

Transformation using floral-dip method

The constructs were mobilized into *Agrobacterium tumefaciens*(GV3101) and the transformations of *Arabidopsis thaliana* (Col-0) were done using floral-dip method (Das and Joshi, 2011). Floral-dipped Arabidopsis were grown to setting seeds in a growth room under 12/12 h day/night cycle at 20 °C.

Kanamycin selection of putative transgenic plants and validation of gene integration

Matured seeds from the mother plants were screen on MS agar media containing kanamycin (50µg/mL) and PCR was used to verify positive transgenic plants (T₀). All T₀ plants were transferred to soil and grown to flowering to produce more seeds (T₁) thus providing more transgenic plants for the study. The same steps were repeated to produce the third transgenic generation (T₂) which would then be used for plant-pathogen test.

Lactonase gene expression validation using RT-PCR

Total RNA samples were extracted and purified from T₂ transgenic Arabidopsis leaves (100mg) using RNazol and cDNAs were synthesized using QuantiTech Reverse Transcription Kit from QIAGEN. The cDNA of lactonase gene was then PCR-amplified by gene specific primers and the fragments were validated on agarose gel based on size (base pair).

Inoculum preparation and plant inoculation

The Arabidopsis pathogen being used in this study is *Pseudomonas syringae* pv *tomato*(*Pst* DC3000). The culture was grown to a bacterial concentration of 1 x 10⁶cfu/mL in low salt Luria Bertani (LB) broth pH 6.90 and resuspended in sterile water (Katagiri *et al.*, 2002) prior to infection. Since this is a preliminary test, the virulence of the pathogen was tested on 2-week old plantlets of wild type Arabidopsis (non-transformants) and a small numbers of transgenic Arabidopsis (T₂). The infiltration of *Pst* DC3000 was done based on Korves and Bergelson (2003) method but with some slight modifications. Inoculum was delivered to the underside of one of the first true leaves using a small blunt-ended syringe until water-soak mark formed. All the plants were let grown under the same conditions mentioned but covered with plastic dome for 2 days to maintain humidity at 70-80%. The disease symptom was observed and scored 4 days after the infection.

Results and Discussion

Selection of seeds on kanamycin agar is really useful for mass screening especially when it involves numerous seeds. Not all survived plants (Figure 1) harbour the foreign gene thus PCR was conducted to molecularly identify positive transgenic plants.



Figure 1. Kanamycin (day 7 post germination) selection at a concentration of 50µg/ml produced putative SP24 (T₀) transgenic Arabidopsis (in circles) that had bigger cotyledons and emerging first two true leaves. These differences became more obvious on day 10 when the non-transformants became stunted, bleached-out and died, while the putative transformants grew even bigger. Kanamycin is toxic to seedlings as it prevents chlorophyll synthesis and root elongation. The selection should not exceed 14 days and survived plants were transferred to soil.

Specific gene amplifications through PCR were performed on putative transgenic plantlets (Figure 2) and a total of 16 transgenic independent lines (T₀) were obtained for both genes *aiiA*-CHB18 (8 lines) and *aiiA*-SP24 (8 lines) transformations. The transformation efficiency obtained for both genes were about 0.21 %. Due to *Arabidopsis* rapid life cycle, second (T₁) and then third (T₂) transgenic generations were produced to obtain more transgenic samples.

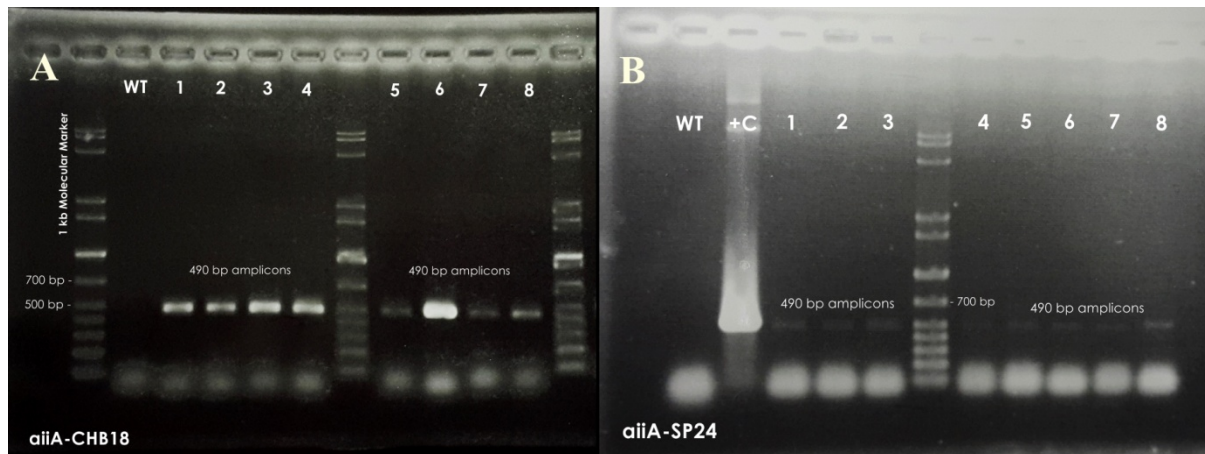


Figure 2. The gene specific primers used in PCR produced 490 base pairs (bp) amplicons from lane 1 to 8 in both genes (A & B), and these confirm the integration of both lactonase (*aiiA*) genes into the genomes of T₀ plants. The absence of band (amplicon) on WT lane indicates the sample did not contain *aiiA* gene. Cloning vector containing the *aiiA* gene acts as a positive control (+C). Faint bands on *aiiA*-SP24 (B) samples were likely due to low gene expression. The actual full length for both genes is 750bp. The primers were purposely designed to amplify only the internal sequence (490bp) of the genes for increased specificity.

Based on phenotypic comparison (Figure 3), T₁ transgenic plants did not possess any growth defect. All plants were continuously grown under neutral light condition (12 hours of daylight and 12 hours of night time) to promote robust rosette growth.



Figure 3. Phenotypic comparison of 4-week-old transgenic plants (T₁) with the non-transgenic reveals that either of aiiA gene does not interfere with the growth of the plants.

In this study, T₂ plants (third generation) were used to validate the gene expression and lactonase function. To validate the expression of the gene, total RNA from T₂ plants were extracted and RT-PCR was conducted. Based on Figure 4, RNA expression of lactonase gene was detected in transgenic lines of second generation. This result revealed the activity of lactonase gene transcription even at the slightest degree. Even though, quantification of the expression should be performed instead, but the data is sufficient to prove the gene cassettes are working in the plants and stable even after a few generations.

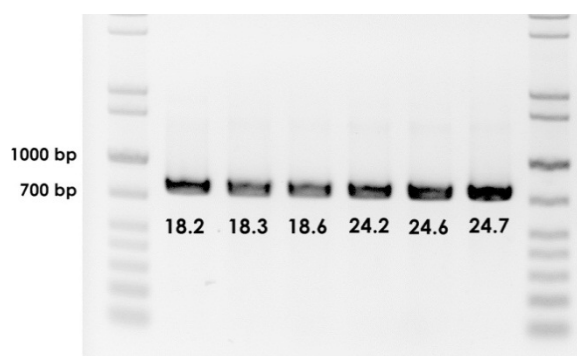


Figure 4. Total RNA was extracted from the Arabidopsis leaves. Reverse transcription was performed and gene specific amplification was conducted using PCR. It shows the presence of lactonase mRNAs in a few transgenic samples. The thick band appeared due to pooled cDNA.

A batch of wild type and transgenic plants (2-week old) were also exposed to pathogen infection. At the moment, it was found out that lactonase gene *aiiA*-CHB18 confer resistance against *Pst* DC3000 infection (Figure 5). This preliminary pathogen test on transgenic *Arabidopsis* verified the plant was able to produce functional lactonase thus exhibit increased resistance towards *Pst* DC3000. However, a bigger scale infection test should be conducted to obtain a more comprehensive and repeatable result.



Figure 5. Both control (left) and transgenic (right) plants were exposed with *Pseudomonas syringae* pv tomato (1×10^6 cfu/mL). After four days, water soak symptom had developed on the susceptible seedling. On the other hand, transformed plant seemed unaffected.

Conclusions

Incorporation of foreign genes of bacterial origin into the *Arabidopsis* genome was successfully accomplished. The plant transformation using floral-dip method was proven to be simple yet able to produce stable transgenic plants even after two generations. The simple gene expression validation through RT-PCR provides evidence that the genes were expressed at least at the transcription level. In addition to these, a preliminary test on transgenic *Arabidopsis* provides evidence that the plants were able to produce functioning lactonase and could confer resistance against the pathogen.

Acknowledgements

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Excision of Selectable Marker Gene from Transgenic Eksotika Papaya by using Heat-inducible FLP/FRT Recombination System

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Introduction

Genetic engineering for crops improvement has been greatly increased over the years. To conduct genetic modification via DNA recombinant technology, the potential gene conferring trait of interest is isolated and constructed into gene cassette along with selectable marker gene (SMG) such as antibiotics resistance gene. SMG is a very useful tool for selection process which enables transformed plants to be differentiated from numerous untransformed plants. This is due to the expression of protein products which able to neutralize toxic compound such as antibiotic in the selection media (Yau and Stewart, 2013).

However, the presence of SMG in the final transgenic product is often undesirable and controversial. Therefore, several marker-free strategies have been proposed and evaluated to address this issue (Yau and Stewart, 2013; Herzog et al., 2012). One of such technique is by utilizing site-specific recombination system for precise removal of SMG from the genome of transgenic plant. By using inducible system, the removal of SMG can be controlled and initiated once it has successfully completed its role during selection stage.

Here, we report the current development of SMG-free transgenic Eksotika papaya by using heat-inducible FLP/FRT recombination system. The system was developed by Herzog et al. (2012) which utilizing site specific recombination system found in *Saccharomyces cerevisiae* and a heat-inducible promoter (HSP) to control the activation of excision system. Transformation of papaya callus was conducted by using *Agrobacterium*-mediated transformation to analyze the efficiency of the system in transgenic papaya.

Materials and Methods

Callus Induction

Immature Eksotika papaya fruits (approximately 90 days post anthesis) were collected at MARDI Pontian . The induction of embryogenic callus was conducted by using immature zygotic embryo as explants on induction media containing half-strength Murashige and Skoog (Murashige and Skoog, 1962) basal salts supplemented with 50 mg/L myo-inositol, full strength MS vitamin (thiamine-HCl, pyridoxine, glycine and nicotinic acid), 60 g/L sucrose, 45.2 µM 2,4-D, 0.14 g/L adenine hemisulfate, 400 mg/L glutamine, 250 mg/L carbenicillin and 3.2 g/L gelrite. The pH of the medium was adjusted to 5.8 prior autoclaving. The calli were cultivated at 28°C in the dark for a month. (Sekeli et al., 2013)

Agrobacterium-mediated Transformation

Agrobacterium tumefaciens (*A. tumefaciens*) strain GV3101 and EHA105 harbouring plasmid vector conferring heat-inducible FLP/FRT recombination system, pB-Npt-Hsp-Flp-Gus, were used to

transform 2000 one-month-old embryogenic calli of Eksotika papaya as described by Vilasini et al. (2000). The transformed calli were selected on half-strength MS medium supplemented with 75 mg/L kanamycin for the first month and 150 mg/L kanamycin for the following 3 months. Surviving calli were transferred onto the De Fossard (De Fossard et al., 1974) regeneration medium supplemented with 0.89 M 6-benzyladenine (BA), 1.1 M 1-naphthaleneacetic acids (NAA). Timentin (500 mg/L) and cefotaxime (250 mg/L) were used during selection and regeneration process to inhibit the growth of *A. tumefaciens*. Untransformed calli were used as a control.

Heat Treatment

Untransformed papaya callus and plantlet were used for incubation at specific temperature and period. Incubation temperatures were set up at 37, 40, 42 and 44°C whereas the incubation period for each temperature was commenced for 2, 4, 6, 8 and 10h. Two replicates of callus and plantlet for each heat treatment were used in this study.

Results and Discussion

Heat treatment study was conducted by using untransformed papaya calli and plantlets to analyze the parameter of effective temperature and incubation period. This preliminary experiment was crucial to assess the effect of heat treatment to the normal development of papaya callus and plantlet.

Normal growth was observed for both callus and plantlet after various incubation parameters up to 44°C for 10 h. Based on our analysis on the rate of callus embryogenesis of heat-treated calli, the optimal incubation condition was ranging from 42-44°C for 4 h.

As for the heat-treated plantlets, the leaves of some plantlets for each heat treatment regime turned yellowish, especially when incubated longer than 6 h (Figure 1). However, all heat-treated plantlets developed new and normal-shaped shoot 3-4 weeks post heat treatment. The results indicated that incubation parameter of 37, 40, 42 and 44°C for 2, 4, 6, 8 and 10h were suitable as both heat-treated calli and plantlets showed normal growth and development. The heat treatment parameter served as reference to induce heat shock promoter (HSP), especially to initiate the expression of flippase (FLP) gene that drives the removal of SMG from the transgenic plant genome (Figure 2).

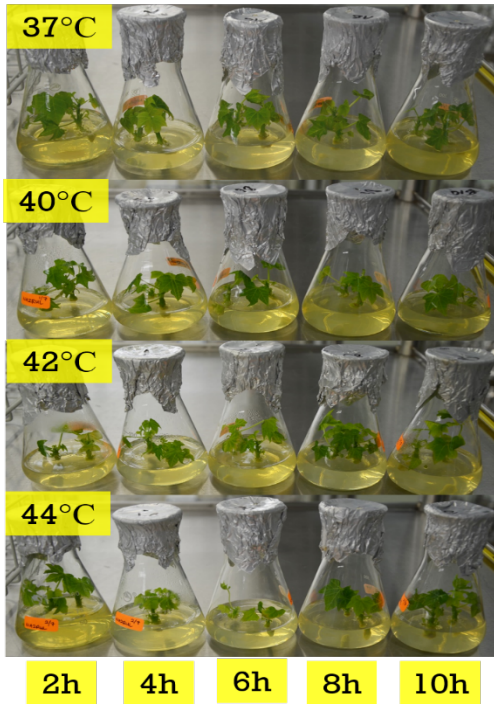


Figure 1. Heat-treated papaya plantlets at 37, 40, 42 and 44°C for 2 to 10 h.

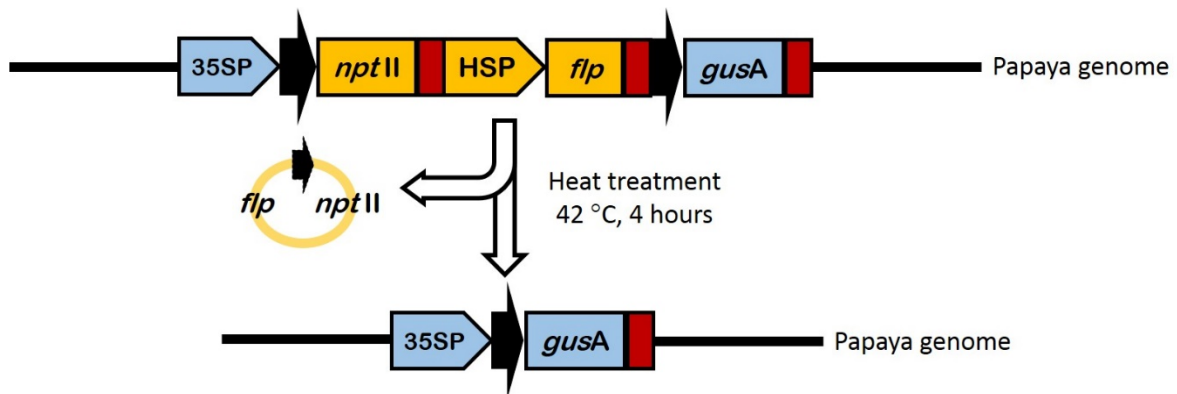


Figure 2. The removal of *FRT*-flanked box containing SMG (*neomycin phosphotransferase II*) from transgenic papaya genome after heat treatment.

One-month-old embryogenic calli were transformed by using two strains of *A. tumefaciens*, EHA 105 and GV3101 to evaluate the transformation efficiency (Figure 3). After four months selection on kanamycin-containing medium, all untransformed calli were completely browning and dead. The putative transgenic calli were then transferred onto the De Fossard regeneration media (Figure 4). 50% out of the total surviving calli for each *A. tumefaciens* strain transformants were heat-treated at 42°C for 4 h to excise *nptII* at callus stage, whereas the remaining calli were regenerated and subsequently heat-treated at plantlet level.

Heat treatment for both callus and plantlet level were proposed, which the former was hypothesized to produce uniform SMG-free lines compare to the latter. This is due to early activation of the excision system in the least differentiated cells and larger surface area per unit volume for HSP induction. Probability of high chimera of GUS expression was expected after heat induction in plantlet level. Based on current analysis of putative transformants, *A. tumefaciens* strain EHA105 showed higher transformation efficiency (4%) than GV3101 (2.25%).

Further validation of transgenic lines will be conducted by using PCR, histochemical GUS assay and heat treatment at plantlet level.

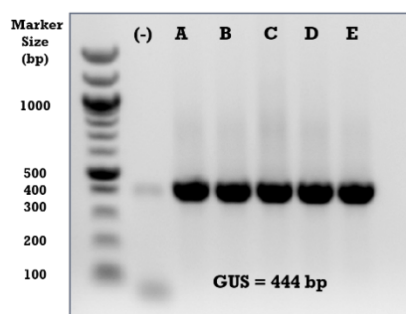


Figure 3. PCR validation of *A. tumefaciens* strain GV3101 and EHA105 harboring pB-Npt-Hsp-Flp-Gus based on GUS reporter gene.

A: Positive control.

B and C: *A. tumefaciens* strain GV3101.

D and E: *A. tumefaciens* strain EHA105.

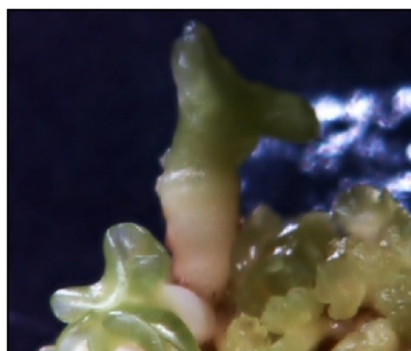


Figure 4. Regeneration and shoot development of transgenic papaya callus.

Conclusions

Transformation of papaya callus with FLP/FRT recombination system was successfully conducted by using *Agrobacterium*-mediated transformation. Range of effective parameter for heat treatment regimes to induce heat shock promoter of both callus and plantlet levels have been identified between 37 to 44°C for 2 to 10 h. Based on putative transformants count, *A. tumefaciens* strain EHA105 harboring pB-Npt-Hsp-Flp-Gus vector showed better transformation efficiency than GV3101.

Acknowledgements

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Extraction of Flavonoids from *Hevea* Leaves Clone RRIM 2001 by Using Supercritical Fluid

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Introduction

Undesirable changes in food quality due to oxidation reactions can be prevented by adding antioxidant compounds into its formulation. The usage of synthetic antioxidant such as butylated hydroxytoluene (BHT) should be replaced by natural antioxidant due to its toxicity (Mandana et al., 2011). Plant antioxidants can be the natural alternatives to synthetic antioxidants especially in the food industry. Flavonoids most commonly known for their antioxidant activity are polyphenolic compounds that are widely distributed in fruits, leaves and medicinal plants (Zhao et al., 2003). It has been proven that the Supercritical Fluid Extraction (SFE) technology can extract the active natural compounds from plant matrices (Casas et al., 2009). Researchers studying phenolics in *Hevea* leaves have identified them as derivatives of kaempferol or quercetin (Sanier et al., 1992). The extraction and identification of these compounds from *Hevea* leaves are important in order to exploit its potential for commercial and medicinal uses. *Hevea* leaves could possibly become a new source of plant antioxidant as they are abundant in source bushes and rubber plantations. The objectives of this study are to extract natural antioxidant compounds such as flavonoids from *Hevea* leaves and investigate the effects of extraction conditions namely temperature and pressure on total extraction yield and antioxidant activity of *Hevea* leaves clone RRIM 2001.

Materials and Methods

The fresh young leaves of *Hevea* Clone RRIM 2001 were obtained from Sourcebush, Sg. Buloh Experimental Station, Selangor. Leaves were dried in an oven and then stored at ambient temperature in the dark. The samples were ground in a dry mill blender (MX-337, Panasonic, Malaysia). Industrial grade liquid carbon dioxide supplied in cylinder with dip tube was purchased from Linde Gases Malaysia. Ethanol (99.5%, analytical grade) was obtained from System. Other reagents used were analytical reagent grade. Extractions were performed using Supercritical Fluid Extraction system (Thar Instruments, Inc., USA). The supercritical carbon dioxide flow rate was maintained at 30 g/min. The extraction time was fixed to 60 min. Extraction was performed at pressure 100 to 300 bar and at temperature 40 to 60 °C. The extracted samples were weighed for total yield determination and the samples were analyzed by UV-Vis Spectrometry for antioxidant assay. The powdered dried leaves have been analyzed for flavonoid profiling by High Performance Liquid Chromatography (HPLC) analysis (Waters Delta 600). Statistical calculations and analysis were performed using statistical software, SAS Version 9.2.

Results and Discussion

The phytochemical screening shows that *Hevea* leaves extracts by SFE contain flavonoids as shown in Table 1. The HPLC analysis revealed the presence of flavonoids skeleton with two λ_{\max} . From the chromatogram (Figure 1), six flavonoids peaks were detected at five wavelengths (200, 254, 290, 300 and 366 nm). The flavonoid peaks can be identified by their characteristic UV/ DAD spectral pattern with two bands, band I with λ_{\max} around 300 – 380 nm and band II with λ_{\max} around 200 – 280 nm (Diagone et al., 2012). However, further analysis of their retention times did not match with that of standards available (rutin, quercitrin, kaempferol, myricetin and fisetin). Further investigation would be required for detail characterization of the flavonoid compounds. Since flavonoids are polyphenolic

compounds that are most commonly known for their antioxidant activity, an attempt was made to determine the antioxidant activity of *Hevea* leaves extracts.

Table 1: Summary of the phytochemical screening

	Alkaloids	Saponins	Flavonoids	Tannins	Triterpenes	Steroids
<i>Hevea</i> leaves	-	+	+	+	+	+
SFE extracts from <i>Hevea</i> leaves	-	-	+	-	+	+

+ = Present, - = Absent

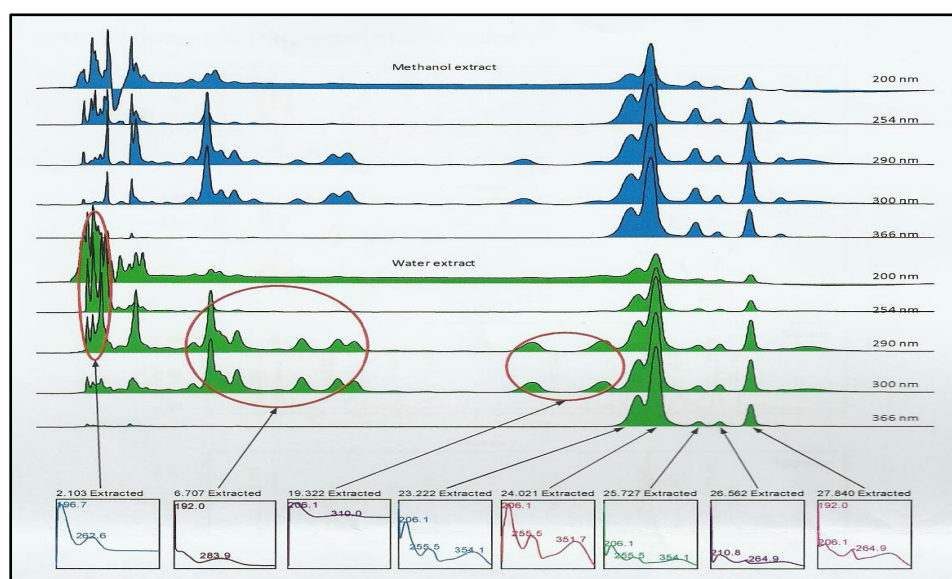


Figure 1. HPLC chromatograms of extracts and UV profiles of flavonoid peak

The effects of pressure and temperature on extraction yield and antioxidant activity were studied. According to ANOVA analysis, pressure and temperature interaction had significant effect ($P < 0.05$) on the extraction yield and antioxidant activity of *Hevea* leaves clone RRIM 2001 extracts. Based on the F value, pressure shows more dominant effect on the extraction yield. Meanwhile temperature shows more dominant effect on the antioxidant activity. The highest total extraction yield (13.10 ± 1.32 mg/g) and antioxidant activity (69.32 ± 0.57 % Inhibition) were achieved at 200 bar and 50°C .

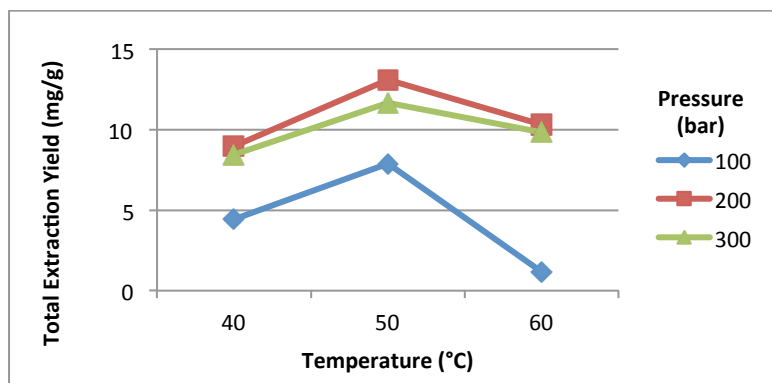


Figure 2. Effect of pressure and temperature on total extraction yield of *Hevea* leaves extracts clone RRIM 2001.

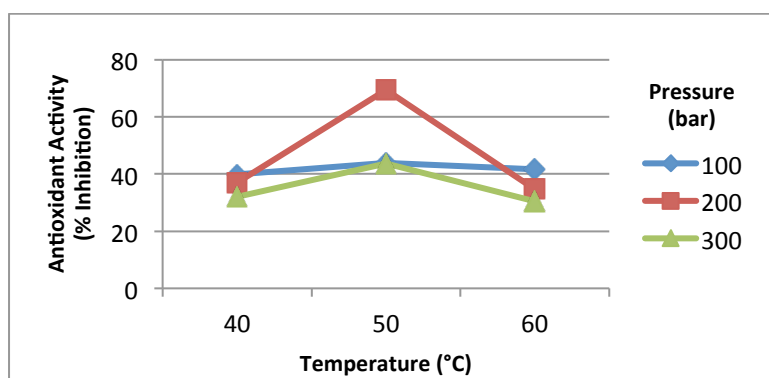


Figure 3. Effect of pressure and temperature on antioxidant activity of *Hevea* leaves extracts clone RRIM 2001.

Figure 2 presents the effect of pressure and temperature on total extraction yield of *Hevea* leaves extracts. The yield was increased from 40 to 50 °C due to the increase in volatility of the analytes. The yield was decreased from 50 to 60 °C due to the reduction in CO₂ density that lead to reduction in solubility of analytes (Casas et al., 2009). Figure 3 illustrates the effect of pressure and temperature on antioxidant activity of *Hevea* leaves extracts. The antioxidant activity was the highest at 50 °C over a temperature range from 40 to 60 °C. This might be due to the thermo-sensitivity of antioxidants at high temperature (Mandana et al., 2011). The extracts obtained in this study had higher antioxidant activity varying from 30.46 % to 69.32 % compared to butylated hydroxytoluene (BHT) as a reference.

Conclusions

Phytochemical screening and HPLC analysis revealed the presence of flavonoids which is an antioxidant compound; in *Hevea* leaves extracts clone RRIM 2001. Flavonoid can be extracted from *Hevea* leaves by using Supercritical Fluid Extraction (SFE) method. The highest total extraction yield (13.10 ± 1.32 mg/g) and antioxidant activity (69.32 ± 0.57 % Inhibition) were obtained at 200 bar and 50 °C. It can be concluded that temperature at 50 °C and pressure at 200 bar are more convenient to be selected for the SFE extraction of flavonoids from *Hevea* leaves clone RRIM 2001. This finding suggests that the *Hevea* leaves are potential source of plant natural antioxidant and SFE process is a promising alternative technique in extracting antioxidant compounds from plant matrices.

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CHAPTER 4

SEED TECHNOLOGY AND QUALITY PLANTING MATERIAL

Evaluation on the Effectiveness of Rapid Drying Method and its Effects on Chilli (*Capsicum annuum*) Seed Quality

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Introduction

Current seed drying process which is sun-drying is very dependable on weather conditions. Drying period has been observed varies from 2 to 7 days. Prolonged sun drying in high humidity may cause reduction in seed quality due to seed deterioration (Nassari et al., 2014). Rapid drying process in high temperature has also been claimed harmful to seed quality due to desiccation damage (Ellis and Roberts, 1980). However, determination of more rapid drying method which is safe for seed would be very useful for high value seed producer.

A new technology for drying seed has been introduced using zeolite beads which it could dry orthodox seeds rapidly depending on beads water-holding capacity and seed volume (Anonymous, 2009). Not much study has been done to evaluate its effectiveness in drying chilli seed and its effects on seed quality. Therefore, this study was aimed to evaluate its effectiveness and effects on seed quality of a potential inbred line chilli as compared to current drying method, sun-drying.

Materials and Methods

Plant materials

A potential inbred line of chilli, Line 5, was used in this study. Six hundred plants were grown on a 12 m x 100 m plot with plant spacing of 1 m x 1 m in plot C6, Klang, Selangor. The plants were raised following the agronomic practices for crop production as recommended by Malaysian Agricultural Research & Development Institute (MARDI). Mature fruits were harvested, extracted and cleaned.

Seed drying

Initial seed moisture content was determined using slow oven method (ISTA, 2014). Then, seeds were dried to target moisture content of 9% that is suitable for medium-term storage using different methods of drying, T1 (sun-drying) and T2 (bead-drying).

I. Rapid drying

Amount of beads required to desiccate the fresh seeds to targeted seed moisture of 9% was estimated by calculating the amount of water to be removed and bead efficiency as following formula (Asbrouck and Taridno, 2014):

i. Water to be removed, W (kg or g) = $A \cdot [A(1-X)/(1-Y)]$, where;

A = Seed weight

X = Initial moisture content measured by oven method

Y = Desired moisture content (9%)

ii. Bead efficiency, Z (%) = $(D-C)/C$, where;

D= Beads weight after stored in 100% relative humidity for 24 hours
C= Initial beads weight

Therefore, amount of beads required, $E = W/Z\%$. Seeds were weighed from time to time to monitor water loss.

II. Slow drying

Another seed lot was dried using sun-drying. To achieve target moisture content, weight of water to be removed (W_f) was estimated using target moisture content formula as per below. Seeds were weighed from time to time and drying was stopped when W_f was reached.

- i. $W_f = W_i \times (MC_i - MC_f) / (100 - MC_f)$, where;
 W_f = Weight that should be reduced from initial weight to obtain MC of 9%
 W_i = Initial weight of seed
 MC_i = Initial moisture content
 MC_f = Target moisture content, which is 9%

Final moisture contents of seeds dried using both methods were determined using slow oven method following ISTA rules (ISTA, 2014).

Seed germination and vigour evaluation

Once the seeds had reached the targeted moisture content, viability was determined by calculating germination percentage while vigour was determined by calculating mean germination time (MGT), germination index (GI), time to obtain 50% germination on total seed tested (T_{50}) and electrical conductivity (EC) test. For germination percentage, four replicates of 100 seeds from each treatment were placed on top of wet tissue paper in sandwich boxes (18.5x13x7 cm), then grown in a germination chamber for 14 days (ISTA, 2014). Germinating seeds were recorded every day for 14 days. Final germination percentage (%) was recorded after 14 days of planting on wet tissue paper. MGT was calculated according to the equation of Ellis and Roberts (1981). GI was calculated as described in Association of Official Seed Analyst (AOSA, 1983). The T_{50} was calculated according to the formula of Coolbear et al. (1984) modified by Farooq et al. (2005). EC of seed was determined using a conductivity meter (CON510, Eutech Instruments, Singapore). Four replicates of 50 seeds from each treatment were weighed and soaked in 50 ml deionized water at 25 °C for 24 hours before the EC readings were taken. Results were expressed in $\mu S \text{ cm}^{-1} \text{ g}^{-1}$ (ISTA, 2014).

Seed analysis

The experiment was carried out in Complete Randomized Design (CRD) with four replications. Statistical procedure was carried out using the SAS software and data were analysed using ANOVA. Treatment means were compared using the Least Significant Difference (LSD) test.

Results and Discussion

Figure 1 showed water loss during seed drying using both methods, sun-drying and bead-drying. Water was absorbed rapidly during the first five minutes of bead-drying with the highest water loss rate of 5.14 g/min. Then, the rate slowly decreased and reached plateau after forty three minutes of drying. Water loss rate was recorded the lowest during plateau stage (0.14 g/min). This pattern of loss in moisture content can be related to the water chemistry whereby the rapid loss is due to loss in free water while the slower rate is attribute to lose in either loosely bound or tightly bound water. Moreover, rapid water loss occurred at the earliest of drying process was due to the ability of zeolite beads to absorb water at the highest efficiency as evidence to declining of RH (38%). Then, a slower

rate was due to decreased in beads efficiency over time when significant amount of water had been absorbed and held tightly in their microscopic pores thus slow down the absorption of water from seeds. Final moisture content was recorded at 11%. In similar study on tomato seeds, Nassari et al. (2014) found that rapid water loss was occurred during desiccation using bead-drying compared to sun-drying under ambient conditions.

This study used the basis that beads would absorb as much water in a bead-seed system as they did when they were placed over water. Besides that, the amount of beads required to reach the targeted moisture content were calculated based on tested beads' water-holding capacity and tested initial moisture content of seeds. However, final moisture content was recorded 2% higher than the targeted moisture content. It might due to loss in beads efficiency when it was exposed to ambient condition right after regeneration or during beads storage. Regenerated zeolite beads easily absorb water from the air, thus slightly reduced its efficiency. Similar basis was used by Hay et al. (2012) in their study on drying rice seeds using zeolite beads. It was recorded that water loss reached plateau stage after 2 days until 28 days of bead-drying, where final moisture content (10.1%) was recorded higher than the targeted moisture content of 6.1%. This discrepancy between final moisture content and target moisture content indicates that the beads did not work to their full efficiency in bead-seed system.

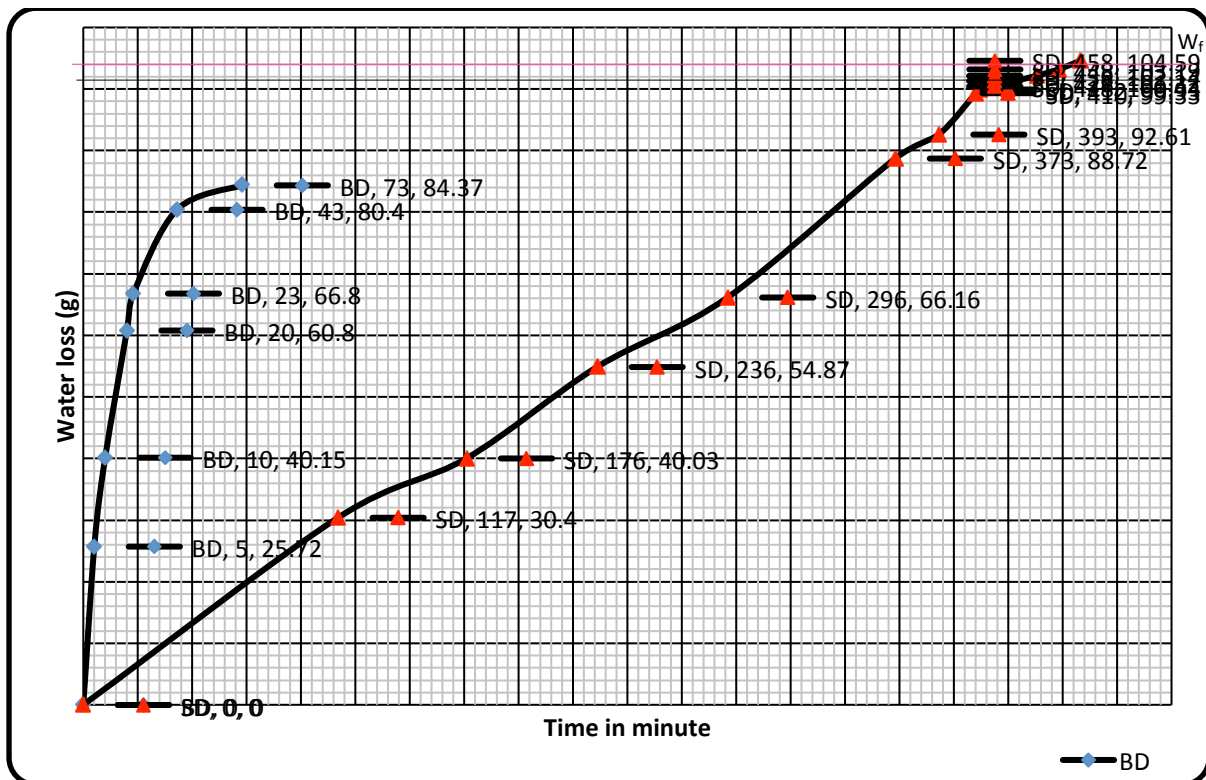


Figure 1. Seed water loss during sun-drying (SD) and bead-drying (BD)

W_f = amount of water should be remove to achieve target moisture content

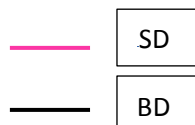


Figure 2 showed water loss of seeds dried using bead-drying after calculated amount of beads required to remove the balance of 2% seed moisture content were added. Water loss rate increased right after additional beads were added. It showed that the amount of beads used earlier was insufficient and the calculation of the amount of beads to use is also not really precise as expected. Modification on protocol for calculating the amount of beads required to achieve target moisture content is recommended to avoid under-drying or over-drying of seeds which may lead to loss in seed viability.

As compared to bead-drying, water loss rate for sun-drying method was recorded very slow, ranging from 0.08 to 0.4 g/min (Figure 1). It might due to high relative humidity of ambient conditions that was observed ranged between 55-78.5% along the drying process. However, the targeted moisture content of 9% was achievable after 458 minutes (7 hours and 38 minutes) of drying.

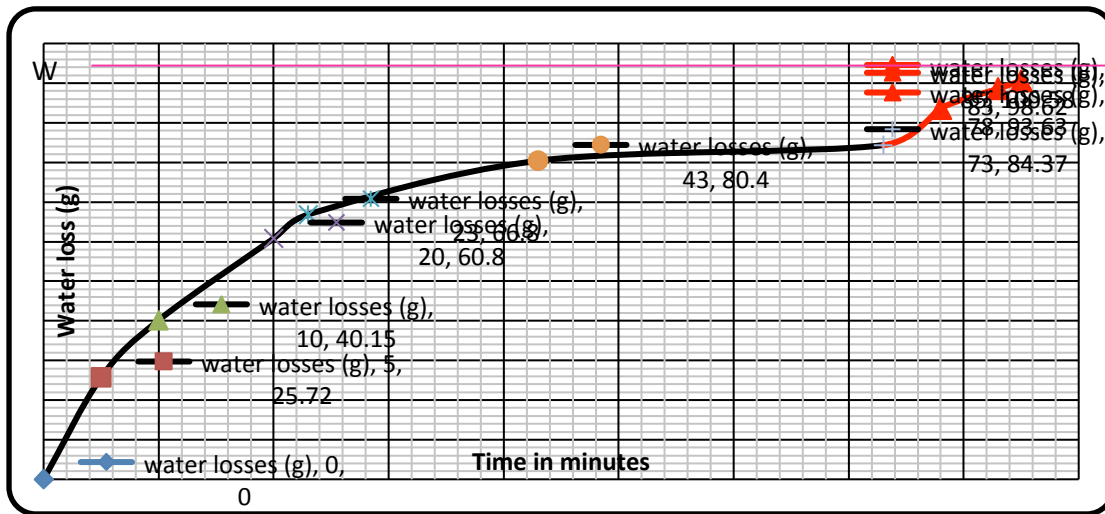


Figure 2. Seed water loss during bead-drying (BD) when after beads were added to achieve target moisture content (red line). W_f = amount of water should be remove to achieve target moisture content

Seed germination and vigour

Table 1 showed that both slow and rapid drying methods have significant effects on seed germination percentage and germination index ($P \leq 0.05$), while not for EC, MGT and T_{50} . Seeds dried by bead-drying method had slightly better seed quality as compared to seeds dried using sun-drying. It was recorded with higher germination percentage (68.3%) and germination index (35.5) as compared to seeds dried using sun-drying (54.3% and 27.8, respectively). As previous study done by Babiker et al. (2010) showed similar result where seed germination percentage significantly affected by different drying methods (sun, shade, silica gel, and seed dryer) on sorghum seeds. In contrast, study done by Nassari et al. (2014) reported that different drying methods (zeolite beads and silica gel) did not significantly affecting seed germination percentage. This difference might due to the absence of exothermic reaction that generally produced by zeolite beads when exposed to wet condition. This is because pre-drying was done to reduce initial moisture content of 60% to 17% before further drying using zeolite beads. Therefore, exothermic reaction that possibly has effects on seed germination was avoided.

Electrical conductivity was not significantly affected by both drying methods with low electrolytes leakages (less than $25 \mu\text{S}/\text{cmg}$) at $P \leq 0.05$. It indicated that no damage on seed membranes caused by these two drying methods. However, low germination percentage (<80%) and low vigour

performance on seeds dried using both methods indicated that they had low seed quality. It may be not only cause by the effects of drying methods but might also by genetic constituents and/or pathogens.

Table 1. Mean comparison of germination and vigour of chilli seed as affected by two different drying methods, slow and rapid.

Drying method, DM	Electrical conductivity, EC ($\mu\text{S}/\text{cmg}$)	Final germination percentage (%)	Mean germination time, MGT (days)	T ₅₀ (days)	Germination index ,GI
Sun-drying	17.63 ^a	54.3 ^b	7.4 ^a	7.8 ^a	27.8 ^b
Bead-drying	21.89 ^a	68.3 ^a	7.3 ^a	6.3 ^a	35.5 ^a

Means with similar letter in the same column are not significantly difference at 5% probability according to LSD test.

Conclusions

From this study, it was confirmed that zeolite beads dry seeds rapidly. However, modification on the protocol for calculating the right amount of beads to be used is required. This is because current protocol fails to calculate the right amount of beads to reach target moisture content. Both slow and rapid drying methods had significant effects on seed germination percentage and germination index. Seeds dried using bead-drying had higher germination percentage and speed of germination compared to seeds dried using sun-drying. However, relatively low seed quality was observed from seeds dried using both methods. It might be caused by other factors such as genetic constituent and/or pathogens.

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Effects of Hormonal and Halo Priming Treatments on papaya (*Carica papaya* cv. Eksotika) Seed Germination

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Introduction

Seed germination is affected by many factors including substrate type, environmental factors such as oxygen, water and temperature and for some species, light (Hartman et al., 2001). The germination of papaya seed is generally slow, erratic and incomplete (Andreoli and Khan, 1993). Gelatinous sarcotesta around papaya seed prevent seed germination. However, seed dormancy is observed even before the sarcotesta layer has been removed (Lange, 1961). Drying the freshly extracted seed however improved seed germination (Yahiro, 1979). To increase germination rate and to break dormancy of papaya seed, attempts such pre-treatment at low temperature (Yahiro, 1979), drying treatment (Chan and Tan, 1990) and potassium priming (Owino and Ouma, 2011) have been made.

Seed priming is commonly used to synchronize seedling emergence and to reduce the time between seed sowing and seedling emergence (Parera and Cantliffe, 1994). Hormonal priming is the pre-treatment with different hormones such as salicylic acid, kinetin and ascorbic acid which promote the growth and development of the seedlings. Combined treatment of matriconditioning and Gibberellin, GA₄₊₇ synergistically promoted germination and seedling emergence (Andreoli and Khan, 1993). Ethylene facilitates seed germination and embryo growth in some crops such as apple, common bean and avocado (Matilla, 2000). Halo priming refers to seed soaking in inorganic salts solution such as NaCl, CaCl₂ and CaSO₄ (Parera and Cantliffe, 1994). The use of potassium chloride (KCl), potassium hydroxide (KOH), potassium nitrate (KNO₃) and potassium sulphate (K₂SO₄) as soaking solution for papaya seed var. Kenya increased germination significantly compared to unsoaked seed (Owino and Ouma, 2011).

The objectives of this study were to evaluate the effects of different priming treatments on papaya seed germination and vigour and to compare which type of priming suitable for papaya seed before drying and for storage.

Materials and Methods

Seed Materials

The hermaphrodite papaya (*Carica papaya* cv. Eksotika) fruits obtained from the seed production plot of Gene Bank and Seed Centre in Malaysian Agricultural Research and Development Institute (MARDI) Pontian, Johor were used in this study. The Eksotika papaya fruits with Maturity Index 2 were collected and kept for postharvest ripening until the fruits reached Maturity Index 5 (Yogeesha et al., 2013). The ripe fruits were cut and seeds were extracted by scooping out using a spoon. Then, seeds were washed under running water to remove the gelatinous sarcotesta by gently rubbing the seed on fine metal mesh to ease removal of sarcotesta. The seeds were then placed in a beaker containing distilled water where the flotation test was carried out. Those that floated were removed as they had low viability (Hartmann et al., 2001). The smaller seeds were removed with a PRADA test sieve with 3.15 mm aperture.

Seed treatments

The washed seeds were divided equally into seven lots and put into a beaker with different solutions. Each beaker was filled with 1 L of distilled water and added with different compound such as gibberellic acid (GA₃), potassium nitrate (KNO₃) and ethephon at different concentrations. Each solution was also added with a fungicide Benex (a.i benomyl) at 2g/L. The treatments were as follows.

- T1 – distilled water (control)
- T2 – 250 mg/L GA₃
- T3 – 500 mg/L GA₃
- T4 – 500 mg/L KNO₃
- T5 – 1000 mg/L KNO₃
- T6 – 500 mg/L ethephon
- T7 – 1000 mg/L ethephon

After an hour, the contents of the beakers were poured into a sieve to remove the remaining solutions. Seeds were then dried at room temperature (75±10% R.H.) until it reached 8-10% moisture content (Yogeesha et al., 2013) before germination test was conducted. Moisture content of the seed was determined with a Moisture Analyzer MX-50 (A&D Company Limited Japan).

Germination test

The dried seeds were then subjected to germination test with four replicates of 25 seeds each. Seeds were placed on paper towel sprayed until saturated with distilled water in transparent container with lid (Salomao and Mundim, 2000). The seeds germinated at 30±2°C under a 12-hour photoperiod (Salomao and Mundim, 2000). The number of seeds germinated daily was recorded for 21 days where only radicle protrusion of length more than 1.0 cm will be considered germinated (Salomao and Mundim, 2000) (Yogeesha et al., 2013). The mean germination time (MGT) was calculated based on the equation of Ellis and Roberts (1981) modified by Moradi Dezfuli (Moradi *et al.*, 2008).

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

Where n is the number of seed, which germinated on day D and D is the number of days counted from the beginning of germination (Moradi *et al.*, 2008).

The time to 50% germination (T₅₀) was calculated according to the formula of Coolbear *et al.* (1984) as modified by Farooq *et al.* (2005).

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

Where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when n_i < N/2 < n_j. (Moradi *et al.*, 2008)

The germination index (GI) was calculated as described by the Association of Official Seed Analysis (AOSA, 1983).

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

Seedling vigour index was calculated by using this formula (Moradi *et al.*, 2008).

Vigour Index (VI) = [seedling length (cm) x germination percentage]

Seedling length was measured with a ruler from seedling apex to the root.

Conductivity test was also conducted to determine the seed vigour after treatments. Samples of 20 seeds per replicate were soaked in 150 mL deionized water at 25°C for 24 hours. Then, the electrical conductivity, EC of seed leachate was measured using EC meter CON 510 (EUTECH Instruments, Japan) and the reading was expressed in $\mu\text{S cm}^{-1} \text{g}^{-1}$. The method followed was as recommended by International Seed Testing Association (ISTA, 1987).

The experiments were carried out in Completely Randomized Design (CRD) in four replicates. Analysis of variance (ANOVA) was conducted using SAS software. Treatment means were compared using Least Significant Difference at 0.05 probability.

Results and Discussion

Seed Germination

The results showed that final germination percentage (FGP) of seed treated with GA_3 and KNO_3 are not significantly different compared to seed treated with distilled water while FGP of seed treated with ethephon was significantly lower than control (Table 1).

Table 1: Effects of hormonal and halo priming treatments on Eksotika papaya seed germination rate.

Treatment	<i>FGP</i> (%)	<i>T</i> ₅₀ (d)	<i>MGT</i> (d)	Seedling length (cm)
Distilled water (control)	89.00 ^a	11.72 ^b	12.10 ^b	3.35 ^c
250 mg/L GA_3	93.00 ^a	6.43 ^c	7.48 ^c	8.93 ^a
500 mg/L GA_3	92.00 ^a	5.39 ^d	6.10 ^d	9.30 ^a
500 mg/L KNO_3	92.00 ^a	6.72 ^c	7.48 ^c	4.05 ^b
1000 mg/L M KNO_3	97.00 ^a	7.05 ^c	7.45 ^c	4.05 ^b
500 mg/L ethephon	34.00 ^b	13.84 ^a	14.35 ^a	2.58 ^d
1000 mg/L ethephon	13.00 ^c	13.94 ^a	14.45 ^a	2.53 ^d
<i>LSD</i> at 0.05	12.45	0.73	0.82	0.50

Means with the same letter in the column do not differ at $p < 0.05$. T_{50} = time to 50% germination; *MGT* = mean germination time; *FGP* = final germination percentage.

Seed Germination Rate

Priming treatment significantly affected the germination rate of Eksotika papaya seed. Early germination occurred as indicated by lower value of T_{50} and *MGT* for seed treated with GA_3 and KNO_3 compared to control. Seeds treated with ethephon had the slowest germination (Table 1). T_{50} and *MGT* values for seed treated with GA_3 and KNO_3 were significantly different indicating the treated seed had higher germination rate compared to control while seed treated with ethephon had highest T_{50} and *MGT* indicating the seed had the lowest germination rate. The effects of GA_3 in accelerating germination may be due to the effectiveness of GA_3 to counteract the chemical inhibitors (mainly phenolic compounds) that may have been present in the papaya seed coat and sarcotesta (Salomao and Mundim, 2000). This result was supported by the finding of muskmelon seed soaked with KNO_3 which showed enhanced activity of dehydrogenase and α -amylase under low temperature (Nawaz et al., 2013). The same reaction probably happened to papaya seed treated with KNO_3 that

improved seed germination rate. Seed treated with ethephon resulted with slowest germination rate. The same outcome was reported where treatment with 5×10^{-7} M and 5×10^{-4} M of chloroethylphosphonic acid (CEPA) or ethephon reduced production of normal seedlings and percentage of radicle emergence (Zanotti et al., 2014) even the concentration used was lower than the concentration used in this experiment.

Insignificant difference of FGP except for ethephon treated seed signified that treatment with GA₃ and KNO₃ do not affect germination percentage but reduced germination time as indicated by T₅₀ and MGT values.

Seedling length

Seedling length of papaya seed treated with GA₃ was the longest and significantly different compared to seed treated with distilled water and followed by the seed treated with KNO₃ while the seed treated with ethephon showed the shortest seedling length (Table 1). The use of GA₃ as plant hormone was known for increasing cell size and stimulating the cell wall to create condition suitable for water absorption hence better cell growth (Shabaq, 2013). This fact was reflected on the length of papaya seedling treated with GA₃ which was longer compared to control. While for seed treated with KNO₃, the result disagreed with Owino and Ouma that the use of potassium salt such as KNO₃ and KCl for papaya seed resulted in no significant difference in plant height compared to control (Owino and Ouma, 2011).

Seed Vigour

Seed vigour was indicated through germination index, GI and vigour index, VI for each treatment. High GI and VI values signified high vigour seed. Maximum GI was recorded for seed treated with 500 mg/L GA₃ followed by seed treated with 250 mg/L GA₃, 500 mg/L and 250 mg/L of KNO₃ and control where seed treated with ethephon had the lowest GI value (Table 2). Vigour index of seed treated with GA₃ were significantly higher compared to seed treated with KNO₃ followed by control and ethephon. However, seed treated with 250 mg/L KNO₃ was not significantly different compared to control (Table 2). Vigour index is positively related to the FGP and seedling length. The higher the FGP value and the longer the seedling length resulted with higher vigour index. The GI value is related to the speed of germination. The quicker the seed germinate, the higher the GI index.

Measurement of electrical conductivity, EC of seed leachate can be used to determine seed vigour. The seed leakages that contribute to electrical conductivity of the water consist of various sugars, amino acids, lipids, organic and inorganic salts (Sørensen et al., 1996). Vigorous seed was determined based on the ability to re-establish the cell membrane integrity faster compared to less vigorous seed thus limiting amount of solutes leak from the seed (Sørensen et al., 1996) consequently low EC value. Seeds treated with KNO₃ had the highest EC reading compared to control seed followed by seed treated with KNO₃ and ethephon (Table 2). The EC values from the experiment do not reflect the vigour of the treated seed except for those treated with GA₃. The only acceptable EC reading indicating high vigour seed was seed treated with GA₃ which has significantly lower EC value compared to control. Seeds treated with KNO₃ had highest EC values indicating low seed vigour but the seeds actually have high GI and VI values. The high EC value could be contributed by traces of left seed treatment. While the seed treated with ethephon had the lowest EC reading indicating least loss of solute hence high vigour but in reality the seed had lower vigour based on GI and VI values. Therefore, EC test results should be interpreted with caution, especially when seed treatment is involved.

Table 2. Effects of hormonal and halo priming treatments on Eksotika papaya seed vigour.

Treatment	<i>GI</i>	<i>VI</i>	EC ($\mu\text{S cm}^{-1} \text{g}^{-1}$)
Distilled water (control)	14.16 ^c	297.60 ^c	30.09 ^c
250 mg/L GA ₃	27.15 ^b	830.00 ^a	26.72 ^d
500 mg/L GA ₃	31.46 ^a	856.40 ^a	21.31 ^e
500 mg/L KNO ₃	26.66 ^b	371.10 ^{bc}	59.66 ^b
1000 mg/L M KNO ₃	26.81 ^b	395.00 ^b	87.52 ^a
500 mg/L ethephon	3.72 ^d	88.88 ^d	8.58 ^f
1000 mg/L ethephon	1.35 ^e	31.70 ^d	7.83 ^f
<i>LSD</i> at 0.05	2.32	77.95	3.10

Means with the same letter in the column do not differ at $p < 0.05$. *GI* = germination index; *VI* = vigour index; EC = electric conductivity.

Conclusions

From this study, it can be concluded that the best priming treatments for seeds of papaya cv. Eksotika are priming with GA₃ and KNO₃ as the seed from these treatments had better germination rate, germinated earlier and high vigour while not affecting the final seed germination percentage.

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Varietal Identification Among Local Rice (*Oryza sativa*) Varieties Through Phenol, Modified Phenol and Ferrous Sulphate Test

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Introduction

In Malaysia, large number of rice varieties have been developed and used by the farmers all over the country based on local preferences. The seed production programme of these varieties has to be strictly monitored to ensure good quality. It often becomes difficult to identify the varieties by using morphological characteristics, especially for those involved in seed certification and quality maintenance. Skilled and experienced seed inspector needs to run standard lab purity test and time duration of one complete season for identification of varieties using grow-out test in which has become one of the major constraints in its implementation. Thus, it is necessary for the development of quick and reliable tests for varietal identification.

Anitalakshimi et al. (2014), Mor et al. (2006) and Vijayalakshmi and Vijay (2009) had reported that some chemical tests are able to distinguish between Indian rice varieties. Currently, there is no 'seed keys' for varietal identification of Malaysian rice seed using chemical tests. Therefore, this study was carried out to develop 'seed keys' for six current local rice varieties using four different chemical tests: (a) Phenol, (b) Modified Phenol with Copper Sulphate (CuSO_4), (c) Modified Phenol with Ferrous Sulphate (FeSO_4), and (d) Ferrous Sulphate (FeSO_4), individually and in combination.

Materials and Methods

Phenol test

Two hundred (50 x 4) seeds were pre-soaked in distilled water for 24 hours at $25 \pm 1^\circ\text{C}$. The seeds then placed on two layers of Whatman No. 1 filter paper moistened with 4 ml of 1 percent phenol solution. After 24 hours, the seeds examined and based on the intensity of colour they were grouped into strong, moderate and no colour change (Walls, 1965).

Modified Phenol with Copper Sulphate (CuSO_4) test and Modified Phenol with Ferrous Sulphate (FeSO_4) test

Modified phenol test was conducted similar to standard phenol test except soaking seeds in 0.5% CuSO_4 and 1.5% FeSO_4 instead of distilled water for 24 hours. Based on colour change of seed coat they were classified as no colour change, light brown, brown, dark brown and black. (Banerjee and Chandra, 1977)

Ferrous Sulphate (FeSO_4) test

Fifty seeds each in four replicates were soaked in 1.5% FeSO_4 solution for 4 hours under ambient condition. Excess moisture was removed before evaluation and the colour reaction is examined. Seeds were grouped based on the colour changed (Gupta and Agrawal, 1987).

Results and Discussion

Based on phenol colour reaction, all the six varieties used in the study were grouped into three classes (Table 1). In the phenol test, phenol was oxidised into dark coloured melanin catalysed by tyrosinase enzyme present in the seeds (Vijayalakshmi and Vijay, 2009). Phenol colour reaction also depends on the quality and quantity of this oxidative enzyme (Walls, 1965). The enzymes present in pericarp, mesocarp or any other seed structure. MR263 and MR269 showed strong colour reaction towards phenol test while MR253 showed moderate colour reaction (Figure 1), probably due to differences in the amount of tyrosinase. The remaining three varieties (MR219, MR220 and MR220 CL2) showed no colour reaction.

Modified phenol test using FeSO₄ and CuSO₄ showed strong black colour reaction to MR263 and MR269. The addition of Cu⁺ and Fe⁺ ions in the modified test greatly enhances the colour reaction for four varieties, MR219, MR220, MR220 CL2 and MR253 (Figure 2 and 3). The presence of metallic ions Fe⁺ and Cu⁺ acts as catalyst that enhances the reaction. Both modified phenol test are unable to distinguish four varieties (Table 1) because most of them show the same colour reaction, which is dark brown colour for modified phenol using FeSO₄ and light brown using CuSO₄.

Based on ferrous sulphate test, all six varieties were grouped into four groups with MR269 showing grey streaks, three varieties showing dark grey spot, one variety showing thick dark grey streak and another one showing thin dark grey streak. Combination of phenol, modified phenol and ferrous sulphate tests would be able to distinguish the six different varieties altogether.

Table 1. Rice varieties showing different colour reactions to chemical tests

Variety	Phenol test	Modified Phenol (FeSO ₄)	Modified Phenol (CuSO ₄)	FeSO ₄
MR269	Strong	Black	Black	GSt
MR263	Strong	Black	Black	DGSp
MR253	Moderate	Dark Brown	Light Brown	DGSp
MR220	No change	Dark Brown	Light Brown	DGSt (t)
MR220 CL2	No change	Dark Brown	Light Brown	DGSt (T)
MR219	No change	Dark Brown	Light Brown	DGSp

GSt: Grey Streak, DGSp: Dark Grey Spot, DGSt (t): Dark Grey Streak (thin), DGSt (T): Dark Grey Streak (thick)

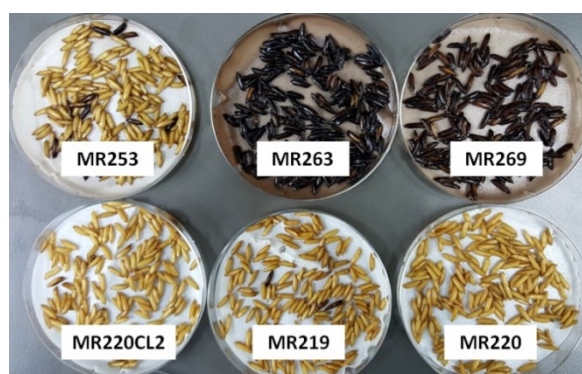


Figure 1: Rice varieties show different colour reactions to phenol tests



Figure 2: Rice varieties show different colour reactions to modified phenol test with FeSO₄

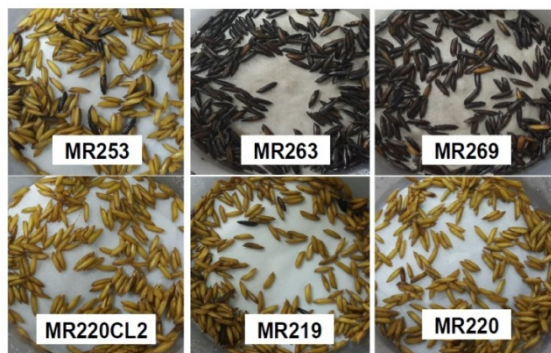


Figure 3: Rice varieties show different colour reactions to modified phenol test with CuSO_4

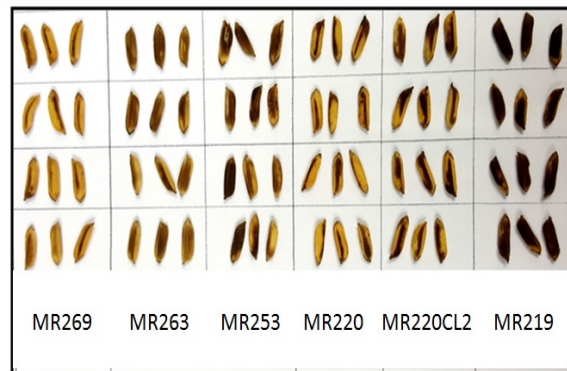


Figure 4: Rice varieties show different colour reactions to ferrous sulphate test

Conclusions

This study suggested that these four chemical tests could be used to identify all six varieties individually when used in combination. It has also proven that there was no single chemical test could distinguish even a single variety. Hence these simple, rapid and reliable tests are immense value for the varietal identification purpose in rice crop. The study also revealed that these tests could be effectively used for determining the varietal purity of rice for routine testing.

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Study on Attributes of *Carica papaya* cv Eksotika 2 for Quality Improvement

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Introduction

Papaya seed germination is often reported to be slow and erratic, and this problem has been associated with inhibitory compounds in the sarcostesta and seed coat, as well as seed dormancy (Andreoli and Khan, 1993). In general, 70% germination via the standard germination method using paper is considered to be satisfactory, in contrast with the germination standards set for other crops, which are generally higher. The Malaysian Standard by SIRIM set an even lower minimum germination percentage of 60%. Experience in MARDI, meanwhile, showed discrepancies among batches of seeds produced by the usual practice of seed production in which seeds are graded by floatation and via separation by sieving. Germination percentage varies in the 60 to 85% range. In addition, another problem with papaya seeds is decreasing viability (germinability) upon storage. Therefore, this study aimed to understand the relationship between seed attributes, in particular, seed mass and anatomy, as well as seed viability and vigour.

Materials and Methods

Plant Material

F1 hybrid Eksotika2 seeds were used. These were produced through controlled pollination of Line 20 with Line 19 pollens. Fruits at Maturity Index 2 were harvested in late 2014, let to ripen to Maturity Index 5, before seeds were extracted.

Seed Extraction and Processing

Fresh seeds were abraded gently on a sieve to remove the gelatinous sarcostesta and graded by floatation in water, in which floating seeds were removed. Seeds were then dried under shade until the seed moisture content reached approximately 9 to 12%. Small seeds were separated using a 3.15mm-aperture Prada sieve. Seeds were further graded and grouped into mass classes of <13, 13, 14, 15, 16, 17 and >17mg. Seeds were counted using a Waiver (Aidex Co. Ltd, Japan) automatic counter.

Seed Analysis

The mass of individual seeds were measured using a 4-decimal balance (Mettler Toledo, Switzerland). Seed moisture content of the seeds was determined by drying samples of 1g in a hot oven (Constance, Germany) at 130°C for an hour.

Germination study was carried out under ambient conditions using peat as the growing substrate. Seeds were considered to have germinated when the looped hypocotyls emerged above the surface of the growing medium (Fig. 1). Germination (emergence) was recorded daily for 11 days.

For electrical conductivity determination, 20 seeds were immersed in 100ml of distilled water for 24 hours at 25°C. Electrical conductivity of the seed leachate was measured with a CON510 conductivity meter (Eutech Instruments, Japan) and readings expressed in $\mu\text{S}/\text{cmg}$.

For anatomical study, seeds were dissected in halves, longitudinally and cross-sectionally. Anatomical study was conducted on seeds using a Dino-Lite digital microscope (Dino-Lite, Taiwan). The presence or absence of the embryo and endosperm was observed.

Seed germination time (MGT) and vigour index (VI) were calculated based on the methods described by Moradi Dezfuli et al. (2008), time to 50% germination (T_{50}) according to the formula of Coolbear et al. (1984) as modified by Farooq et al. (2005), and germination index (GI) was calculated using the formula described by the Association of Official Seed Analysts (AOSA, 1983).



Figure 1. A seed is considered germinated, in this study, when the looped-hypocotyl emerged above the surface of the growing medium.

Results and Discussion

Seed Weight Distribution

Weight of individual seeds ranged from 3.0 to 18.2mg in terms of mass (weight). Mean seed weight was 14.9mg. Seeds were grouped into classes (Fig. 2), and most of the seeds fell into the 13 to13.9mg, 14 to14.9mg, 15 to 15.9mg, and 16 to 16.9mg classes. In general, the majority of seeds have a mass between 14 to 17mg. and measured 4.56 to 16.03mm in length and 3.17 to 4.45mm in width (diameter). In addition, moisture content ranged from 7.7 to 11.10%.

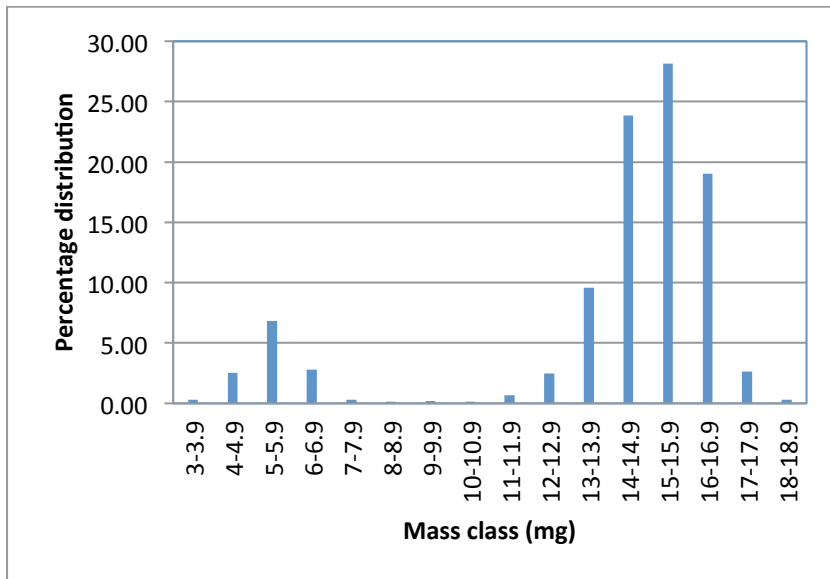


Figure 2. Percentage distribution of seeds vs seed mass (mg)

Seed Germination and Vigour

Germination of the different classes of seed is presented in Table 1. Seeds underwent epigeal germination (Jimenez et al., 2014). Our results showed that most of the seeds in all mass classes germinated between Day 5 and Day 7, almost synchronously (Fig. 3) and very much to the contrary of what was reported by many (Andreoli and Khan, 1993). Mean germination time (MGT) was almost similar in all mass days classes, ranging from 5.9-6.9 days, while time to 50% germination, T_{50} was approximately 6 days.

High germination percentages of 88 to 98% were recorded for seeds weighing more than 13mg (Table 1). Lighter seeds, i.e. those weighing <13mg, fared worst at 30%. Anatomical study showed the absence of embryos in the lighter seeds, as also reported by Nagao and Furutani (1986), and those with embryos (cotyledon) visible were void of the endosperm. The heavier and larger seeds, therefore, seemed to be well-endowed with nutrient, hence the high germination success. Similar results were also reported by Manzoor et al. (2007) in rice seeds.

Seed vigour as shown by the germination index (GI), i.e. 1.3 in the lighter seeds and 3.4 to 4.0 in the heavier seeds was also in agreement with the germination performance. Meanwhile, electrical conductivity (EC) test results were quite inconclusive.

Results from this study showed that including a further grading step based on seed mass could enable us to produce seeds with higher germination percentage and hence, higher quality. It is envisaged that the germination performance of those seeds might be further increased if seeds were subjected to treatments with compounds such as KNO_3 (Nurhazwani et al., 2014) or priming with gibberellic acid (Mohd Nizam, personal communication).



Figure 3. Excellent germination performance of seeds in the 16 to 16.9mg class

Table 1. Seed vigour measurements for selected seed classes

Seed weight (mg)	Germination (%)	MGT (days)	GI	EC ($\mu\text{S}/\text{cmg}$)
<13	30	5.9	1.3	12.35
13-13.9	84	6.2	3.6	31.15
14-14.9	91	6.0	4.1	33.79
15-15.9	86	6.3	3.6	31.83
16-16.9	98	6.5	4.0	36.20
>17	88	6.9	3.4	32.60

Conclusions

Seed quality in terms of germination rate may be determined by the seed weight. The heavier seeds seemed to have higher germination ability, compared to the lighter ones. Seeds below 13 mg could not germinate due to lack of endosperm or fully developed embryos. Eksotika2 seed with a seed mass of 13mg or more were shown to have high viability. Results also indicated that seed quality may be further improved, and significantly too, with an improved grading procedure based on seed mass. However, this may require the development or the modification of existing grading machines to suit the rough surface of the papaya seeds. Results of this study also showed that the minimum germination percentage set in the seed standards for papaya might also reflect the ability to produce higher quality seeds, as in other crops.

Acknowledgements

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Effects of Polymer Coating on Rice Seed Germination

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Introduction

Seed companies strive to add value to seeds and protect them from counterfeiting. On the other hand, buyers look for high quality and guaranteed seeds. Therefore, seed coating technology offers various possibilities for enhancing seed quality. It also enables identification and traceability. Three techniques of coating usually used for vegetable crop seeds; film coating, encrusting and pelleting.

The seed coating process involves all aspects of sticking materials onto the surface of seeds without significantly increasing the size or weight of seed. The application of polymers to seed serves as an extra exterior shell in order to give the desired seed characteristics viz., quick or delayed water uptake and enhanced germination that would be beneficial for better emergence and establishment in the given condition (Taylor *et al.*, 1998). Therefore, a study to determine the effects of polymer coating on rice seed germination was conducted.

Materials and Methods

All experiments were carried out in the Seed Quality Control Laboratory, Genebank and Seed Centre, MARDI Headquarters, Serdang, Selangor. Rice seeds (*Oryza sativa* var. MR 269) obtained from MARDI Seberang Perai were used in this study.

Polymer (Agritec Blue Lite) coating was prepared at different concentrations (60, 70, 80, 90 and 100%) in distilled water. Then, the polymer was applied at different volumes (1, 2, 3, 4 and 5 ml) onto 20 g of rice seeds and mixed using a rotary coater. Seeds were dried back to its initial moisture content after coated with the polymer for 8 hours at room temperature. Non-coated seeds used as a Control. Prior to coating, initial seed moisture content and weight were determined. Then, multispectral imaging of the coated seeds was analysed using VideometerLab to determine the optimal seed coating coverage.

Two replicates of five seeds (total of 5.0 g) used for moisture content determination. The moisture content of all samples was determined on a fresh weight basis using the oven method at $130\pm 2^{\circ}\text{C}$ for 1 hour as recommended by ISTA (2011). Seeds were germinated on moistened paper towel in a germination box (100 seeds per replications) and placed in a growth chamber at a temperature of $25\pm 2^{\circ}\text{C}$. Seed emergence was recorded every day for 14 days based on the radicle protrusion ($>0.5\text{cm}$). The mean germination time (MGT) was calculated based on the equation of Ellis and Roberts (1981) modified by Moradi *et al.* (2008). The time to 50% germination (T50) was calculated according to the formula of Coolbear *et al.* (1984) modified by Farooq *et al.* (2005). Germination index (GI) was calculated as described by Association of Official Seed Analysts (AOSA, 1983).

The experiments were carried out in a Completely Randomized Design (CRD) with four replications. The SAS software was used for analysis of variance (ANOVA). Treatment means were compared using the Tukey's Studentized Range (HSD) Test.

Results and Discussion

Seed coating coverage

Results show that coverage by polymer coating on rice seed surface was >99% when coated with 5 ml of 60, 70 and 80% polymer (Tab. 1). Suitable coating formulation provides an even coating on seed surface (Fig. 1). Polymer adheres well and gives an attractive appearance to seeds when coating coverage was high (>99%).

Table 1. Coating coverage (%) of rice seeds after coated with different concentrations and volume of polymer.

Polymer \ Volume	1ml	2ml	3ml	4ml	5ml
Control	-	-	-	-	-
60%	38.4	70.8	88.9	96.9	99.7
70%	53.3	76.9	88.3	98.1	99.6
80%	59.2	78.7	89.3	96.0	99.0
90%	60.6	81.3	88.0	94.6	98.8
100%	54.7	74.0	81.7	87.2	95.2



Figure 1. The visual of polymer coating coverage on rice seed surface.

Seed germination

The final germination percentage for both non-coated and coated seeds was high (>85%) (Fig. 2). It shows that polymer coating does not cause any phytotoxicity effects on seeds.

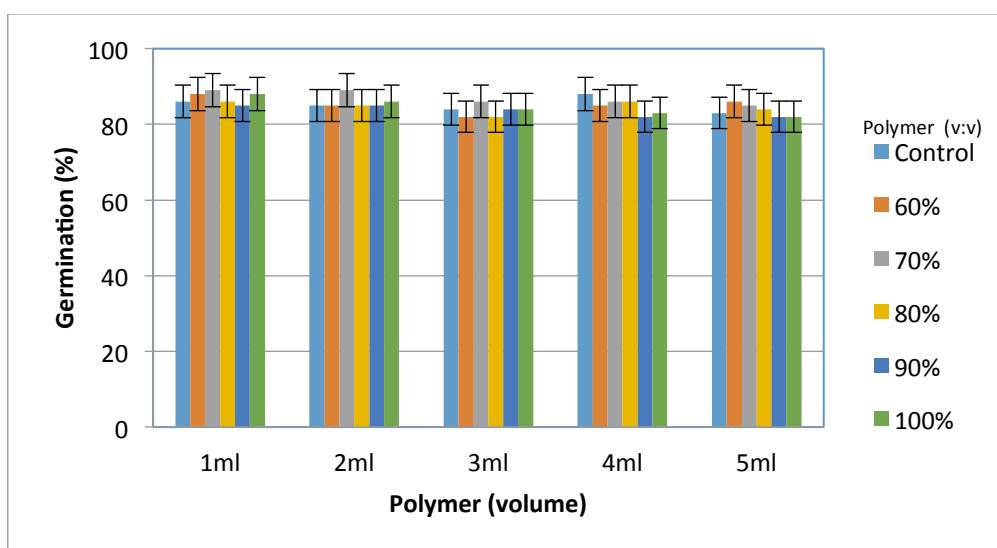


Figure 2. Rice seed germination after dosed with different concentration and dosage volume of polymer.

Seed germination rates

Seeds coated with 60% and 70% polymer have similar germination rates (MGT \approx 4.0, T50 \approx 1.5 and GI \approx 22.2) with the control (Tab. 2). However, seeds coated with higher polymer concentrations (80, 90 and 100%) has lower germination rate (MGT $>$ 4.0, T50 $>$ 1.5 and GI $<$ 22.2) as compared to the control. It shows that germination rate was affected by the coating coverage. The lower germination rates might be due to the high concentrations of polymer that creates a thicker layer of coating on seed surface that slows down the water uptake.

Table 2. Mean germination time (MGT), the time was taken to 50% germination (T50) and germination index (GI) of coated rice seed as compared to the control.

Polymer Concentration (Volume)	MGT	T50	GI
Control	4.0c	1.5b	22.3a
60% (1ml)	4.2bc	1.5b	21.8ab
60% (2ml)	4.1bc	1.5b	22.7a
60% (3ml)	4.1bc	1.5b	21.7ab
60% (4ml)	4.2bc	1.5b	22.4a
60% (5ml)	4.3b	1.5b	21.7ab
70% (1ml)	4.1bc	1.5b	22.1ab
70% (2ml)	4.1bc	1.5b	22.7a
70% (3ml)	4.1bc	1.5b	22.4a
70% (4ml)	4.0c	1.5b	22.7a
70% (5ml)	4.2bc	1.5b	22.2a
80% (1ml)	5.1a	2.0a	19.2c
80% (2ml)	4.9a	2.0a	19.6c
80% (3ml)	4.9a	2.0a	19.6c
80% (4ml)	5.0a	2.0a	18.9c
80% (5ml)	5.0a	2.0a	18.5c
90% (1ml)	4.9a	2.0a	20.1bc
90% (2ml)	5.0a	2.0a	19.5c
90% (3ml)	5.0a	2.0a	18.4c
90% (4ml)	5.0a	2.0a	18.7c
90% (5ml)	5.1a	2.0a	18.7c
100% (1ml)	4.9a	2.0a	19.3c
100% (2ml)	5.0a	2.0a	19.4c
100% (3ml)	5.0a	2.0a	18.7c
100% (4ml)	5.0a	2.0a	18.5c
100% (5ml)	5.0a	2.0a	18.3c

Means with the same letter in the column do not differ significantly ($p < 0.05$).

Conclusions

Based on the results above, polymer coating does affect rice seed germination rates when coated with higher concentrations and volumes. Coating coverage of 60% and 70% polymer was suitable, as germination rate and physiological quality of rice seeds were not affected.

Acknowledgements

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CHAPTER 5

POST HARVEST TECHNOLOGY AND QUALITY CONTROL

Impact of Tapping Intensity on the Physiological Changes of *Hevea brasiliensis* in Clone RRIM 2025

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Introduction

The improvement in productivity of a rubber tree is determined by latex harvesting technologies which are the tapping systems for rubber yield. Tapping enables rubber yield, which in turn may be limited by the metabolism of the latex vessel system. However, the continuous decrease in the size of rubber area has affected the production of natural rubber. Therefore, many smallholders has gone to the adoption of more intensive frequencies than alternate daily tapping (d2), i.e. daily tapping (d1), two days out of three (2d/3) or three days out of four (3d/4), with only one day of tapping rest to increase productivity. These intensive tapping systems may result in over exploitation with high rates of tapping panel dryness, thus reduce the life-cycles of the tree. To cope with that, it is essential to improve latex harvesting techniques by a length of cut, frequency, and stimulant application in order to reduce stress to rubber tree. For that purpose, several intensities tapping systems of latex harvesting have been tested to evaluate the physiological effect and yield response of rubber tree.

Materials and Methods

The study was conducted in Field 1, Permatang Division, Rubber Research Institute Experimental Station (RRIES) Kota Tinggi, Johore, Malaysia. The study was carried out on RRIM 2025 on panel B0-1. The design was based on a complete randomized design (CRD) which consisted of nine treatments laid out randomly and repeated in four replications. Trees of uniform size were selected. Every replication contained 10 trees and total trees per treatment were 40 trees. The treatments are;

(T1) S/2 d3 no stimulation = Half spiral cut downward, tapped third daily, without stimulant application

(T2) S/2 d3 Mtx 2.5% La 6/y = Half spiral cut downward, tapped third daily, stimulated with MORTEX 2.5% concentration, six times a year

(T3) 2xS/4 d3 Mtx 2.5% La 6/y = Two quarter spiral cut downward each tapped alternately at every tapping, tapped third daily, stimulated with MORTEX 2.5% concentration, six times a year

(T4) S/2 d2 no stimulation = Half spiral cut downward, tapped alternate daily, without stimulant application

(T5) S/2 d2 Mtx 2.5% La 6/y = Half spiral cut downward, tapped alternate daily, stimulated with MORTEX 2.5% concentration, six times a year

(T6) 2xS/4 d2 Mtx 2.5% La 6/y = Two quarter spiral cut downward each tapped alternately at every tapping, tapped alternate daily, stimulated with MORTEX 2.5% concentration, six times a year

(T7) S/2 d1 2d/3 no stimulation = Half spiral cut downward, tapped daily on alternate tapping two out of three days, without stimulant application

(T8) S/2 d1 2d/3 Mtx 2.5% La 6/y = Half spiral cut downward tapped daily on alternate tapping two out of three days, stimulated with MORTEX 2.5% concentration, six times a year

(T9) 2xS/4 d1 2d/3 Mtx 2.5% La 6/y = Two quarter spiral cut downward each tapped alternately at every tapping, tapped daily on alternate tapping two out of three days, stimulated with MORTEX 2.5% concentration, six times a year

The average yield of the treatment was expressed in term of dry rubber yield. The unit used for tree production was weight in gram per tree per tapping ($gt^{-1}t^{-1}$). The incidence of panel dryness was expressed in term of percentage of length total dry over total length of cut for the respective treatments. For bark moisture content, tapped collected bark was dried in oven at 70°C for 48 hours. The bark moisture reading was obtained by subtracting the bark dry weight from the bark fresh weight. Meanwhile, the pH was determined using the pH meter HANNA 8417. Total solid content (TSC) was determined by dried the dish filled with latex at 60°C until constant weight, and the calculation was obtained by subtracting the dry weight from the fresh weight. In dry rubber content (DRC), the latex was immersed in a coagulant mixture containing methanol and acetic acid (RRIM, 1973). The coagulum was pressed into a thin film, wash thoroughly and the glass plate was steam dried for 15 minutes. The calculation was obtained from the ratio of dry to wet weight. The sucrose concentration was verified by pipette trichloroacetic acid (TCA) serum with TCA 2.5% and 3 ml anthrone solution. The mixture was heated, cooled and measured at 620 nm using UV-Vis spectrophotometer.

Results and Discussion

The yield of tree productivity from the different treatments is shown in Table 1. High tapping intensity contributes to low tree productivity, where the 132% intensity shows the lowest from 30.25 to 26.76 g/t/t compared to 100% and 67% intensity. It is generally accepted that in trees tapped less intensity, the yields will be better in view of a long period of rest from tapping. The application of shortcut system, i.e. two quarter spiral cut downward each tapped alternately at every tapping, which tapped alternate daily and stimulated with MORTEX 2.5% concentration in six times a year (2xS/4 Mtx 2.5% 6/y) gave an encouraging yield and resulted no differences ($p>0.05$) between S/2 cut systems. The high yield obtained from the 2xS/4 Mtx 2.5% in high tapping frequency was reflected with intensity of tapping equally divided between the two panels at each tapping. The time available for latex regeneration will be much longer by splitting the tapping into two different tapping cuts. Thus, the establishment of equilibrium maintained between latex extraction and latex replenishment in the vessels of each drainage area of the cut. Moreover, the application of Mtx 2.5% helps to increase the drainage area and reduces plugging, thus increase the latex flow.

Table 1: Average in yield of clone RRIM 2025 applied with difference tapping systems

Treatment	Tapping intensity (%)	Tree productivity (g/t/t)
S/2 d3 no stimulation	67	46.21±5.88a
S/2 d3 Mtx 2.5% La 6/y (C)	67	46.24±6.23a
2xS/4 d3 Mtx 2.5% La 6/y	33(2)	34.70±5.83ab
S/2 d2 no stimulation	100	30.63±2.56ab
S/2 d2 Mtx 2.5% La 6/y	100	34.69±2.80ab
2xS/4 d2 Mtx 2.5% La 6/y	50(2)	30.93±4.11ab
S/2 d1 2d/3 no stimulation	132	27.93±1.84ab

S/2 d1 2d/3 Mtx 2.5% La 6/y	132	30.25±2.12ab
2xS/4 d1 2d/3 Mtx 2.5% La 6/y	66(2)	26.76±3.02b

Data are expressed as means ± S.E. Mean values in columns with same superscripts are not significantly different ($\alpha=0.05$)

Currently, no incidence of panel dryness had been detected (Table 2). This might due to a period of study which still in early period and also the effectiveness of mortex 2.5 %. The insignificant incidence of tree dryness suggests that the latex extraction rate was in equilibrium with latex production. In addition, the effect of palm oil as a carrier in the mortex could be another factor.

High tapping intensity contributes to the low of moisture content (Table 2). Results also showed that the stimulated trees were consistently lower moisture content than the unstimulated trees. The decrease bark moisture could due to heavy yield extracted from the tree. In addition, the low level of moisture suggested that the tree is in stressful condition and could lead to panel dryness. This implies the competition for vital growth substance in the tree which may be lost in the latex serum, and this reflected in poor growth and moisture content. This pattern also leads to a low of DRC and TSC value. Meanwhile, the short cut tapping obtained higher moisture content compared to long cut tapping although in high tapping intensity. It could due to the latex extraction rate was in equilibrium with latex synthesis in the vessels, thus gave constant of water transfers within latex vessels and less stress to the tree.

Commensurate with the rubber yield, the intensive tapping showed significantly depressed DRC and TSC (Table 2). The short cut tapping maintain higher contents compared to S/2 cut systems. It's due to the fact that the constant of water transfers within latex vessels in short cut tapping thus the latex extraction rate was in equilibrium with latex synthesis in the vessels. In addition, the stimulated trees showed lower contents compared to unstimulated trees in the same length of cut. This fall of contents is due to the fact that stimulation induces an increase of water transfer within latex vessels (Lacrotte, 1991). This process leads to dilution of the latex in the long run, which is the cause of lesser contents.

The pH of latex normally fluctuates within a very narrow range. However, the clear pattern is observed when high tapping intensity contributes to the low of pH (Table 2). While the decrease in latex pH with stimulant was observed, the difference was not significant compared to without stimulant. Moreover, the pH of latex in short cut tapping remain higher compared to long cut systems. Generally, latex with low pH had low yield. It because pH related to carbohydrate catabolism and low of pH will slow the isoprene biosynthesis.

Results have shown that trees applied with stimulation and intensive tapping had fallen the sucrose content (Suc) (Table 2). The unstimulated trees gave higher sucrose compared to stimulated trees. Indeed, this content reflects the activation of latex production metabolism since sucrose is the basic molecule of isoprene biosynthesis. The low level of sucrose suggested that the tree is in stressful condition and could affect the isoprene biosynthesis which will decrease latex yield in the future. However, the shortcut can maintain higher sucrose compared to long cut systems. These suggested that the tree applied to short cut tapping was less stress and indicate a good supply of latex vessels in relation to the productivity of rubber trees, although the high tapping frequency had been applied.

Table 2: Average in the latex physiology of clone RRIM 2025 applied to difference tapping systems

Treatment	Dryness (%)	Bark moisture (%) ^a	DRC (%)	TSC (%)	pH ^b	Suc (mM) ^a
S/2 d3 no stimulation	0	54.00±1.62	28.53±0.48ab	32.50±0.64b	6.86±0.09	2.85±0.16
S/2 d3 Mtx 2.5% La 6/y	0	53.98±0.52	27.08±0.88a	30.13±0.86ab	6.86±0.07	2.59±0.10
2xS/4 d3 Mtx 2.5% La 6/y	0	54.94±1.28	29.57±0.57b	31.77±1.10b	6.90±0.07	2.92±0.04
S/2 d2 no stimulation	0	52.66±0.71	25.64±0.64c	28.76±1.00ac	6.86±0.04	2.48±0.16

S/2 d2 Mtx 2.5% La 6/y	0	51.34±1.47	24.57±0.67c	27.38±0.72ac	6.85±0.07	2.40±0.12
2xS/4 d2 Mtx 2.5% La 6/y	0	52.17±0.68	26.66±0.41c	29.59±0.61ac	6.88±0.06	2.55±0.21
S/2 d1 2d/3 no stimulation	0	51.71±3.23	25.37±0.68c	27.46±0.77ac	6.84±0.08	2.53±0.18
S/2 d1 2d/3 Mtx 2.5% La 6/y	0	51.15±0.91	24.54±0.64c	26.46±0.83c	6.82±0.10	2.52±0.12
2xS/4 d1 2d/3 Mtx 2.5% La 6/y	0	54.75±1.06	25.95±0.57c	28.95±0.66ac	6.89±0.07	2.58±0.09

Data are expressed as means ± S.E. Mean values in columns with same superscripts are not significantly different ($\alpha=0.05$)

Conclusions

The fact that the physiological changes from low to high tapping intensity to be related to over exploitation leading to exhaustion of the laticifer system, thus lead to physiological stress to the tree. However, by manipulating the tapping cut and stimulation, seem that short cut tapping gave steady results compared to long cut tapping although in high intensity tapping. Therefore, in high intensity, short cut tapping could be attractive features to be applied by smallholder compared to convectional system i.e. S/2 cut system due to stable yield and physiological aspects.

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Effects of Maturity Stages, Storage Temperatures and Storage Durations on Chilling Injury and Antioxidant Activity of Fresh Ginger (*Zingiberofficinale*)

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Introduction

Ginger (*Zingiberofficinale*Rosc.) is an herbaceous perennial which has been used as traditional medicines and spices all over the world. Ginger contains secondary metabolites such as terpenes, flavonoids and phenolics. The phenolic components which are 6-shagaol, 6-gingerol, 8-gingerol, 8-gingerol, 10-gingerol and curcumin have been identified as major antioxidant components in ginger (Yehet al., 2014). However, ginger is susceptible to chilling injuries. Different maturity stages, varieties of rhizomes, temperature management, environmental circumstances and storage durations influenced the occurrence of chilling injuries in rhizomes. Chilling injury affects physical, physiological and chemical characteristics of the rhizome which bring down the quality of the rhizome. Delaying harvest will reduce the rhizome quality, increase fiber content, decrease storage life and increase the incidence of sprouting (National Agricultural Research Institute, 2004). Studies have been conducted on storage temperatures and storage durations effect on the physico-chemical changes of fresh ginger. However, comparisons between maturity stages of gingers towards chilling injury and antioxidant activity are still scarce. Thus, the objective of this study was to determine the optimum maturity stages and optimum storage temperatures for optimum antioxidant activities and minimal browning effects on fresh ginger.

Materials and Methods

Plant material

Fresh ginger rhizomes (*Zingiberofficinale*Rosc. cv. Bentong), harvested at 7-8, 9-10 and 11-12 months after planting, were purchased from a farmer in Bentong, Pahang. The ginger rhizomes were transported to the Postharvest Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (UPM) within 2-3 hours to maintain freshness and quality of the rhizomes.

Extraction

Ginger extraction was conducted by using a method established by Maizura et al. (2011) with some modifications. Ginger rhizomes were washed under tap water to remove all the foreign matters. The gingers were peeled and surface dried using an oven at 37 °C for 30 mins. The ginger was extracted by using a juice extractor (HU-100, Korea). The ginger extracts were filtered by Whatman No.1 filter paper and centrifuged at 8,000rpm for 20 mins at 4 °C (Maizura et al., 2011).

Storage treatments

The fresh ginger rhizomes at three maturity stages (7-8, 9-10 and 11-12 months after planting) were selected and stored at three storage temperatures (4, 13 and 25 °C) for six storage durations (0, 4, 8, 12, 16 and 20 days). At the end of each storage duration, the ginger samples were characterized physically (chilling injury, sprouting and browning) and assessed for antioxidant activity by DPPH assay.

Browning Index (BI)

The ginger rhizomes (20 g) were extracted by a juice extractor. The ginger extracts were centrifuged at 8,000rpm for 20 mins at 4 °C and then filtered through Whatman No.1 filter paper. The absorbance values of the ginger juice were measured immediately by a spectrophotometer (UV 1601, Shimadzu-Japan) at 420 nm to determine the BI (Jeonget al., 2008).

Antioxidant Activity (AA)

The antioxidant activities of the ginger extracts were determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical (Maizura et al., 2011). The chemical reaction was based on the electron transfer reaction between DPPH reagent and the plant extracts. Firstly, 400 ml ethanol was added into 100 µl ginger extract. Then, 1 ml of 0.1mM DPPH was added into the sample and then the sample was incubated at 37°C for one hour. The absorbance value was measured by a spectrophotometer at 517 nm. Percentage of DPPH inhibition was calculated by $[(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100$.

Statistical analysis

The experiment was conducted using RCBD in a three factorial arrangements of treatments, with four replicates. Data were analyzed using ANOVA, DMRT and regression (SAS version 9.3).

Results and Discussion

Browning Index (BI)

The BI was higher at 4°C compared to 13°C and 25°C storage temperature for all maturity stages except for 9-10 months gingers (Figure 1). BI was highest at 7-8 months (1.14), followed by 9-10 (1.12) and 11-12 months (1.06) at 4 °C. This was supported by Medlicott et al. (1990), who reported that chilling injury (internal browning, surface pitting and increased susceptibility to decay) and other storage disorders occurred more on fruits harvested at earlier maturity stage than older maturity stage under same storage temperature. Furthermore, 7-8 months ginger showed lower BI at day 20 as compared to 9-10 and 11-12 months ginger (Figure 3). BI increased as the storage durations increased for 7-8, 9-10 and 11-12 months gingers. Clark and Burmeister (1999) reported that browning effects on fruits increased when the storage duration increased. BI also showed quadratic relationship with storage temperature and duration (Figure 5). There was a slight increase in BI at day 16 of storage as compared to initial day of storage for 4°C, 13°C and 25°C. According to Omidiji and Okpuzor (1996), degree of browning was maximal at 30 °C but later declined with the rise in storage temperature. Proper storage temperature tends to control the undesirable enzyme activities and eliminate one or more of the essential components (oxygen, enzyme, copper or substrate) from the enzymatic reaction (Egwin *et al.*, 2013). In addition, ginger rhizomes sprouted after two weeks when stored at 25°C but those stored at 4°C and 13°C were apparently free from sprouting due to chilling injury.

Antioxidant activity (AA)

There were significant interactions between maturity stages and storage temperatures, maturity stages and storage durations, and storage temperatures and storage durations on AA. The 9-10 and 11-12 months ginger rhizomes stored at 13°C showed higher AA as compared to those stored at 4°C and 25°C (Figure 2). The 11-12 months ginger showed the highest AA at 13 °C which was 37.90% and 30.90% higher, respectively, than 7-8 and 9-10 months gingers. This was similar to the finding by Policegoudra and Aradhya (2007), who reported that storing the mango ginger at moderate low temperature tends to minimize the biochemical changes, maintained or increased the AA and

prolonged the shelf life. Storage durations negatively affected the AA of 7-8, 9-10 and 11-12 months gingers (Figure 4). Longer storage durations of fruits and vegetables are often associated with loss of antioxidant compounds in the crops (Hounsomet al., 2009). According to Naithanietal. (2006), the antioxidant capacity of herbal teas, including ginger, declined as the storage period increased. On day 20, 9-10 months ginger showed the lowest AA as compared to 7-8 and 11-12 months gingers. Moreover, AA decreased as storage durations increased under different storage temperatures (Figure 6). However, AA started to increase at day 8 for different storage temperatures and at day 20, ginger stored at 4°C retained higher antioxidant activity compared to those stored at 13°C and 25°C. An increased of AA associated with the accumulation of bioactive compounds at the day after storage could be used to determine the optimum time for storage of ginger rhizome. A decrease in AA with increasing temperature is very common in many crops (Pokorny, 1986).

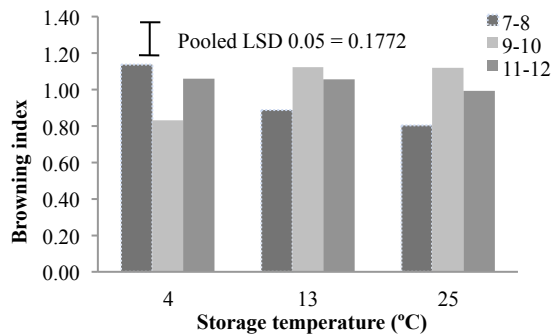


Figure 1. Effects of three maturity stages (7-8, 9-10 and 11-12 months) of gingers at different storage temperatures on the browning index of ginger

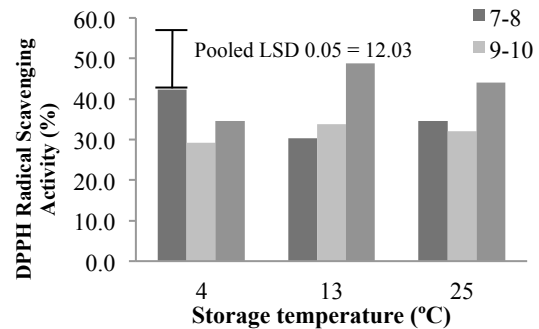


Figure 2. Effects of three maturity stages (7-8, 9-10 and 11-12 months) of gingers at different storage temperatures on the antioxidant activity (%inhibition) of gingers

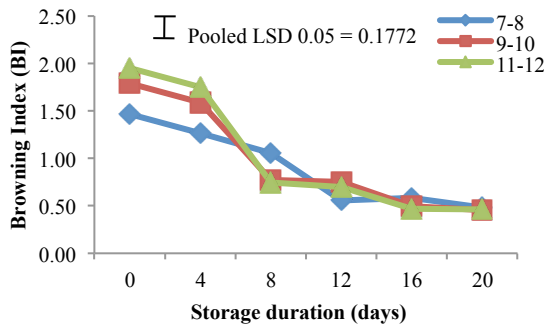


Figure 3. Effects of three maturity stages of gingers at different storage durations on the browning index of ginger

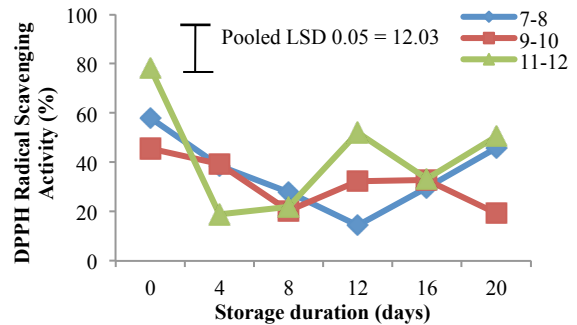


Figure 4. Effects of three maturity stages of gingers at different storage durations on the browning index of ginger

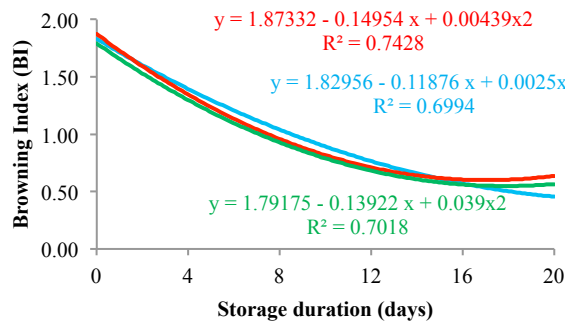


Figure 5. Browning index of ginger at different storage temperatures [4°C (-), 13°C (-), 25°C (-)] as affected by storage durations

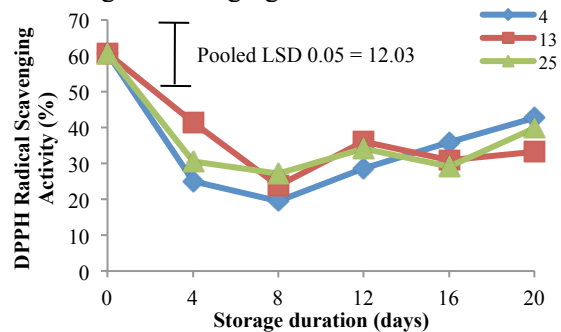


Figure 6. Antioxidant activity of ginger at different storage temperatures as affected by storage durations

Conclusions

The results of this study showed that 11-12 months old ginger was chosen as the optimum maturity stage due to higher antioxidant activity after storage and minimum browning effect as compared to gingers in the other maturity stages. The optimum storage temperature was recommended at 13 °C as there was no chilling injury and sprouting of the ginger rhizomes at this storage temperature.

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Effects of New Soilless Growing Media and Arbuscular Mycorrhiza on the Growth Performance of Lowland Cherry Tomato (*Solanum lycopersicon* var. *Cerasiforme*)

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Introduction

Cherry tomato (*Solanum lycopersicum* var. *Cerasiforme*) is an important horticultural crop grown commercially worldwide and available all-year-round. In Malaysia, the demand for healthy and safety tomatoes has increased both, export and domestic markets. However, insufficient supplies of tomato due to intensive demand from both markets entail more tomato cultivation. Currently, tomatoes are widely cultivated using the fertigation systems where coco peat (CP) is extensively used as the growing medium. This may due to the facts that it is cheaper, readily available and hold large quantities of water. However, CP contains less mineral nutrients. Thus, new alternatives should be discovered to replace or enhance the performance of CP by combining it with other agricultural wastes that abundantly available such as Oil Palm Fruit Bunches (OP) and rice husk biochar (B). Ain Najwa et al. (2014) reported that the combination of CP and OP resulted in higher yield of cherry tomatoes as compared to CP alone. In addition, combination of CP and B also showed a similar trend in the production of cherry tomato (Ain Najwa et al., 2014). Thus, B and OP could be a good medium in keeping nutrient readily available that helps the plant to absorb the nutrients. Among the significance of using CP and OP as the growing media, it is believed can maximize the utilisation of waste products. It is well documented that CP and OP are categorized as the waste products of the agriculture industry. Hence, by utilising these products, agricultural waste can be reduced as well as its pollution. In fact, the application of Arbuscular Mycorrhiza (AM) is believed to increase the growth performance of the plant due to its symbiosis characteristics with the plants root. It is renowned that AM plays a key role in enhancing growth performance of various crops. Thus, combining AM with the newly developed growing media such as CP, OP, and B could be the best alternatives to increase the growth, yield and quality of cherry tomato. Therefore, this study aimed at evaluating the effects of new soilless growing media and AM either alone or combination on the growth performance of lowland cherry tomato.

Materials and Methods

The experiment was conducted at the Greenhouse, School of Food Science and Technology, Universiti Malaysia Terengganu. The cherry tomato seeds and CP were purchased from Green World Genetics Sdn Bhd and Bumi Agro Maju Enterprise, Kuala Terengganu, respectively. Meanwhile, the rice husk biochar (B) and OP were obtained from Kilang Bernas, Tumpat, Kelantan and Oil Palm Factory in Kuala Terengganu respectively. Meanwhile, AM was a gift sample from Malaysian Agri-Hi Tech, MTDC-UPM, Selangor.

Thirty six of cherry tomato seeds were sown in seedling tray containing peat moss as the growth medium. After 25 days, the mature seedlings were transferred into polybags (18 cm x 15 cm) containing different growth media. Fertilizers used in this study were type A and B. Irrigation was equipped with droplet system and scheduled for 5 min per day (1000 mL). Irrigation water filled with fertilizers then applied at every 0800 h and 1700 h per day. The experiment was arranged in the randomized complete block design (RCBD), with treatments comprising different combinations of soilless growing media. Three replications were used for each treatment. The treatments were: i) combination of CP (2.85kg) + B (150g) (CB without AM); ii) CP (2.85kg) + B (150g) + AM (100g)

(CB with AM), iii) OP (2.85kg) + B (150g) (OPB without AM), iv) OP (2.85kg) + B (150g) + AM (100g) (OPB with AM), v) CP (1.425kg) + OP (1.425kg) + B (150g) (COPB without AM), vi) CP (1.425kg) + OP (1.425kg) + B (150g) + AM (100g) (COPB with AM). All treatments received similar agricultural practices such as watering, trellising, pruning and pesticide sprays. Growth performance of the plant which includes stem diameter and plant height, postharvest qualities such as soluble solids concentration (SSC), titratable acidity (TA), firmness and colour, yield of cherry tomatoes which includes number of cherry tomato, cumulative fresh weight, and volume of cherry tomato, and the spore count as well as percentage of root infection were measured throughout the experimental period.

The experimental data collected were subjected two-way ANOVA using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

No interaction between the two factors was observed on the growth performance of cherry tomato planted in soilless growing media. This was in agreement with the report by Dasgan et al. (2008) where vegetative plant growth was not significantly increased for tomato plant. In contrast, Wan Zaliha et al. (2015) claimed that AM inoculated roselle plants grown in soilless culture system showed significantly increased vegetative growth mainly below the ground level only. Possibly, the changes in responsiveness towards mycorrhizal colonization occurred with plant development stages that positively affected the plant in later stage which influence the reproductive characteristics such as fruit and seed development (Bryla and Koide, 1998). However, irrespective of AM, different growth media resulted in a significant influenced of vegetative growth of cherry tomato (Figures 1 and 2). Similarly, Ain Najwa et al. (2014) claimed that the combination of CP and biochar increased the growth of lowland cherry tomato planted in soilless media. Besides, the effect of biochar could improve the soil quality as well as plant growth (Chan et al., 2007), which possibly explain the rapid increase of plant height and stem diameter of cherry tomato grown on CB. As reported by Elad et al. (2010) biochar amended treatments in tomato were less susceptible to two foliar fungal pathogens (*Botrytis cinerea* and *Leveillula taurica*).

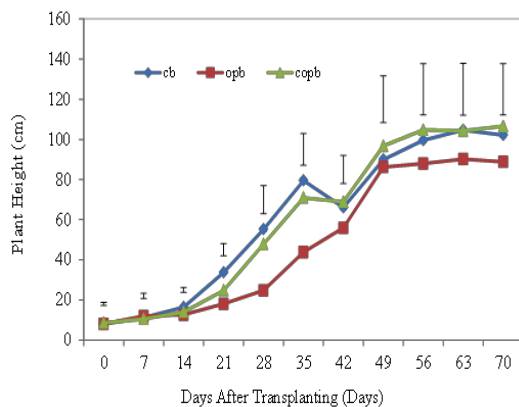


Figure 1. Effects of different growth media on plant height of cherry tomato. Vertical bars represent LSD ($P \leq 0.05$).
 CB= cocopeat+biochar, OPB= oil palm fruit bunches+biochar, COPB=cocopeat+biochar+oil palm fruit bunches

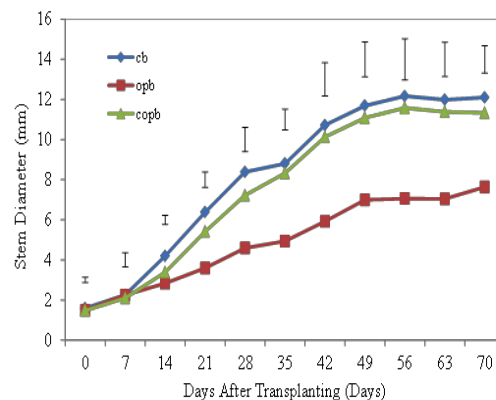


Figure 2. Effects of different growth media on stem diameter of cherry tomato. Vertical bars represent LSD ($P \leq 0.05$).
 CB= cocopeat+biochar, OPB= oil palm fruit bunches+biochar, COPB=cocopeat+biochar+oil palm fruit bunches

The suppressive effect of biochar on foliar diseases indicates that biochar induced systemic resistance towards disease and thus, promote plant growth (Elad et al., 2010).

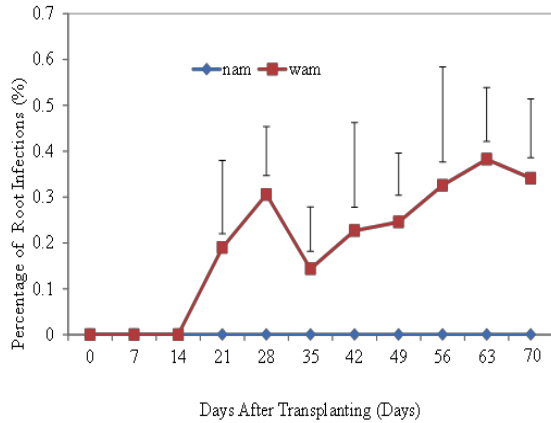


Figure 3. Effects of AM on percentage of root infection of cherry tomato. Vertical bars represent LSD ($P \leq 0.05$). nam=without AM, wam=with AM)

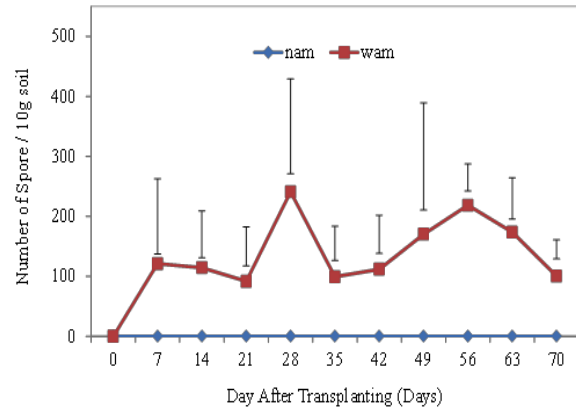


Figure 4. Effects of AM on percentage of root infection of cherry tomato. Vertical bars represent LSD ($P \leq 0.05$). nam=without AM, wam=with AM)

It is well documented that the inoculation of AM assists plant in the uptake of nutrients and to improve the plant growth (Douds et al., 2005). However, in the present study, the fertigation process that was done twice daily may affect the composition of the growing media which eventually caused the suppression of the AM association. Biermann and Linderman (1983) reported that the suppression of AM may be due to the heavy rates of fertiliser application to the mixed of the growing media, but other factors might also be involved. Moreover, Linderman and Davis (2003) claimed that media containing peatmoss as a major component have been shown to suppress AM fungal establishment. This was due to different peats have been shown to cause varied extent of suppression of the AM association (Linderman and Davis, 2003). In line to the above, spore numbers and percentage of root infection resulted in insignificant interaction ($P \geq 0.05$) for both factors, growth media and AM. However, percentage of root infection and spore numbers were significantly affected ($P \leq 0.05$) by AM inoculation only, but the growth media were not (Figures 3, 4 and Plates 1 and 2). Probably, the difference of root colonization in the plant does occur due to the variability of the host plant responsiveness towards the mycorrhizal colonisation (Bryla and Koide, 1998). In fact, the wide variation in the effect of these fungi on the root colonisation which in turn promoting the plant growth has not only been reported between different plant species but also between genotypes of the same species (Graham and Syvertsen, 1985).

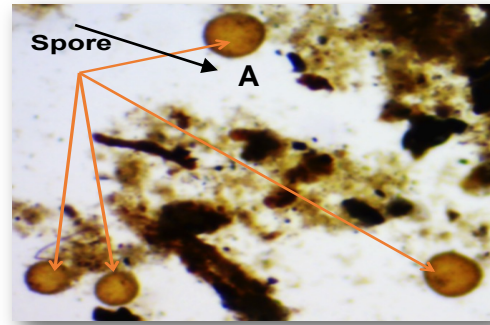
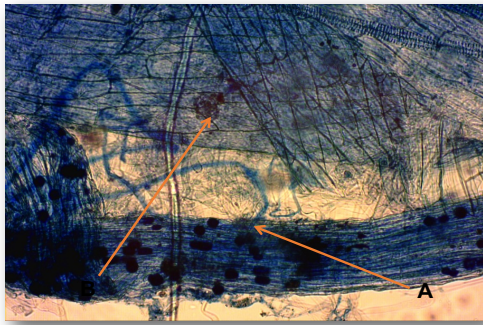


Plate 1. AM infection in cherry tomato root. A: Vesicle and B: hyphae

Plate 2. AM spore in cherry tomato root.

Meanwhile, the yield of cherry tomato which include number and cumulative fresh weight did not significantly affected by the interaction of the two factors and also irrespective of growth media (data not included). In contrary, Wahb-Allah et al. (2014) revealed that AM inoculated tomato plant showed a significant increase in the tomato fruit set, fruit number, early and total fruit yield compared to uninoculated tomato plant. This could be due to the ability of AM to increase the supply of available phosphorus and other immobile nutrients by translocating them from the soil through their hyphae to the plants (Plenchette et al., 2005). Nevertheless, there was a significant effect in term of growth media only towards the yield of cherry tomato (Figure 5 and 6). In general, cocopeat (CO) and rice husk biochar (CB) tend to have a higher fruit number and fresh weight while combination of CO, OPB and biochar (COPB) showed a higher value for volume of cherry tomato. In addition, the application of biochar which can improve the retention of nutrient (Lehmann et al., 2003) as well as to alter the soil microbial populations and functions (Pietikainen et al. 2000) may also act in concert to result in the significant effect of soilless growing media towards the yield of cherry tomato.

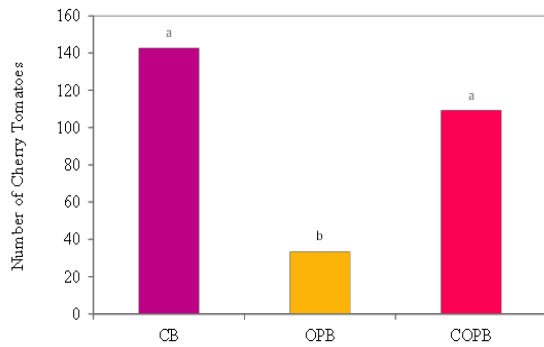


Figure 5. Effects of different growth media on the number of cherry tomato fruits.
 CB= cocopeat+biochar, OPB= oil palm fruit bunches+biochar, COPB=cocopeat+biochar+oil palm fruit bunches.

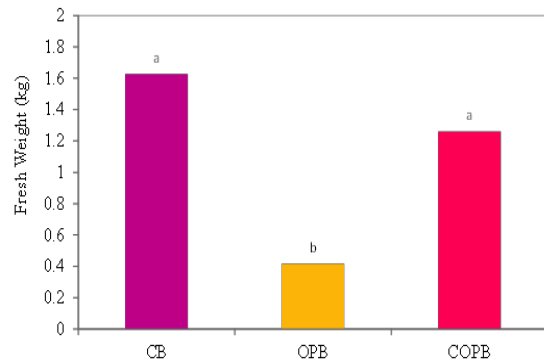


Figure 6. Effects of different growth media on fresh weight of cherry tomato fruits.
 CB= cocopeat+biochar, OPB= oil palm fruit bunches+biochar, COPB=cocopeat+biochar+oil palm fruit bunches

In general, all postharvest parameters (fruit colour, TA, firmness and SSC) exhibit a non-significant results neither in term of interaction between AM and growth media, inoculation of AM nor the soilless growing media (Table 1 and 2). This was in agreement with the reports of Ain Najwa et al. (2014). They reported that soilless growing media such as coco peat and biochar did increase the growth and yield of cherry tomato without adversely affecting the postharvest qualities of cherry tomato.

Table 1. Effects of AM and different growth media on fruit colour of soilless grown cherry tomato

Growth media treatment	Without AM			With AM		
	Lightness (L*)	Chromaticity a*	Chromaticity b*	Lightness (L*)	Chromaticity a*	Chromaticity b*
CB	41.87aA	35.81aA	26.68aA	42.45aA	37.82aA	27.31aA
OPB	36.31aA	31.49aA	23.85aA	35.60aA	30.69aA	30.69aA
COPB	41.79aA	37.14aA	26.82aA	41.87aA	33.79aA	25.93aA
LSD (P≤0.05)	15.97	16.27	10.248	16.623	13.89	13.23

Means followed by the same letter in capital letter (between AM treatments) and in small letter (between growth media) are not significantly different at $P \geq 0.05$. CB= cocopeat+biochar, OPB= oil palm fruit bunches+biochar, COPB=cocopeat+biochar+oil palm fruit bunches.

Table 2. Effects of AM and different growth media on fruit colour, firmness, titratable acidity and soluble solids concentration of soilless grown cherry tomato

Growth media	Without AM			With AM		
	TA (%malic acid)	Fruit firmness (N)	SSC (%)	TA (%malic acid)	Fruit firmness (N)	SSC (%)
CB	0.43aA	4.52aA	7.51aA	0.52aA	4.55aA	8.96aA
OPB	0.45aA	3.98aA	9.82aA	0.37aA	3.53aA	9.78aA
COPB	0.40aA	4.54aA	8.48aA	0.38aA	4.92aA	9.29aA
LSD (P≤0.05)	0.07	0.96	2.33	0.17	0.74	2.51

Means followed by the same letter in capital letter (between AM treatments) and in small letter (between growth media) are not significantly different at $P \geq 0.05$. CB= cocopeat+biochar, OPB= oil palm fruit bunches+biochar, COPB=cocopeat+biochar+oil palm fruit bunches

Conclusions

In conclusions, the inoculation of arbuscular mycorrhiza was capable to increase the number of spore and percentage of root infection. However, it was not able to enhance the growth performance of cherry tomato as well as the post-harvest quality attributes. On the other hand, the newly developed soilless growing media had the potential to improve the yield of cherry tomato. Irrespective of AM application, CB and COPB treated plants had higher fresh compared to other treatments. CB and COPB proved to be the best treatment to be applied as its increase yield and maintained other post-harvest quality attribute of cherry tomato grown on fertigation system. In addition, light and biodegradable growing media were also developed and able to replace current commercial soilless growing media, coco peat.

Acknowledgements

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Essential Oil in Combination with Coatings in Controlling Postharvest Rot in Berangan Banana

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Introduction

Crown rot of deheaded banana and anthracnose are two primary postharvest rot of banana (*Musa* spp.) that cause losses during storage and marketing of banana. The diseases usually appear on ripening fruits either at points of sale (farmer's markets, grocery stores) or later after purchase. The fungus *Collectotrichum musae* can cause both crown rot and anthracnose. In addition, crown rot diseases may also be caused by fungal pathogens *Lasiodiplodia theobromae* and *Fusarium proliferatum* (Anthony et al., 2004).

Application of systemic fungicides is the most common practice for commercial control of banana crown rot (Cox, 1996). Benlate (benomyl) was widely used as a postharvest dip to control crown rot disease until recently (Perera and Karunaratna, 2001). However, this fungicide has been banned by many countries due to its carcinogenicity and reproductive toxicity on human health.

Recently, researchers have shown an interest in the application of non-toxic alternatives or supplements to synthetic fungicides. Plant extracts, including essential oils (EO), have been investigated as alternative measures against pathological breakdown (Klieber et al., 2002; Ahmed et al., 2007). Incorporation of EO in based edible coatings has gained interest in the agricultural science owing to the bactericidal and fungicidal properties associated with these volatile compounds. The incorporation of EO in coatings has been regarded as an alternative option for the control of postharvest rot and it is a postharvest tool that is worthy of further exploration. Therefore, this study aims to enrich fruit coating with EO, for the additive antimicrobial benefit and to test it as a potential postharvest treatment against postharvest rot of banana.

Materials and Methods

Preparation of edible coating and EO

Coating solution (palm oil based) was prepared. Then, 0.3% (v/v) and 0.5% (v/v) *Cymbopogon nardus*, *Piper betel* and *Citrus hystrix* EO were respectively added to coating solution to prepare a total of six different solutions. To ensure incorporation of EO in coating, the solution was mixed using a homogenizer for 10 min set at 1,500 rpm. Fruits were dipped into respective solution for 2 min and allowed air dried with the assistance of a standing fan. These treatments were compared with the coatings without EO and control fruits without any coating treatment.

Efficacy of antifungal assay of edible coating and EO

A bunch of mature unripe banana fruits purchased from banana farm in Gurun, Kedah, Malaysia, was deheaded by cutting through the crown very close to the main stalk and selected for uniformity in size and shape, and absence of external injury. Fruits were washed with tap water, followed by treatment with 1% aluminium potassium sulphate and air drying at ambient temperature with the assistance of a standing fan. Control fruits were washed with water and air dried. Then, the fruits were packed in corrugated fiberboard and stored at 25 °C (RH 85-90%) for 10 days. Physical quality was based on appearance of the surface for disease incidence (DI) and severity (DS). The percentage of crown rot incidence was recorded at the end of the storage period. DS was scored following the scale (1=0%,

2=1-25%, 3=26-50%, 4=51-75% and 5=76-100% rotten fruit surface, respectively) (Sivakumar et al., 2002).

At the table ripe stage, bananas were evaluated for their sensory properties of treated and control samples by trained taste panellists. A questionnaire was used to record the data and taste, odour, flavour and overall acceptability were evaluated by the taste panel using a scale of zero to 100 (1=0-25%=Poor, 2=25-50%=Fair, 3=50-75=Good and 4=75-100=Excellent, respectively) (Sarananda and Wijeratnam, 1994). Zero indicated off taste and the absence of characteristic and therefore, non acceptability and 100 indicated banana characteristic, flavour, odour, taste and high acceptability.

The experiment designed was Complete Randomized (CRD). Data were analyzed using SAS (Statistical Analysis System) programme (SAS Institute, 1999) for analyses of variance (ANOVA) and when treatments were significant, separation of means were done using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Efficacy of EO with coating treatments against postharvest rot disease incidence and severity during storage

After dipping with treatment solution, bananas were stored for 9 days at ambient temperature and then examined for postharvest rot incidence and severity. Results presented in Table 1 show that postharvest rot incidence and severity, especially crown rot, were reduced by increasing concentration of EO. *Cymbopogon nardus* oil at 0.3% and 0.5% and 0.5% *C. hystrix* oil caused above 90% postharvest rot incidence of banana fruits, followed by *P. betel* oil of 0.5% and coating without EO which reduced disease incidence by 60% and 50%, respectively. A similar trend was also observed concerning postharvest rot severity (Table 1). Banana treated with *C. nardus* oil (0.3% and 0.5%) and 0.5% *C. hystrix* oil caused 1-25% postharvest rot disease severity. EO of *C. nardus* have been used to control postharvest diseases of banana while maintaining quality (Anthony et al., 2003). Moreover, EO of *C. nardus* contains antimicrobial compounds such as citronellal that can control postharvest fungi. This study also showed that higher concentration of 0.5% *C. hystrix* oil also inhibited growth of postharvest rot fungi. This was attributed to high terpine -4-ol and also citronellol contents as antimicrobial compounds in *C. hystrix* oil (Weikedre et al., 2010).

Table 1. In vivo postharvest rot disease incidence and severity in response to fruit coating with different concentrations of some EO

EO / Coatings	EO Concentration (%)	Disease incidence (%)	Reduction (%)	Disease Severity (%)
<i>Cymbopogon nardus</i> + coating	0.30	8c	90	2c
	0.50	5c	93.75	2c
<i>Piper betel</i> + coating	0.30	80a	0	5a
	0.50	32b	60	3bc
<i>Citrus hystrix</i> + coating	0.30	80a	0	5a
	0.50	8c	90	2c
Coating	---	40b	50	4a
Control fruits	---	80a	---	5a

Figures in each column with the same letter are not significantly different ($P \leq 0.05$).

Sensory evaluation

Sensory evaluation of organoleptic properties is shown in Table 2, e.g. odour, flavour, taste and overall acceptability of EO treated banana when stored at 25 °C. Banana treated with higher concentration of *P. betel* oil showed significant lower odour comparing with other EO applied. Odour was reduced with reduced concentration of EO. Meanwhile, *C. nardus* and *C. hystrix* oil at 0.3% had no significant effect on odour properties of ripe stored banana or control. The values pertaining flavour, taste and overall acceptability of bananas treated with 0.5% *C. hystrix* oil were significantly lower when compared with other treatments. Bananas treated with *C. nardus* oil at 0.3% and 0.5% showed no significant differences as compared with the control. This means that banana treated with natural compound will be more acceptable to consumer than banana treated with fungicide. In addition, volatile compound from plants can inhibit growth of fungal pathogens before evaporating without leaving any residue. Some of them are normal constituents of the human diet and are unlikely to be of any health risk (Hamilton-Kemp et al., 2000).

Table 2. Sensory evaluation of bananas after treatment with different EO stored at 25 °C for 9 days

EO / Coatings	EO Concentration (%)	Odour Acceptability	Flavour	Taste	Overall Acceptability
<i>Cymbopogon nardus</i> + coating	0.30	88.6a	93a	93a	93a
	0.50	78b	88.2a	90a	90a
<i>Piper betel</i> + coating	0.30	80b	80b	80b	75b
	0.50	60c	71.2b	85a	85b
<i>Citrus hystrix</i> + coating	0.30	87a	75b	80b	78b
	0.50	75b	75b	85a	80b
Coating	---	90a	93a	93a	92a
Control fruits	---	90a	92a	94a	94a

Different letters in the same column denote a significant difference ($P \leq 0.05$).

Conclusions

Hence, *C. nardus* oil treatments combined with coating developed during the current study could be recommended as a safe method for treating bananas to control postharvest rot disease. Coating treatment only delayed the ripening process need for such alternative control or antifungal agent for controlling post harvest diseases.

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Effects of removing stalk and storage temperatures to overcome short shelf life of *Capsicum frutescens*

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Introduction

Capsicum frutescens is one of the popular vegetables that has been used as cooking ingredient or served fresh. About 224 hectares land have been grown with *Capsicum frutescens* in Malaysia in 2009 producing 1,400 metric ton valued at RM 6.2 million. Johore was the largest producer of *Capsicum frutescens* (75 ha) followed by Sarawak and Kelantan. *Capsicum frutescens* is easily affected with browning that starts from the stalk. This causes the shelf life of chilli to become shorter which is due to the infection by fungus. Deterioration on chilli can be solved by performing proper postharvest handling. Minimally processed operation which consists of trimming, washing and packing may help to prolong the shelf life of chilli. Minimal processing describes non-thermal technologies to process food in a manner to guarantee the food safety and preservation as well as to maintain as much as possible the fresh-like characteristics of fruits and vegetables (Manvell, 1997). Among others, visible properties of fresh-cut fruit and vegetable commodities are one of the most important parameters to evaluate the total quality of the product by consumers. Suitable packaging helps to reduce discoloration such as enzymatic browning of cut surfaces, yellowing of green vegetables and pale color of bright vegetables, mechanical damage including foiled lettuce leaves and absence of cutting damage, as well as decay (Bolin and Huxsoll, 1991; Jacxsens et al., 2003; Varoquaux and Wiley, 1994). Storage temperature is the single most important factor affecting spoilage of minimally processed fruit and vegetables. Chilling injury is one of the factors that affect the quality of *Capsicum frutescens*. The optimal storage temperature for vegetables is 10°C whereas 5°C is the temperature suitable for keeping products in chiller during sale. However, the suitable temperature for storage of minimal processing product is 2°C. Therefore, the objective of this study was to determine the effect of removing stalk towards quality of *Capsicum frutescens* and identify the suitable storage temperature for chilli.

Materials and Methods

Capsicum frutescens was purchased from wholesale market Pasar Borong, Puchong, Selangor. The chillies were preconditioned at 10°C overnight. The chillies were then sorted out by choosing those with no visible microbial growth and free from visible physical defects with index 3. Then the chillies were divided into two groups which were 1) with stalk (control) and 2) without stalk. The chillies were washed with tap water followed by rinsing with filtered water. They were then dipped in 1 % CaCl₂ for 30 seconds. The samples were drip-dried and packed in LDPE 0.04 mm (70 g for each pack). The chillies were stored at three different temperatures which were 2°C, 5°C, and 10°C. Statistical analyses of the results were conducted using Analysis of Variance (ANOVA) and Duncan Multiple Range Test to determine whether the comparison between different treatments and different storage durations show significant differences ($p < 0.05$).

Results and Discussion

During storage, chillies could be stored until 21 days at 2°C with the treatment with and without stalk. However, the chillies stored at 2°C had chilling injuries after 28 days. At 5°C, the chillies without stalk showed less browning with retarded deterioration. By removing the stalk, storage life could be prolonged from 21 days to 28 days compared to samples with stalk. By increasing the storage

temperature from 5 to 10°C, the chillies could be stored only until 14 days. The factors that limited storage life were stalk browning, fungal growth and fruit rot.

Weight Loss

Percentage of weight loss was significantly increased with increasing storage temperature which were <0.25%, 0.35% and <0.8% at 2°C, 5°C and 10°C, respectively (Figure 1) Weight loss of chillies with stalk was significantly higher ($p < 0.05$) compared to chillies without stalk at 10°C (Figure 1).

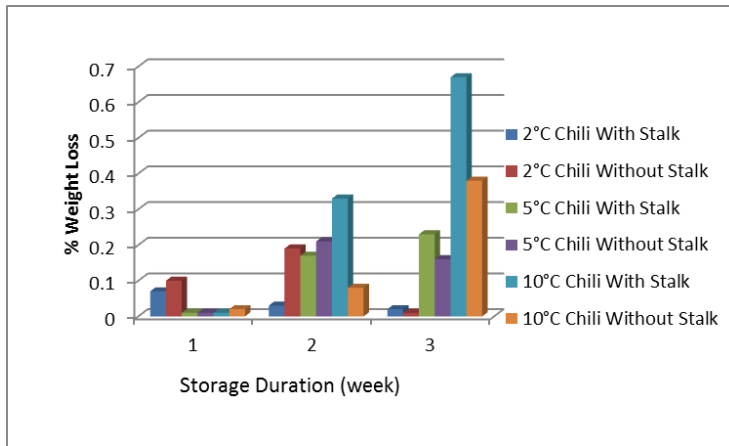


Figure 1. The effect of storage temperature and treatments towards percentage of weight loss in chilli

pH Content

pH was found to decrease with increasing storage temperature but none of the treatment was significantly affected by the pH change (Figure 2). Storage of chillies at 2°C showed significantly higher pH compared to other temperatures.

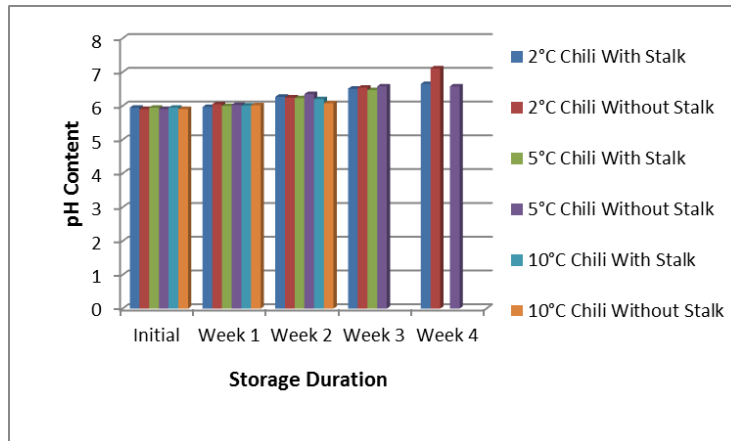


Figure 2. The effect of storage temperature and treatments towards pH content in chilli

Acidity

The acidity was not significantly ($p > 0.05$) affected by the increasing storage temperature while upon treatments, the acidity of chilli was significantly higher by trimming the stalk at $p < 0.05$ (Figure 3). Storage at the low temperature of 2°C has higher total titratable acidity content compared to 5°C and

10°C. By trimming the stalks of the chilli resulted in high acidity content compared to the chillies without trimming.

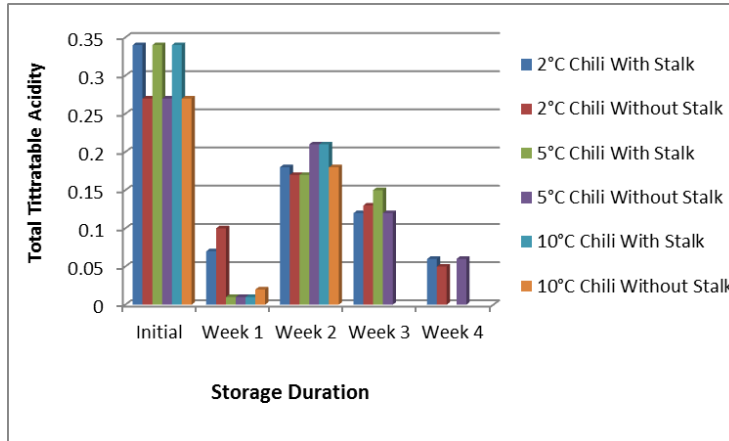


Figure 3. The effect of storage temperature and treatments towards acid content in chilli

Ascorbic Acid

Increasing the storage temperature was found to reduce the content of ascorbic acid significantly. There was no significant ($p > 0.05$) difference on ascorbic acid content for chillies stored at 2°C and 5°C but at 10°C the results were significantly ($p < 0.05$) lower (Figure 4). The content of ascorbic acid in treatment was significantly higher by trimming the stalk ($p < 0.05$) compared to chillies without trimming.

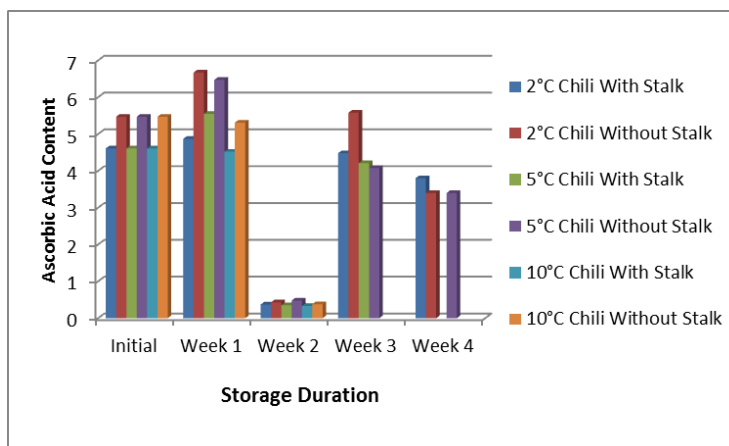


Figure 4. The effect of storage temperature and treatments towards ascorbic acid content in chilli

Microbial Count

Chillies without trimming showed slightly higher ($p > 0.05$) number of microbial count compared to chillies with stalk. Storage at lower temperature (2 to 5°C) significantly reduced ($p < 0.05$) mesophilic aerobes and coliform but not significantly ($p > 0.05$) affected yeast and mould (Figure 5). Due to low temperature storage, the microbiological population on vegetables was expected to be dominated by

psychrotrophic microorganisms (Zagory, 1999). Nevertheless, Kang and Lee (1997) reported that shelf-life of fresh cut peppers at 5°C (4 days) was determined by chilling injury rather than by microbial quality.

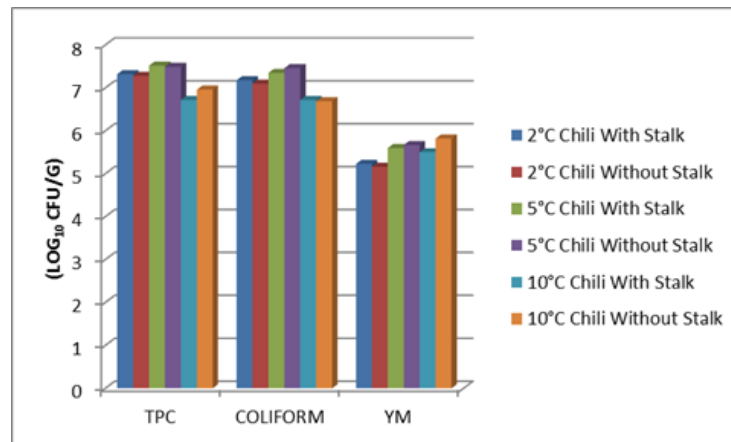


Figure 5. The effect of storage temperature and treatments towards microbial count in chili

Conclusions

By removing the stalks of chillies, it slightly helped to delay the growth of fungi (mesophilic aerobes and coliform). Storage at 2°C caused the growth of fungi to slow down but shortened the shelf life because of chilling injuries. Therefore, storage at 5°C and removing the stalk could prolong the shelf life of chilli for up to 28 days.

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Bamboo (*Gigantochloa albociliata*) Shoot: From Smelly Pickle to New Fresh Cut Form

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Introduction

Bamboo (*Gigantochloa albociliata*) is a group of evergreen plants belonging to Poaceae or grass family. Bamboo shoots are the immature and edible culms arising from the rhizomes. Shoots emerge in early spring, can grow quickly at over 1 m per day, and usually become lignified (woody) in 2–3 days (Luo et al., 2002, Wang, 2002, Zhang et al., 2000). For these reasons, bamboo shoots have limitations for consumption and storage. In local markets of southern China, bamboo shoots are usually stored and sold at ambient temperatures and unpackaged, which easily leads to bamboo shoot browning and lignification (Qun et al., 2006). In Malaysia, minimal processing of bamboo is done in small quantities by the local people, and this results in rapid deterioration under ambient conditions with lignification and browning occurring on the cut surfaces. In addition, browning at the cut surface of minimally processed (MP) bamboos is also a limiting factor for shelf life and consumer preference. The perishability of bamboo shoots requires the development of technologies that reduce their postharvest deterioration and extend their shelf life.

Suitable storage temperature is an important factor in maintaining the quality and extending the shelf life of MP vegetables (Osornio and Chaves, 1998). It is reported that the shelf life of bamboo shoots was limited to one day at ambient temperature (20-25 °C), while the shelf life could be extended to 28 days by combination of low temperature and packaging with low-density polyethylene (LDPE) bags (Kleinhenz et al., 2000). According to Luo et al. (2002), the bamboo shoots usually lignified in 2-3 days at ambient temperature and became inedible. Therefore, this study was conducted with the aim to determine the suitable temperature for storage of MP bamboo shoots.

Materials and Methods

Bamboo shoot was harvested from a local farm in Selangor. They were selected, being of uniform size and free of physical damage and fungal infection. The bamboo shoots were washed with tap water and peeled by sharp knife. Then, the bamboo shoots were sliced 2 mm vertically and immersed in chilled water containing 1% calcium chloride (CaCl₂) for about 1-2 minutes. Samples were drip-dried to free the excess water. The cut samples were packed in 0.04 mm thick polyethylene bag (LDPE) containing water absorbance. Samples were stored at different temperatures; 2, 5 and 10 °C for 21 days. The bamboo shoots were sampled weekly to determine the change of quality.

Postharvest quality evaluation included physical (visual appearance, texture, colour in terms of L, hue, chroma) and chemical (pH, TSS, TA, ascorbic acid). The quality of bamboo shoots was judged visually and the criteria used were retention of original colour, freshness and damage severity. The colour of bamboo shoots was measured using a Chromameter (Model CR-300 Minolta, Japan). Each color value for Hunter L (lightness), chroma, and Hue angle (h°) was expressed as the mean of three measurements. Total soluble solids (TSS) were determined with a digital refractometer (Atago, CO., LTD, Japan and Model DBX-55). The pH values were measured using a pH meter (Hanna Instruments pH 211 Inc. RI-USA, Microprocessor pH Meter). Total titratable acidity (TTA) was determined by titrating 20 ml of extraction with 0.1 mol l⁻¹ NaOH to pH 8.2. Ascorbic acid was determined by extraction of 10 gm sample with the addition of 100 ml of 3% metaphosphoric acid.

Then, 10 ml extraction was titrated immediately with a standard dye solution to first permanent pink endpoint.

All data were analysed by analysis of variance (ANOVA) using SAS from Statistical Analysis. To determine difference between treatments, Duncan tests were applied and significant differences were established at $P \leq 0.05$.

Results and Discussion

MP bamboo shoots started to change colour, soften, show browning and lignification after seven days for storage at 10 °C, 14 days for stored at 5 °C and 21 days for storage at 2 °C. Kleinhenz et al. (2000) also reported that shelf life of bamboo shoots was possible for no more than seven days at storage temperatures above 8 °C and using packaging of semi-permeable materials (micro-perforated LDPE bag and LDPE film), shelf life of bamboo shoots could be extended to 28 days when stored at 1 °C. The colour of MP bamboo shoots changed from white to brown and it obviously appeared in 10 °C and 5 °C samples. The browning of bamboo shoots is enzymatic browning and the main phenolic substrate is chlorogenic acid (Zhang et al., 2000). Storage time significantly affected the colour parameters. Lightness and chroma of MP bamboo shoots increased linearly during storage (Table 1). MP bamboo shoots' colour became yellowing to browning during storage. However, no significant differences were observed on hue of MP bamboo shoots during storage. In addition, no significant differences were observed on colour among temperature treatments.

One of the main factors used to determine quality and postharvest shelf life is the loss of firmness during storage. Comparisons in MP bamboo shoot firmness between temperatures are shown in Table 1. During storage, there were significant differences in firmness but no significant differences were observed among temperature treatments.

Table 1. Effect of different temperature on shelf life and quality of MP bamboo shoots (*Gigantochloa albociliata*)

Factor	Colour of bamboo shoot			Firmness (N)	Ascorbic acid content (mg/100ml)	TTA (% citric acid)	pH
	L	C	Hue				
Temperature (T)							
2°C	74.41	18.69	93.97	12.04	2.16	0.24a	5.53b
5°C	73.01	18.49	92.62	11.17	2.05	0.20b	5.83a
10°C	73.57	18.35	91.71	12.21	2.11	0.18b	5.75a
F-Test significance	NS	NS	NS	NS	NS	**	*
Storage day (D)							
0	41.83b	52.99a	91.96	4.35a	3.13a	0.09c	5.80
7	80.91a	10.89b	92.87	5.81b	1.87c	0.21b	5.67
14	80.98a	10.40b	91.75	5.72b	1.88c	0.21b	5.69
21	80.31a	11.25b	93.96	5.20b	2.24b	0.24a	5.71
F-Test Significance Interaction	*	*	NS	*	**	**	NS
T x D	NS	NS	NS	NS	NS	*	NS

Each value was the mean of three replicates. *Means within columns and factors followed by the same letter are not significantly different based on DUNCAN at $P \leq 0.05$. NS, *, ** denote non-significant, significant and highly significant at $P < 0.05$ and $P < 0.001$, respectively.

Ascorbic acid content of the MP bamboo shoots was not affected by storage temperatures (Table 1). However, ascorbic acid content was slightly reduced during storage. The loss of ascorbic acid depends on storage temperature rather than on the length of storage period (Adisa, 1986). All the treatments significantly affected the total titratable acidity (TTA) and pH of MP bamboo shoots. TTA and pH was significantly affected ($p < 0.05$) during storage. Acidity was higher with storage at 2 °C as compared to other temperatures.

Conclusions

Temperature was the most significant factor influencing the shelf life of MP bamboo shoot. MP bamboo shoots stored at 10 °C had short shelf life (7 days) as compared to 21 days when stored at 2 °C. It was due to rapid change in the chemical and physical properties. Storage at 2 °C provided the best condition to extend storage life of MP bamboo shoots.

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Postharvest Quality of Mangosteen and Mesta at Different Maturity Stages

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Introduction

Mangosteen (*Garcinia mangostana* L.) or commonly referred as manggis in Malaysia, is native to tropical Southeast Asia and commonly grown throughout Thailand, Malaysia, Philippines and Indonesia. Mangosteen, like durians and rambutans, are seasonal fruits with its peak fruiting season occurring during June-August and November-February (Osman and Milan, 2006). However, the fruits could still be harvested at an earlier stage of maturity for the market. Mangosteen requires about 3 months from successful pollination to fruit development. For mangosteen, the main determination of harvest maturity is peel colour. Immature fruit are entirely pale green with speckles of pink (Index 1). As the fruit matures, spots of pink would start to appear on the peel surface. The fruit will continue to turn from light pink to purple, eventually ripening to a completely dark purple (Index 6). Fruits harvested at the immature pale green stage (Index 1) will not ripen properly and the fruit will taste bland. Recommended harvesting maturity indices for mangosteen are Index 3. Flavour and taste improved in this stage as the fruit continue to turn deep purple within few days after harvest.

Mesta is the lesser known variety of the mangosteen group and could be found mainly in Pahang and Sabah. Its tree is smaller compared to mangosteen tree thus, making harvesting easier. Fruits of mesta are smaller and ovoid in shaped with a slightly pointed distal end whereas fruits of mangosteen are slightly bigger and with flat distal ends. According to Paul and Ketsa (2014), mangosteen have 4-8 segments of edible white aril, including 1 or 2 larger segments containing apomictic seeds. As for Mesta, most of its edible white arils are seedless or have undeveloped seed.

The storage life and quality of horticultural produce are greatly influenced by maturity at harvest. Appropriate physiological maturity at harvest is crucial for proper quality and shelf-life. Changes that occur during fruit developmental processes in terms of physico-chemical, physiological and sensory aspects also need to be taken into consideration for the development of optimum maturity index. There is lack of harvesting information on the mesta fruit compared to mangosteen. Therefore, a study on the postharvest quality of mesta and mangosteen at six different stages of maturity were conducted.

Materials and Methods

Fruits of mangosteen and mesta were selected for colour and size uniformity. Mangosteen was obtained from MARDI Kluang, Johor whereas mesta was from a grower's farm in Temerloh, Pahang. Fruits from each variety were separated by peel colour into six stages, using a colour index developed by MARDI; light greenish yellow with traces of red (Index 1), reddish-yellow with patches of red (Index 2), reddish with patches of bright red (Index 3), reddish brown (Index 4), purplish red (Index 5) and dark purple (Index 6). Rind colour of fruit was determined by using a portable chromameter (model CR-400 Minolta Corp., Osaka, Japan). The colour was expressed as lightness (L^*), chroma (C) and hue (h°) values.

Fruit was peeled and pulp was blended using a kitchen blender (Pensonic, Malaysia). Soluble solids concentration (SSC) was measured using a digital refractometer (ATAGO RX-5000, Japan). The pH was measured using a pH meter. Total titratable acidity (TTA) was determined using the titration method as reported by Nerd et al. (1999) and expressed in % citric acid. Ascorbic acid content was determined by titration with 2, 6 dichlorophenolindophenol until a faint pink colour persist and

expressed in mg/100 mg fresh weight. Antioxidant activity for mesta and mangosteen was studied through the evaluation of free radical scavenging effect on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Lim et al., 2007). Total phenolics content of mesta and mangosteen were determined using the Folin-Ciocalteu method and gallic acid was used as standard (Singleton and Rossi, 1996). The experiment was conducted using the completely randomized design (CRD). Data were analysed using ANOVA (SAS ver. 9.3) and means were separated using least significance difference (LSD).

Results and Discussion

The rind colour of both mangosteen and mesta fruits, changed from green to dark purple after harvest as the fruit ripens. L*, C* and h° values of mesta and mangosteen fruits decreased significantly (P<0.05) as fruit maturity progressed (Table 1). At stage six, fruits of mesta were found to have a lower value of L*, C and h° values indicating that its rind possessed a deeper shade of purple compared to mangosteen which is more vivid in colour. The changes in rind colour from green to purple colour of the mangosteen fruit pericarp is mainly due to anthocyanins (Palapol et al, 2009) with the decrease of chlorophyll as maturation progressed. At ambient temperature, fruits of mesta and mangosteen from index 3, 4 and 5 were able to developed rapidly into index 6 (dark purple) within 4 days in storage.

Table 1. Rind colour (L*, C* and h°) of mangosteen (Ma) and mesta (Me) fruits harvested at six maturity stages.

Maturity stages	L*		C*		h°	
	Ma	Me	Ma	Me	Ma	Me
Index 1	58.24 aA	58.83 aA	33.93 aA	35.26 aA	97.58 aA	103.21aA
Index 2	52.55 aA	52.93 aB	31.07 aA	29.90 aB	92.87 aA	88.64 aB
Index 3	45.73 aB	45.77 aC	23.93 aB	23.23 aC	53.50 aB	46.89 aC
Index 4	37.75 aC	39.33 aC	19.54 aC	19.94 aC	35.90 aC	36.69 aD
Index 5	32.70 aD	31.89 aD	11.71 aD	11.76 aD	17.58 aD	18.70 aE
Index 6	30.93 aD	29.76 aD	7.27 aE	4.13 bE	19.03 aD	11.55 bE

Values represent means (n=12 three replicates of four fruits per maturity stage)

Mean values within the same maturity stage followed by the same lower case lower case letter are not significantly different (P<0.05), using LSD test

Mean values within the same column followed by the same capital letter are not significantly different (P<0.05), using LSD test.

The pulp colour for mangosteen and mesta fruits showed no significant difference in L* values except for C* and h° values (Table 2) which decreased as fruit maturity progressed. The decrease of C* and h° values of both varieties might be due to white arils becoming more translucent as maturity advanced. There were no significant differences in L* and C* values in both mangosteen and mesta fruits except for h° values.

The pH readings of the mangosteen and mesta fruits depends on the concentration of free H⁺ ions (Wills et al., 2007). For both mangosteen and mesta, the pH increased as maturity progressed (Table 3). As pH decreased, the reading for titratable acidity would decrease. Usually during ripening, organic acid declined as they are respired and converted to sugar (Wills et al., 2007).

Table 2. Pulp colour (L*, C* and h°) of mangosteen (Ma) and mesta (Me) fruits harvested at six maturity stages.

Maturity stages	L		C		h°	
	Ma	Me	Ma	Me	Ma	Me
Index 1	66.12 aA	68.47 aA	12.99 aA	16.21 aA	97.38 aA	89.40 bA
Index 2	66.05 aA	70.38 aA	12.02 aA	13.89 aA	97.32 aA	91.04 bA
Index 3	68.27 aA	70.70 aA	12.73 aAB	14.29 aAB	95.92 aA	87.27 bAB
Index 4	73.43 aA	71.57 aA	10.62 aAB	11.77 aAB	90.93 aB	87.07 bAB
Index 5	73.52 aA	70.33 aA	10.90 aB	11.91 aB	90.86 aB	83.50 bB
Index 6	74.95 aA	70.11 aA	10.84 aAB	11.40 aAB	91.67 aB	82.30 bB

Values represent means (n=12 three replicates of four fruits per maturity stage)

Mean values within the same maturity stage followed by the same lower case lower case letter are not significantly different (P<0.05), using LSD test

Mean values within the same column followed by the same capital letter are not significantly different (P<0.05), using LSD test.

There was a continuous increase of SSC in mangosteen and mesta as fruit maturation advanced. Mesta fruits showed a gradual increase of SSC at maturity stage four at maturity stage six (Table 3). Mangosteen fruit tends to be sweeter than mesta fruit at every maturity stage and % SSC was achieved at maturity stage six (Table 3). Mangosteen fruits had significantly higher % SSC and SSC:TA ratio compared to mesta as fruit maturity progressed (Table 3). Fruit usually reached its optimum eating stage when its SSC had reached the highest reading. Similar findings were reported for mango (Jha et al., 2006) and mangosteen (Palapol et al., 2009).

Table 3. Chemical properties of mangosteen (Ma) and mesta (Me) fruits harvested at six maturity stages

Maturity stages	pH		TA (% citric acid)		%SSC		AA (mg 100mL ⁻¹)		SSC:TA	
	Ma	Me	Ma	Me	Ma	Me	Ma	Me	Ma	Me
Index 1	4.06 aB	3.98 aA	0.52 aBC	0.48 aE	15.6 aC	11.7 bC	1.52 aA	1.56 aA	30.00 Ab	24.38 bA
Index 2	4.15 aA	3.77 bB	0.50 bC	0.58 aCD	16.3 aC	14.2 bB	1.43 bA	1.57 aA	30.60 aB	24.48 bA
Index 3	3.76 aE	3.81 a,B	0.59 aA	0.54 aD	16.8 aB	14.9 bB	1.47 bA	1.57 aA	28.47 aB	27.59 bA
Index 4	3.8 aD	3.61 bCD	0.56 bAB	0.62 aBC	17.5 aB	16.6 bA	1.36 bB	1.56 aA	31.25 aB	26.77 bA
Index 5	3.88 aC	3.73 bBC	0.56 bAB	0.67 aAB	17.5 aB	16.8 bA	1.37 bB	1.52 aA	31.25 aB	25.07b ,A
Index 6	3.89 aC	3.55 bD	0.47 bC	0.69 aA	19.5 aA	17.0 bA	1.25 bB	1.46aB	41.49 aA	24.64 bA

Values represent means (n=12 three replicates of four fruits per maturity stage)

Mean values within the same maturity stage followed by the same lower case lower case letter are not significantly different (P<0.05), using LSD test

Mean values within the same column followed by the same capital letter are not significantly different (P<0.05), using LSD test.

There is a gradual decrease of AA content for mangosteen fruits as maturation advanced (Table 3). AA content for mesta fruits only decreased at when fruit matures to stage six. The decrease in ascorbic acid coincided with the initiation of ripening, as indicated by color change, and with an increase in the activity of ascorbate oxidase as found in tomato and bell pepper fruits (Yahia et al., 2001).

There were no significant different in antioxidant activity in the arils of both mangosteen and mesta (Table 4). All fruits exhibited high DPPH radical scavenging activity regardless of maturity indices. On the other hand, total phenolics content were found to be significantly higher for mesta than mangosteen fruits at all stages (Table 4).

Table 4. Antioxidant activity (DPPH) and total phenolic content (TPC) of mangosteen (Ma) and mesta (Me) fruits harvested at six maturity stages

Maturity stages	%inhibition		TPC	
	Ma	Me	Ma	Me
Index 1	88.33 aA	87.29 aA	1.24 bA	1.52 aA
Index 2	88.17 aA	87.15 aA	0.77 bB	0.81 aBC
Index 3	89.28 aA	87.94 aA	0.64 bC	0.85 aB
Index 4	88.49 aA	88.80 aA	0.60 bC	0.86 aB
Index 5	88.72 aA	88.33 aA	0.66 bC	0.81 aBC
Index 6	88.33 aA	88.88 aA	0.62 bC	0.76 aC

Values represent means (n=12 three replicates of four fruits per maturity stage)

Mean values within the same maturity stage followed by the same lower case lower case letter are not significantly different ($P<0.05$), using LSD test

Mean values within the same column followed by the same capital letter are not significantly different ($P<0.05$), using LSD test.

Conclusions

In this study, results showed that mesta had similar characteristics like mangosteen such as colour changes, pH, TA and antioxidant activity. Mesta tended to be less sweet but had higher AA content and total phenolic content than mangosteen. Further studies still need to be carried out to fully understand the postharvest qualities of mesta fruits.

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Ripening Stages and Fruit Parts Affect MD-2 Pineapple Eating Quality

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Introduction

One of the most popular pineapple varieties is 'MD-2'. MD-2 pineapple fruit has glossy and green-golden shell colour with a uniform and attractive cylindrical shape. The fruit is high in vitamin C while the flesh is more yellow, less acidic, sweeter, more aromatic and slightly firmer than other varieties. As a result, MD-2 pineapple fruit is a preferable variety among consumers as compared to other varieties (Syahrin, 2011).

Malaysia is one of the important pineapple fruit producing and exporting countries. However, the production of MD-2 pineapple fruit only started in 2009. The ripening process of pineapple fruit is started from its slip end and then progressed upwards to its crown. It is experienced that slip end part of pineapple fruit is sweeter than its crown end. However, there was no scientific evidence to prove this. Therefore, this study was carried out with the aim to determine firmness and soluble solids concentration of three different parts of MD-2 pineapple fruit harvested at five ripening stages.

Materials and Methods

MD-2 pineapple (*Ananas comosus* L.) fruits were collected from JTP Trading Sdn. Bhd., Johor, Malaysia, at five different ripening stages. The fruit was transported to the laboratory within 5 h. Fruit of stage 1 (mature green), stage 2 (25% yellow), stage 3 (50% yellow), stage 4 (75% yellow) and stage 5 (100% yellow) were used. There were five fruits used in each ripening stage.

The fruits were divided into three parts, i.e. crown end (near to crown), equatorial (middle) and slip end (near to fruit peduncle). Each part of the fruit was analyzed for flesh firmness and soluble solids concentration (SSC). Flesh firmness was measured as penetration force using a texture analyzer, Instron Universal Testing Machine (Model 5543, Instron Co., USA), with Merlin Software version M12-13664-EN. The penetration force was expressed in Newtons (N). The percentage of SSC in juice extracted from flesh samples was determined using a digital refractometer PAL-1 (Atago Co., Ltd., Tokyo, Japan).

The experiment was a randomized complete block design with three replicates. Data was analyzed using ANOVA while least significant difference (LSD) was used to separate the means when F-values showing significance at 5%.

Results and Discussion

There was significant interaction between ripening stage (RS) x fruit part (FP) in fruit firmness but this did not occur in SSC of MD-2 pineapple fruit (Table 1). There was no significant difference in fruit firmness among ripening stages of pineapple fruit when determined using slip end and equatorial parts of fruit (Figure 1). However, firmness of fruit crown end at ripening stage 1 showed highest firmness as compared to other ripening stages. As fruit ripened from ripening stage 1 to 5, the firmness decreased gradually with ripening stage 5 has the lowest firmness.

Fruit ripening affected SSC of MD-2 pineapple fruit where SSC increased as ripening progressed to stage 4, then SSC decreased (Table 1). For fruit part, the highest fruit part was found at slip end of fruit while lowest SSC was found at crown end.

Table 1. Main and interaction effects of ripening stage and fruit part on firmness and soluble solids concentration of MD-2 pineapple fruit.

Factor	Firmness (N)	Soluble solids concentration (%SSC)
Ripening stage (RS)		
1	8.53 a ^z	14.59 c
2	6.98 b	16.51 b
3	6.54 bc	17.18 ab
4	5.59 cd	18.01 a
5	5.30 d	16.41 b
Fruit part (FP)		
Crown end	9.00 a	14.36 c
Equatorial	5.03 b	16.99 b
Slip end	5.73 b	18.26 a
Interaction		
RS x FP	**	NS

^zMeans followed by the same letter in the same column are not significantly different by LSD at $P \leq 0.05$. ns, non-significant difference at $P > 0.05$, Significant difference at $**P \leq 0.01$.

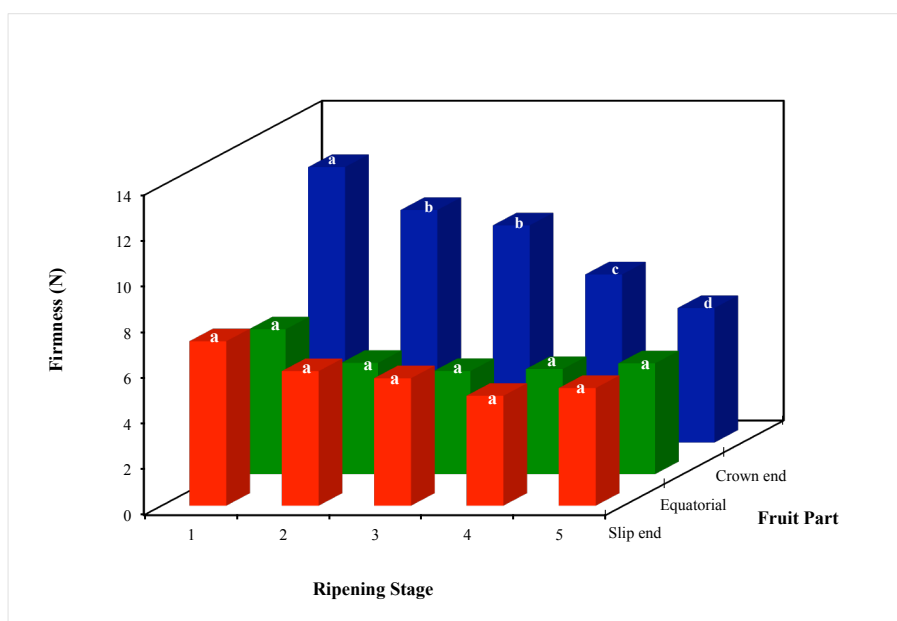


Figure 1. Effects of ripening stage x fruit part on fruit firmness of MD-2 pineapple fruit. Mean separations pertaining to each fruit part followed by the different letter are significantly different by LSD at $P \leq 0.05$.

Flesh firmness and SSC are important quality characteristics in determining organoleptic quality of a fruit. Firmness affects fruit texture and it is affected by its cell wall chemical composition. The main cell wall component of pineapple fruit is hemicellulose (41.8%), cellulose (33.6%) and pectin (21.2%) (Vidal-Valverde et al., 1982). SSC is perceived as sweetness and from this study MD-2 pineapple fruit has higher SSC than other varieties. The SSC of 'Comte de Paris' and 'Smooth Cayenne' variety

of pineapple fruit was 12.20°Brix (Hong et al., 2013) and 12.53°Brix (Joomwong, 2006), respectively. This indicated MD-2 pineapple fruit has rather high SSC than other varieties.

Conclusions

The present finding indicated that the firmness of MD-2 pineapple fruit at crown end is affected by interaction between ripening stage and fruit part. While SSC of MD-2 pineapple fruit is affected by ripening stage and its part. For those prefer high sweetness with reasonable texture of fruit, flesh of slip end will be the choice.

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Effects of Arbuscular Mycorrhiza and Rice Husk Biochar on the Growth Performance and Postharvest Quality of Sabah Snake Grass (*Clinacanthus nutans* Lindau) Grown on BRIS Soil

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Introduction

The herbal industry is a new source of wealth and has the potential to become a significant industry in Malaysia. This industry is estimated to grow at the rate of 15 percent per annum, with the market value rising from 7 billion ringgit in 2010 to some 29 billion ringgit in 2020 (ECER, 2011). Under the 9th Malaysia Plan (RMK9), the government has focused on developing the herbal products such as tongkat ali, pegaga, kacip fatimah, ginger, hempedu bumi, misai kucing, citronella, mengkudu, and dukung anak as a new target to increase the economic growth. There also other local herbs that are not listed in the group such as *Clinacanthus nutans* Lindau which is highly potential in preventing various chronic diseases such as cancers, AIDS and possesses analgesic, anti-inflammatory activities and anti-viral activities (P'ng et al., 2012). The production of Malaysian local herbs has increased tremendously year by year from 2008 until 2010. However, the planting density of these local herbs decreased year by year as frequent harvesting has resulted into reduction and even extinction of these herbs. Besides, lack of postharvest management and technology in handling these local herbs also contributes to significant losses in herbal industry. Therefore, there is an urgent need to revitalize these local herbs mainly belalai gajah.

In Terengganu, belalai gajah is not yet exploited. To encourage the farmers in Terengganu to exploit this herb as new source of wealth, the application of Arbuscular Mycorrhiza (AM) fungi and rice husk biochar (RHB) could be the best approaches to be implemented. To the best of my knowledge, the information on pre- and postharvest performance of this local herb planted on Beach Ridges Interspersed with Swales BRIS soil is scarce. Besides, the application of AM could also contributes to the short planting period, higher yield and quality of these local herbs grown mainly on BRIS soil. In addition, rice husk can significantly improve soil properties by decreasing soil bulk density, adding organic carbon, enhancing soil pH, increasing available nutrients and removing heavy metals from the system, ultimately increasing crop yields has well documented. In line to the above, the objective of this study is to investigate the effect of AM and RHB on pre- and postharvest performances of belalai gajah, grown on BRIS soil.

Materials and Methods

The experiment was conducted in a greenhouse at the School of Food Science and Technology Universiti Malaysia Terengganu. Belalai gajah was obtained from the MARDI Headquarters, Serdang. Meanwhile, the BRIS soil was obtained from Department of Commodity Centre, Setiu, and Arbuscular Mycorrhiza (AM) was a gift sample by Malaysian Agri Hitech Sdn. Bhd., Selangor while rice husk biochar (RHB) was obtained from Tumpat, Kelantan. All postharvest parameter assessments were assessed at the Postharvest Technology Laboratory, Universiti Malaysia Terengganu (UMT).

Sixty two-node stem cuttings (10-15 cm) were propagated in seedling tray containing peat moss. All cuttings were dipped in auxin to promote rooting. The mature stem belalai gajah, age 4-5 weeks were transplanted into polybag (15 cm x 15 cm) containing different treatments as described below. Each polybag contain 2 kg of growth media either alone or combinations of AM and RHB. The experiment was arranged in a randomized complete block design (RCBD) with two factors which are the different amount of AM and with or without RHB. The treatments i.e. (i) control (without AM and without RHB), (ii) 50g AM, (iii) 100g AM, (iv) 150g AM, (v) 100g RHB, (vi) 50g AM + 100g RHB, (vii) 100g AM + 100g RHB and (viii) 150g AM + 100g RHB. The treatments were replicated three times and two plants were represented as single experimental unit. Parameter evaluations were stem diameter, spore numbers, root infection and postharvest attributes (fresh weight and mineral nutrients of individual organs).

The data collected were subjected to two-way ANOVA using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

There was no significant interaction between the amount of AM and RHB for all parameters evaluated on belalai gajah except spore numbers. On day 77, the inoculation of 150g AM without RHB resulted in the highest number of AM spore (394 spores) while the lowest in control and RHB only (0 spore) (Figures 1 and 2). This was in agreement with Nur Amirah et al. (2013) who reported that misai kucing plants inoculated with AM had higher number of spores and percentage of root infection as compared to non inoculated plants due to the availability of vesicle and hyphae. As shown in Figure 3, the vesicle and hyphae of AM in belalai gajah Gajah root which reflected to the increased of AM spore numbers.

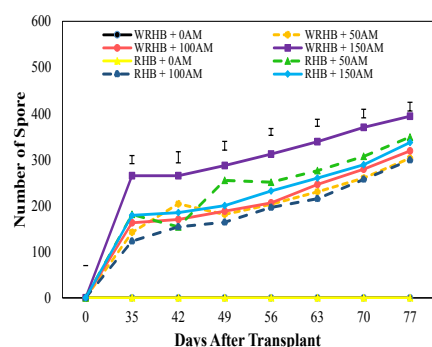


Figure 1. Effects of spore count of belalai gajah with the combination of AM and RHB. Vertical bars represent $LSD_{0.05}$. RHB=rice husk biochar, WRHB=without rice husk biochar.

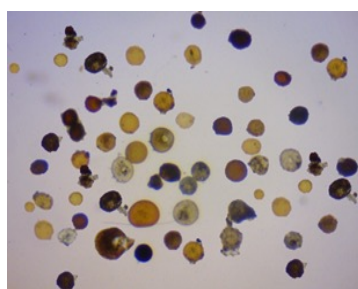


Figure 2. Spore of arbuscular mycorrhiza fungi

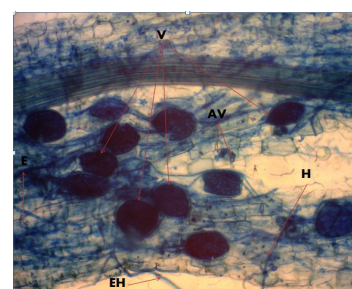


Figure 3. Arbuscular mycorrhizal fungi infection in root of belalai gajah showing (H) hyphae and (V) vesicle

However, for root infection, no interaction of two factors were recorded. However, irrespective of RHB, the percentage of root infection in belalai gajah increased with the increased of AM inoculation on day 77 only which was 13.5 % (Figure 4). According to Ishi and Kadoya (1994), they observed that the increase of root infection in citrus closely related to the number of AM spores. Meanwhile, regardless of AM inoculation, there was no effect of RHB on the root infection (Figure 5). Although, no significant effect was observed on application RHB but tend to have higher percentage of root infection. Azizah (1999) also states that a higher percentage of organic matter present in the AM treated BRIS soil would produce a higher number of mycorrhizal fungal spores that could eventually increase root colonization. Similar findings have also been reported by Nur Shuhadah et al. (2012).

For stem diameter, there was no significant interaction between AM and RHB. However, the stem diameter of belalai gajah Gajah only significant on day 77 with different amount of AM (Figure 6). A 150g AM inoculated plant tend to have biggest stem diameter (4.35mm) compared without AM inoculated (3.80mm). On day 77, bigger stem diameter was observed which may be attributed to the higher of spores number and root colonization in belalai gajah Gajah root. Similarly, Nur Amirah et al. (2013), also reported that stem diameter of misai kucing was significantly affected with AM inoculation. In addition, Wan Zaliha et al. (2015) found that large numbers of fungal spores indicate better performance of AM symbiosis with the roselle plant through AM interaction with the root.

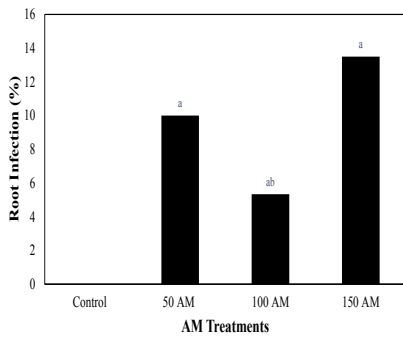


Figure 4. Effects of different amounts AM on percentage root infection of belalai gajah on day 77 after transplanting. Means with different letters are significantly different at the 5% level according to LSD test

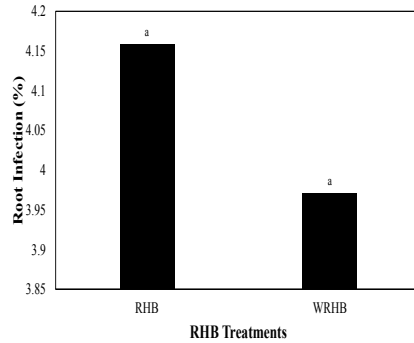


Figure 5. Effects of RHB on percentage root infection of belalai gajah on day 77 after transplanting. Means with different letters are significantly different at the 5% level according to LSD test

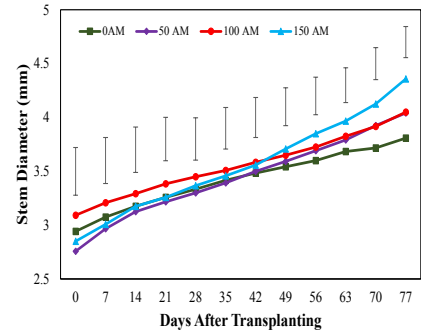


Figure 6. Effects of different amounts AM on stem diameter of belalai gajah on day 77 after transplanting. Vertical bars represent $LSD_{0.05}$.

On the other hand, the RHB factor did not show any significant different on stem diameter among treatments but exhibited an increasing trend and tend to greater stem diameter as compared the control (Figure 7). Cumulative fresh and dry weight of belalai gajah Gajah includes the weight of individual organs such as leaf, stem and root. The interaction effect of cumulative fresh and dry weight between the amounts of AM and RHB on belalai gajah Gajah were not significant ($P \geq 0.05$). Regardless of RHB application, there was a significant different among AM inoculation on cumulative fresh and dry weight of belalai gajah plants with 150 g AM inoculation had the highest value, 6.53g and 1.4 g respectively (Figure 8). As revealed by Gianinazzi et al. (1990) the present of AM fungi in plant root system is a key factor in order to improve growth performance of plant in agricultural practices. Meanwhile, application of RHB did not show any difference of fresh cumulative weight (Figure 9). Similarly, Ain Najwa et al. (2014) claimed that the application agricultural wastes such cocopeat (CP) and oil palm fruit bunch (OPFB) resulted in a small stem diameter, low number of fruits and fresh weight of cherry tomato.

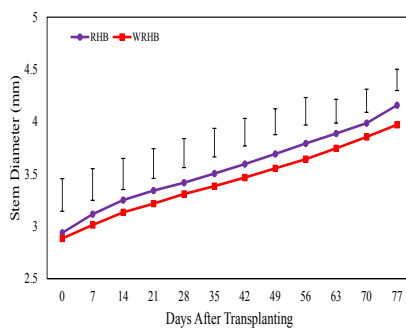


Figure 7. Effects of different RHB on stem diameter of belalai gajah on day 77 after transplanting. Vertical bars represent $LSD_{0.05}$.

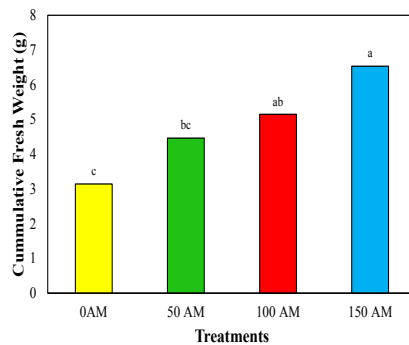


Figure 8. Effects of different amounts AM on cumulative fresh weight of belalai gajah. Means with different letters are significantly different at the 5% level according to LSD test

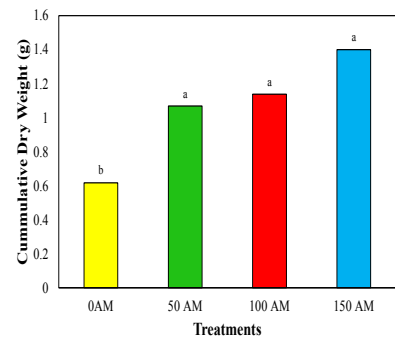


Figure 9. Effects of different amounts AM on cumulative dry weight of belalai gajah. Means with different letters are significantly different at the 5% level according to LSD test

Two factors, different amounts of AM and RHB resulted in no significant interaction ($P \geq 0.05$) on chlorophyll a, chlorophyll b, carotenoid, total chlorophyll, macro and micro nutrients (leaf, stem and root) of belalai gajah. Moreover, chlorophyll a, chlorophyll b, carotenoid and total chlorophyll also not affected either with AM inoculation or RHB application (data not included). In contrast, chlorophyll a, chlorophyll b, carotenoid and total chlorophylls had significant changes in cotton (Arya and Buch, 2013) and cowpea (Arumugan et al., 2010) with AM inoculation. This increased maybe due to an increase stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Sampanth and Ganesh, 2003). The inconsistent results could be due to different effects of AM depending on the type of crop, the soil type and its fertility, environmental conditions, and the water irrigation system applied as claimed by Aulia et al. (2009). Meanwhile, for mineral nutrients, AM fungi inoculation did not affect phosphorus (P) content, probably due to low mycorrhizal root colonisation (data not included). Nur Amirah et al., (2013) claimed that the application of AM inoculation increased the branching of misai kucing roots and this help to improve nutrients absorption which later being translocate to the above ground parts. Moreover, Jamaluddin et al. (2001) stated that that mycorrhizal infection increase nutrient uptake in the absorbing surface area of roots.

Conclusions

As a conclusion, higher inoculation of AM at 150g was pronounced in enhancing growth performance of belalai gajah as well as its colonization. While RHB was effective only in increasing the content of mineral nutrients of belalai gajah

Acknowledgements

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Effects of Exogenous Spray of Prohexadione-Calcium on Pre- and Postharvest Performance of Roselle (*Hibiscus sabdariffa* L.)

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Introduction

Roselle (*Hibiscus sabdariffa* L.) is relatively a new cash crop in Malaysia which belongs to the Malvaceae family. It is an annual or herbaceous shrub widely grown in tropical and subtropical region mainly for its stem fibres, edible calyces (fruits), leaves and seeds. Roselle fruits were generally preferred due to their bioactive compounds, anthocyanin concentration and vitamins as reported by Mahadevan et al. (2008). The red colour of roselle is determined by the type and concentration of anthocyanins which are water soluble. The individual anthocyanins found in roselle fruit such as delphinidin-3-sambubioside, cyanidin-3-sambubioside, cyanidin-3-glucoside and delphinidin-3-glucoside which are the non-methylated type (Castaneda-Ovando et al., 2009). Apart from contributing to the fruit colour, anthocyanins and other phenolic compounds have been reported to reduce chronic disease such as various cancers, cardiovascular diseases, asthma and type II diabetes (Boyer and Liu, 2004) by acting as anti-oxidative, anti-mutagenic, anti-microbial and anti-carcinogenic. However, poor management of vigorous growth of roselle plant resulted in reduction in light penetration, reduce tree productivity, poor fruit colour development, reduce fruit quality, profit and negatively influence pest control. Thus, the application of prohexadione-calcium (ProCa), a new growth retardant could enhance the pre- and postharvest performances of roselle plant including its fruit colour and other quality attributes.

ProCa has been reported to improve red colouration in various fruit skin (Mata et al., 2006; Medjdoub et al., 2005) by reducing shoot growth and allowing greater light penetration into tree canopies. In addition, ProCa has a short-lived, and its application always been made in early season (Mata et al., 2006), thus it is not translocated into the growing fruit (Halbwirth et al., 2003). ProCa application has been reported to reduce vegetative growth of various fruit crops (Basak and Rademacher, 2000; Byers and Yoder, 1999; Evans et al., 1997; Miller and Tworowski, 2003). Currently, no information is available on the effects of different concentrations of ProCa sprays in improving colour and accumulation of anthocyanins in roselle fruit. Therefore, these observations prompted us to investigate the effects of different concentrations of ProCa on pre- and postharvest quality of roselle fruit.

Materials and Methods

A field experiment was conducted at the Department of Agriculture, Rhu Tapai, Kuala Terengganu. Seventy-six roselle plants were used in the experiment. Adjacent plots were separated by guard plants. Eighteen plants were used as guard plants. The experimental plants were planted at the distance of 0.6 m x 1.0 m on Beach Ridges interspersed with Swales (BRIS) soil. Various concentrations of ProCa spray were used namely control (without ProCa), 50 mg L⁻¹, 100 mg L⁻¹ and 150 mg L⁻¹. The experiment was performed according to a randomized complete block design with four replications. Six trees were treated as an experimental unit. ProCa (Regalis 10% a.i., BASF, Carl-Bosch-Straße, Lamburgerhof, Germany) was a gift sample from BASF (Carl-Bosch-Straße, Lamburgerhof, Germany). An aqueous solution containing different concentrations of ProCa and a non-ionic surfactant Tween®20 (0.125% v/v, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was sprayed

onto the fruit of whole tree till runoff. The spray treatments were applied at 3 to 5 cm shoot length (40 days after transplanting, DAT). A knapsack sprayer was used for spraying an aqueous solution. Unsprayed trees served as control. Shoot length was recorded on 20, 30, 40, 50, 60 and 70 DAT. Fifteen roselle fruits were randomly chosen from all parts of the tree canopy for fruit quality assessment. Fruits were harvested on 74 DAT. The parameters for evaluation were fresh weight, number of fruit, fruit colour and firmness, titratable acidity (TA), soluble solids concentration (SSC), and total anthocyanin concentration as described by Wan Zaliha (2009).

The experimental data collected was subjected to an analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

Various concentrations of ProCa spray significantly reduced shoot length of roselle plants grown on BRIS soil. As shown in Figure 1, the application of different concentrations of ProCa spray significantly ($P \leq 0.05$) inhibited shoot growth of roselle plants. The reduction of shoot length was noticeable with the application of ProCa only. A comparable level of inhibition of shoot growth after 30 days spray application recorded in shoot treated with all ProCa (50, 100 and 150 mg L⁻¹) treatments. After 20 days spray application, the plants with ProCa (100 mg L⁻¹) inhibited the shoot growth (37%) as compared to control (48%). The reduction of shoot length was more pronounced after an initial spray at higher concentration of ProCa which was 10% of control plants (Figure 1). Similarly, the reduction of shoot growth after the first spray has been reported (Byers and Yoder, 1999; Medjdoub et al., 2005; Rademacher and Kober, 2003). The gradual reduction in shoot length after two and more spray applications have also been reported (Miller, 2002). Similar observation on the relative increase in the reduction of shoot length recorded up to 33%. In the present study, single application of ProCa was not enough to prevent shoot growth of this cultivar. This may be due to the rapid degradation of ProCa in the leaf (Evans et al., 1997; Rademacher and Kober, 2003) thereby, the second application of ProCa (Medjdoub et al., 2005) or more were essential to retard the shoot regrowth (Rademacher and Kober, 2003). The efficacy of ProCa in reducing shoot length was dependant on the concentrations, times and number of sprays application. The reduction in shoot length with the spray application of ProCa may be ascribed to the reduction in the endogenous concentrations of the biologically active GA₁ and increase in the concentrations of inactive GA₂₀ via interference of the 3-β hydroxylation of GA₂₀ to GA₁ (Lo Giudice et al., 2004; Rademacher and Kober, 2003).

Meanwhile, ProCa spray at 100 and 150 mg·L⁻¹ resulted in significantly higher total anthocyanin concentration in roselle fruit as compared to control (Figure 2). The intensification of red skin colouration on fruit skin has also been reported after ProCa treatment in apple (Byers and Yoder, 1999), 'Seyval' grape berries (Lo Giudice et al., 2004) and 'Forelle' pear (Smit et al., 2005). The increase in fruit colour may be ascribed to the increased concentration of total anthocyanins due to the spray application of ProCa that reduced the shoot elongation. ProCa induced red skin colour (lower lightness) with more saturated (higher chroma) on the blush side of 'Fuji' and 'Cripps Pink' apple and also higher total anthocyanin concentration (Wan Zaliha, 2009; Medjdoub et al., 2005). Similarly, the higher concentration of total anthocyanins was found in 'Fuji' apple with the application of ProCa (Mata et al., 2006). This may be attributed to the reduced vegetative growth, and consequently improves light penetration into tree canopy (Basak, 2004). The increased light penetration into tree canopy has been reported to upregulate the activities of enzymes involved in the biosynthesis of anthocyanins such as phenylalanine ammonia-lyase (PAL) and UDP galactose:flavonoid 3-O-galactosyltransferase (UFGaT). In addition, light also has been reported to upregulate the activity of UFGaT in 'Fuji' apple (Ju et al., 1999) and PAL activity in 'Royal Gala' apple (Dong et al., 1995).

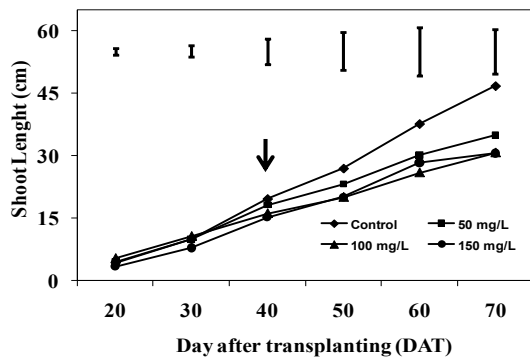


Figure 1. Effects of different concentrations of Prohexadione-calcium spray on shoot growth length of roselle plant. Vertical bars represent LSD. LSD ($P \leq 0.05$) on 20 DAT = 1.43, 30 DAT = 2.75, 40 DAT = 6.08, 50 DAT = 9.13, 60 DAT = 11.56, 70 DAT = 10.73. Thick arrow = spray application on 40 DAT.

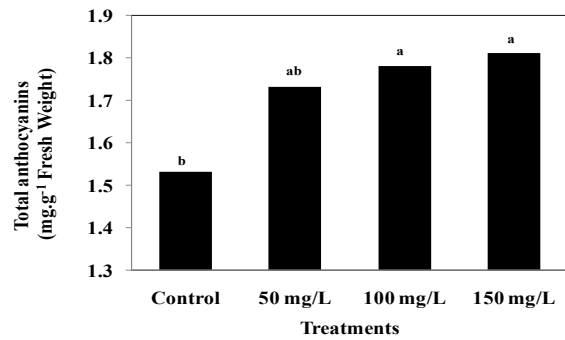


Figure 2. Effects of different concentrations of Prohexadione-calcium spray on total anthocyanins concentration in roselle calyx. Means followed by the same letter are not significantly different at $P \leq 0.05$.

For fruit colour development, the reduction in hue angle (h°), chromaticity value b^* , lightness (L^*) and higher chromaticity value a^* indicates redder fruit skin colour. In general, roselle plants sprayed with higher concentrations of ProCa had higher chromaticity value a^* , chroma and lower lightness on the fruit surface as compared to control (Table 1). The increase in fruit colour and its parameters may be ascribed to the increased concentration of total anthocyanins due to the spray application of ProCa that reduced the shoot elongation as discussed above. Meanwhile, fresh weight and number of roselle fruits were not significantly affected with the application of ProCa (Table 2). However, all plants treated with ProCa (50, 100 and 150 mg L^{-1}) tended to have higher fresh weight (344.0, 369.7 and 276.8 g, respectively) and number of fruits (34, 36 and 27, respectively) as compared to control. Similar findings were also reported on no apparent different of ProCa on fruit set or yield of fruit crops (Byers and Yoder, 1999; Miller, 2002).

Table 1. Roselle fruit colours indices as affected with different concentrations of Prohexadione-calcium spray.

Treatment	Chromaticity a^*	Chromaticity b^*	Lightness (L^*)	Hue angle (h°)	Chroma (C^*)
Control	9.69b	3.09a	20.54a	18.41a	10.22b
ProCa 50 mg L^{-1}	17.01a	2.94a	20.76a	17.01a	17.80a
ProCa 100 mg L^{-1}	19.19a	3.43a	20.70a	16.93a	20.15a
ProCa 150 mg L^{-1}	15.62ab	3.9a	18.02b	15.18a	16.75ab

Means followed by the same letter within column are not significantly different at $P \geq 0.05$.

Table 2. Fresh weight and number of fruits as affected with different concentrations of Prohexadione-calcium spray

Treatment	Fresh weight (g)	Number of fruit
Control	130.8a	16a
ProCa 50 mg L^{-1}	344.0a	34a
ProCa 100 mg L^{-1}	369.7a	36a
ProCa 150 mg L^{-1}	276.8a	27a

Means followed by the same letter within column are not significantly different at $P \geq 0.05$. Fresh weight and number of fruits were recorded only once on 74 DAT.

Conclusion

In conclusion, exogenous spray application of ProCa (100 and 150 mg·L⁻¹) effectively reduced shoot growth, increased anthocyanins accumulation and fruit colour without adversely affecting other quality parameters of roselle grown on BRIS soil.

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CHAPTER 6

INNOVATIVE PRACTICES

Effects of Chlorophyll Content and Stage of Maturity on Total Phenolic, Total Flavonoid and Antioxidant Activity of Miracle Tree Leaf of (*Moringa oleifera* Lam.)

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Introduction

Many studies revealed that *Moringa oleifera* is an excellent source of natural anti-oxidants that can be used to prevent the progression of various diseases (Iqbal and Bhangar, 2006). Some medicinal values of *Moringa oleifera* has been known, more than a decades, many countries in the world uses different part of Moringa plant in one way or the other. These include to cure certain diseases such as ascites, rheumatism, venomous bites and also act as a cardiac and circulatory stimulant¹ (Guenera, Vergas and Milagros, (1996). In Jamaicans, the sap has been utilised as blue dye, Malaysia and Puerto Rico-to get rid of intestinal worms, and Philippines as the remedy for lactating problems, glandular swelling and anaemia. Some of these antioxidant compounds are the substances that prevent cell damage and cancer caused by free radicals that may eventually cause damage to DNA and leads to the possible development of cancer (Pong, 2003). This oxidative damage was also found to cause many chronic human diseases, such as cancer, neurode-generative diseases, diabetes mellitus, arthritis, atherosclerosis and the ageing process (Pong, 2003; Brewer, 2011). The most useful antioxidants are those that can interfere the free radical chain reaction and avoid the damages that can be cause from their consequences where free radicals are extremely reactive that can easily react with DNA, lipids, proteins, and even carbohydrate to cause injury to the cell due to the deficiency of one electron which make them unstable compounds (Badarinath et al. 2010; Dudonne et al., (2009). It has also been reported that reported that under certain conditions, the reactive oxygen species (ROS) eg oxygen (O_2^-) or hydrogen peroxide (H_2O_2) that are produced as free radicals in the body are purified by the natural antioxidants present within the body, this may lead to a balance between the ROS formed and the antioxidants already exist (Kohen and Gati,2000). However, abundance of ROS and or lack of sufficient antioxidant that can neutralised the ROS, may lead to oxidative stress. Many studies also revealed that *Moringa oleifera* is an excellent source of natural anti-oxidants that can be used to prevent the progression of many diseases (Iqbal and Bhangar, 2006). Some of the vital phenolic compounds which are usually present in medicinal plant, performing antioxidant activities are flavonoids, alkaloids, phenols and tannins (Bako et al., 2010). However, it was also stated that antioxidants activity of Moringa leaves differ with the stage of maturity (Sreelatha and Padma, 2009). Hence the main aim of this research is to study the effect of chlorophyll content as well as different stages of maturity on antioxidant activity of *Moringa oleifera*.

Methodology

Chemicals

DPPH, DMSO, quercetin, tripyridyltriazine, Folin-Ciocalteau (F-C), Hexane and $Na_2 CO_3$ were used in this experiment, However the percentage of antioxidant activity (AA%) of each substance used were assessed through DPPH free radical Assay while the DPPH scavenging activities were measured, according to the method by Clarke et al.(2013) which was a standard techniques of measuring DPPH scavenging activity.

Plant material

Fresh leaves of *Moringa oleifera* Lam. were collected from Gongbadak district in Terrengganu, Malaysia.

Extract preparation

The leaves samples were washed thoroughly and dried at 40-43 °C. The dried samples were extracted with 100% methanol. The extracts were collected and filtered through filter paper (Whatman 1) and then concentrated on rotary evaporator (Buchi, Flavil, Switzerland) at 45 °C, followed by subsequent dried and kept at -20 °C till used for the assay. The mass ratio of sample and solvent was 2: 1 during extraction. The extracts were dissolved in DMSO or methanol to get the final concentration as per requirement based on assessment method by Luqman et al (2012).

Total phenolic compounds Assay

The total phenolic content of the extract was determined based on the method of Ainsworth and Gillespie (2007) but with some modifications. Folin-Ciocalteu reagent was used throughout the experiment, 250µL of extract diluted appropriately in methanol was put in a test tube and subsequent mixed with 1.25ml of F-C reagent diluted in distilled water 1:9, it is then incubated for 10 minute, 1ml of 7.5% Na₂CO₃ solution was then added, followed by incubation for 30minute in dark prior to measurement at 650nm in spectrophotometer. Galic acid solution was used as a standard.

Total flavonoid content

The total flavonoid content was determined using the modified methods of Sankhalkar (2014), aluminium chloride (AlCl₃) assay mixture consisting of plant extract (0.5ml), 0.3ml distilled water; 0.03ml of 5% NaNO₂ was incubated for 5 min at 25°C. After 5 minutes 0.03 ml of 10% AlCl₃ was added and further incubated to another 5 min. The reaction mixture was then treated with 0.2 ml of 1mM NaOH. Finally, the reaction mixture was diluted to 1ml with water and the absorbance was measured at 510 nm. Quercetin was used as standard.

DPPH Assay

Effects of *Moringa oleifera* extracts on DPPH was tested based on the method of Clarke (2013) but with some modification. For this DPPH radical scavenging assay, 96-well plate was used, whereby 60 µL of Moringa extract diluted in DMSO was mixed with 200 µL of DPPH in methanol (0.1Mm), to form a total volume of 300µL per well. The plate was kept in the dark for 30 min, after which the absorbance of the solution was measured with Multiskan Ascent plate-reader (Thermo Electron Corporation, Basingstoke, UK) at 540 nm. Blanks (DMSO) and standards (quercetin solutions in DMSO) were run concurrently. Extracts were first tested at a single concentration of 0.1mM, followed by subsequent serial dilution which resulted to a range of concentrations using the formula:

$$\text{DPPH scavenging effect (\%)} = [A_0 - A_1] / A_0 \times 100$$

Where, A₀ is the observance of the control reaction and A₁ is the observance of the sample.

Results and Discussion

Total phenolic content

The total phenolic content of *Moringa oleifera* leaf was expressed in terms of GAE of the extract. It was calculated using linear equation obtained from the calibration curve of standard gallic acid as follows

$$Y=0.0097X+0.1439 \quad R^2=0.9968$$

Where Y is the average absorbance of the sample, X represent amount of gallic acid in µg/ml.

Among the extract used, tender leaf and high chlorophyll leaf (HCIL) were found to have high phenolic content with an average value of 35.5052 ± 0.0073 mg GAE/g and 32.8284 ± 0.0031 mg GAE/g as compare to Low chlorophyll leaf (LCIL) with 30.83 ± 2.21 mg GAE/g (Table 1). Thus it was reported that the phenolic compounds in plant extract are more often linked with other molecules like chlorophyll, proteins, polysaccharides, terpenes and other inorganic compounds (Tatiya et al., 2011). Tender leaf was found to have high phenolic content than matured leaf, contrarily to the result obtained by Srelatha and Padma (2009) that revealed high phenolic content from mature leaf extract compared to tender leaf extract. The result of total phenolic content observed by Masum *et al.*, (2012) was higher than current experiment, particularly from ethyl acetate fraction of Moringa leaf having 107.209 mg/g GAE, whereas chloroform fraction and pet ether fraction were found to have lower than the current experiment with 7.79 and 7.209 mg/g GAE respectively. Hence this variation may be related to concentration and solvent differences.

Table 1: Total phenolic and total flavonoid contents of *Moringa oleifera* with regard to leaf maturity and chlorophyll contents

Samples	TPC (mg/g GAE)	TFC (mg/g QAE)
Tender leaf	35.51±1.07	50.69±1.28
High chlorophyll methanol (HCIL)/Matured leaf	32.83±1.19	98.67±2.10
Low chlorophyll leaf (LCIL)	30.83±2.21	32.74±1.036

Total flavonoid content

Total flavonoid contents of the samples were calculated from the quarcetin acid standard curve obtained from the following equation

$$Y = 00.19X + 0.0446 \quad R^2 = 0.9771$$

Where Y is the average absorbance of the sample, X is the amount of gallic acid in µg/ml.

The result of the flavonoids content from different samples used varied significantly with maturity and chlorophyll content ($P < 0.05$) each, where Methanolic high chlorophyll leaf (HCIL) or matured leaf showed almost two times flavonoids content found in tender leaf, and three times than methanolic low chlorophyll leaf (LCIL) (table 1). The highest flavonoids was observed from (HCIL)/Matured leaf 98.67 ± 2.10 mg/g QAE. This result was found to be higher than that obtained by Saikia and Upadhyaya (2011) whom reported 37.0 mg/g QAE although Masum *et al.*, (2012) discovered higher flavonoid contents than current experiment, especially from ethyl acetate fraction of Moringa leaf with (359.53 mg/g QAE), while chloroform fraction and pet ether fraction was found to be lower than the current result having 3.721 and 47.326 mg/g QAE respectively. These differences may be attributed to the differences of solvent, growing under environment and genetic variability. Flavonoids is among the most important secondary compounds, mainly founds in medicinal plants which revealed antioxidant property.

DPPH

The result from this experiment shows that (LCIL) has higher percentage inhibition of DPPH radicals than (HCIL), with 75.73±1.10% and 58.62±1.13% respectively. However in terms of leaf maturity, tender leaf was found to have high percentage inhibition with 74.07±0.46%. The potential of the samples were compared by the amount of antioxidant needed to scavenge 50% of DPPH free radicals (IC₅₀) at 517nm (table 2). Pattanayak *et al.*, (2013) relate a higher DPPH radical-scavenging activity to be attributed with lower IC₅₀ value. Among the extract used tender leaf revealed low inhibitory concentration IC₅₀ 305 µg/ml followed by (HCIL) with 320µg/ml. lower than (LCIL) with 380 µg/ml (table 3). The result contrasts with the findings of (Sreelatha and Padma 2009) whom reported low IC₅₀ from matured leaf. However Oloyede *et al.*, (2013) reported the increase antioxidant activities from matured leaf of *Amaranthus cruentus*, whereas antioxidant activity of *Celosia argentea* also increased at the 5th week and declined on reaching the 6th week of maturity. Nevertheless, Chapman (2002) revealed no differences in secondary compounds content between young and mature leaves from wild trees. Leaves changed to yellow due to inadequate chlorophyll which can be-caused by insufficient soil nutrient, pathogens, or soil pH. While iron which is an essential component of photosynthesis is also responsible for green colour of the leaf. Hence, any insufficient iron may cause chlorosis (CMG 2013). The IC₅₀ of the whole sample found in this research was found to be lower than the result obtained by Saikia and Upadhyaya (2011) whom reported 429.31 µg/ml (Table 3).

Table 2: DPPH radicals scavenging activity of *Moringa oleifera* with respect to stage of maturity and chlorophyll contents of the leaf

Samples	Mean ⁿ (%) inhibition of DPPH radicals ± SD	IC50 (µg/ml)
Standard Quarcetin	87.49±0.18	13
High chlorophyll leaf (HCIL)/matured leaf	58.62±1.13	320
Low chlorophyll Leaf (LCIL)	75.73±1.10	380
Tender leaf methanol	74.07±0.46	305



A B C
Figure 3(A) - Low chlorophyll leaf (LCIL), (B) - Tender leaf and (C) - High chlorophyll leaf (HCIL)/matured leaf.

Conclusions

Antioxidant activity of medicinal plant largely depends on the availability of many major compounds such as phenolic, flavonoids and tannins . It is concluded that all the extract obtained are good source of natural antioxidant, which can be used as a substitute to produce synthetic antioxidant.

Acknowledgements

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Synthesis of Hydroxyapatite (HA) for the Development of Slow Release Fertilizer

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Introduction

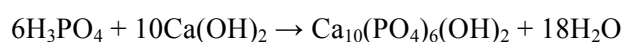
In order to sustain crop yields, fertilizers have to be applied to soils to provide plants with essential nutrients. Conservative estimates show that 30–50% of crop yields are attributed to natural or synthetic commercial fertilizers. As modern agriculture relies increasingly on non-renewable fertilizer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

In this study, Hydroxyapatite (HA) nanoparticles were synthesized by wet chemical precipitation method (Kottegoda N. 2011). This technique was chosen because large amount of HA can be produced in absence of organic solvent at a reasonable cost. HA nano-particles have ready surface modification with different organic and inorganic materials due to their nano size and high surface area. Various studies with different method to synthesized HA by wet chemical precipitation method. This method used because can produce large amount of HA with lower cost without the presence of other materials. Temperature and pH of the solution, reagent addition velocity, stir speed and stirring time influenced the morphology and size of HA formed.

Materials and Methods

Synthesis of Hydroxyapatite (HA) nano particles

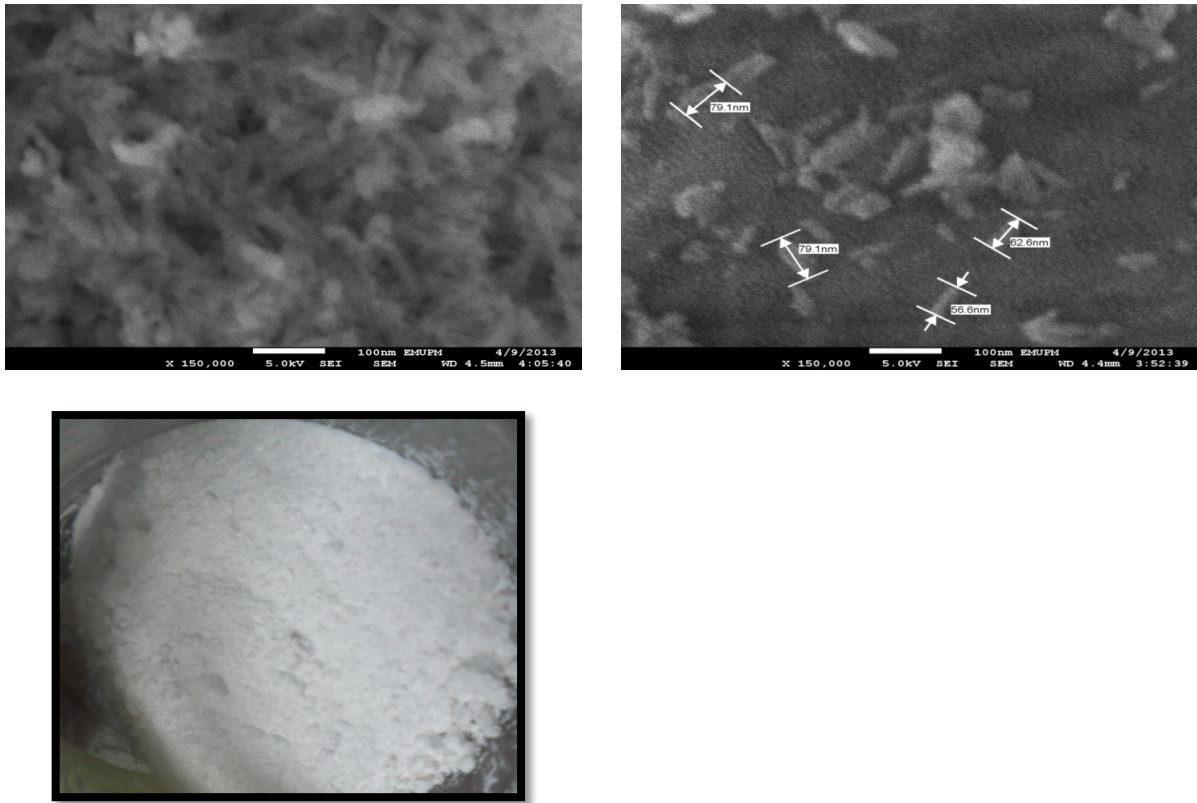
HA nanoparticles were synthesized using aqueous solutions (wet chemical precipitation method) of calcium hydroxide (Ca(OH)₂) and orthophosphoric acid (H₃PO₄), 85%. 0.6M H₃PO₄ (250ml) was added drop wise into suspension of (Ca(OH)₂) while stirring vigorously. The suspension was then stirred for 24 hours. The reaction was according to the following equation.



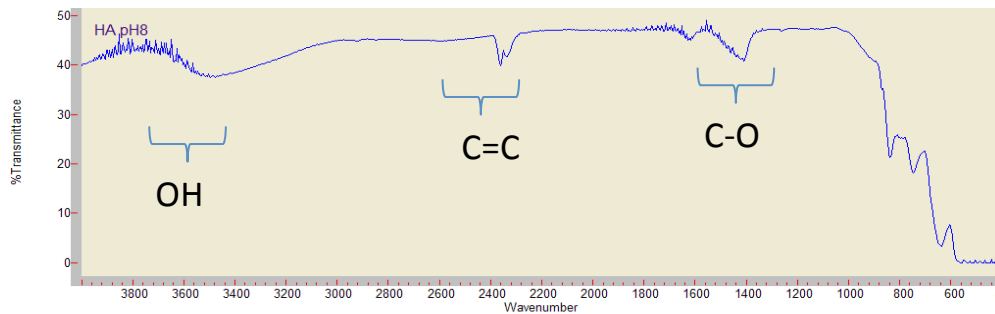
HA nanoparticles were allowed to settle and decanted the supernatant. The resulting HA nanoparticles were washed thrice with distilled water. The solid thus obtained was dried at 100°C for 2 hours. The product was then characterized using SEM and FTIR.

Results and Discussion

Picture 1 shows SEM images of HA nano-particles. It shows aggregate and rod-like morphology with diameter less than 100nm. Different properties of the final products such as particle size, shape and its purity would be controlled by as sonication output power, temperature, the solvent, the chemical species and their concentrations in the reaction mixture. The size, shape and pH obtained at the end of the synthesis of HA are sensitive to the orthophosphoric acid addition rate. The size of HA will uniform and smaller if the addition of acid is added drop-wise. The size of HA also influenced by stirring time. By stirring the solution for 24 hours will obtain particles sizes of HA less than 100nm⁷. Dry HA nano-particles exhibit white powder appearances.



Picture 1: (A) SEM image of synthesized HA nanoparticles at pH 5 (x150) (B) SEM image of HA nanoparticles at pH 8 (x150) (C) Dry HA nanoparticles



Picture 2: FTIR spectrum of the modified HA

FTIR spectrum of the modified HA, it is confirm hydroxyl group exist in the newly developed compound. Therefore, binding site is available for the conjugation of urea with HA. As a result, we have modified a the developed HA with urea for development of slow release fertilizer.

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CHAPTER 7

PLANT PRODUCTION

Improving Corn Forage Production and Quality through Legume Intercropping: A Comparison of Bambara nut and Groundnut

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Introduction

Cereal and legumes constitute a major source of energy and protein for livestock (Yilmaz *et al.*, 2015). Corn is one of the most important cereal crops that could substantially be used to improve livestock feeding. Corn-legume intercropping is also known to be one of the sustainable practices in an agricultural production system that could increase forage quality and quantity, and decrease requirements for protein supplements in livestock feeds, as well as improve the utilisation of agricultural inputs. Corn has high nitrogen requirements but is low in crude protein content, hence animals have to be supplemented with other protein source in their feed. In order to reduce nitrogen fertilizer requirement as well as to increase protein in the feed, planting corn with legumes may be a solution. Goran and Guessan (1999) reported an increased grain yield of corn as a result of nitrogen contribution of the companion groundnut (*Arachis hypogea* L) in intercrop. Bambara nut (*Vigna subterranean* L. Verdc) was also reported to increase the grain yield of millet and sorghum in intercrop (Karikari *et al.*, 1999; 2002). The benefit of groundnut and bambara nut in intercrop with corn for forage production has not been studied in the humid tropical climate. Therefore, the study aims to evaluate the contribution of bambara nut and groundnut in intercrop with corn.

Materials and Methods

A field experiment was set up using the randomised complete block design (RCBD) involving six treatments [sole corn with nitrogen (SCN), sole corn without nitrogen (SC), sole groundnut (SG), sole bambara nut (SB), corn/bambara nut intercrop (CB), and corn/groundnut intercrop (CG)] and replicated three times at Universiti Putra Malaysia, Serdang (latitude 3° 2'N; longitude 101° 42'E; elevation 31 m ASL) to determine and compare the contributions of two different grain legumes: bambara nut and groundnut towards the yield and quality of combined forage with corn. Nitrogen fertiliser was applied as urea (200 kg/ha) to SCN plots in two split doses (Amjad *et al.*, 2014) while phosphorus (65 kg/ha of TSP) and potassium (200 kg/ha of MOP) fertilisers were applied to all treatments during land preparation (Dahmadeh, 2013). Weeds were controlled by covering the soil with plastic mulch (Bonanno, 1996; George, 2002). Seeding was done on ridges at alternate rows between corn and legume for the intercropped plots. Crop growth rate was measured on a dry weight basis in accordance with (Kumar and Kumar, 2008). Leaf chlorophyll content was measured in the field using the SPAD portable chlorophyll meter (SPAD 502- Minolta Inc.). Leaf area index (LAI) was measured with a plant canopy analyser (LI-COR 2200). Photosynthesis was measured at 1.15 pm on a cloud clear day at 6 weeks after sowing using LI-COR (LI-6400 portable photosynthesis system). Forage nutritive qualities: neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and crude protein CP) were determined by Van Soest chemical method of fibre analysis (Van Soest and Wines, 1991), and digestibility was determined by in-vitro digestion (gas production) method as described by Festus *et al.*, (2010). Data obtained were subjected to analysis of variance (ANOVA) using SAS version 9.4. Means were separated using LSD at $P \leq 0.05$ (Gomez and Gomez, 1984).

Results and Discussion

Figure 1 explains the total dry matter yield (DMY) of forage. Intercropping increased the total dry matter yield (corn+legume) in the corn-groundnut intercrop by 64.3% and 69.5% as compared to sole groundnut and sole corn with no fertilization respectively. Intercropping also increased the total dry matter yield in the corn-bambara nut intercrop by 75.4% and 60.6% as compared to sole bambara nut and sole corn without fertilizers respectively (Figure 1). Shiva (1984) noted that legumes differ in their N-fixing capacity and N-requirements for their growth, and therefore vary in their N-contribution to the growth and development of companion cereal crop in intercrop. The increase in the total dry matter yield could be due the contribution of corn as well as that of the nitrogen fixed by legumes which benefitted the nitrogen need of the companion corn. The application of nitrogen to SCN increased the dry matter yield of corn by 17.5% and 25.4% when compared to the intercrops of groundnut and bambara nut respectively (Figure 1).

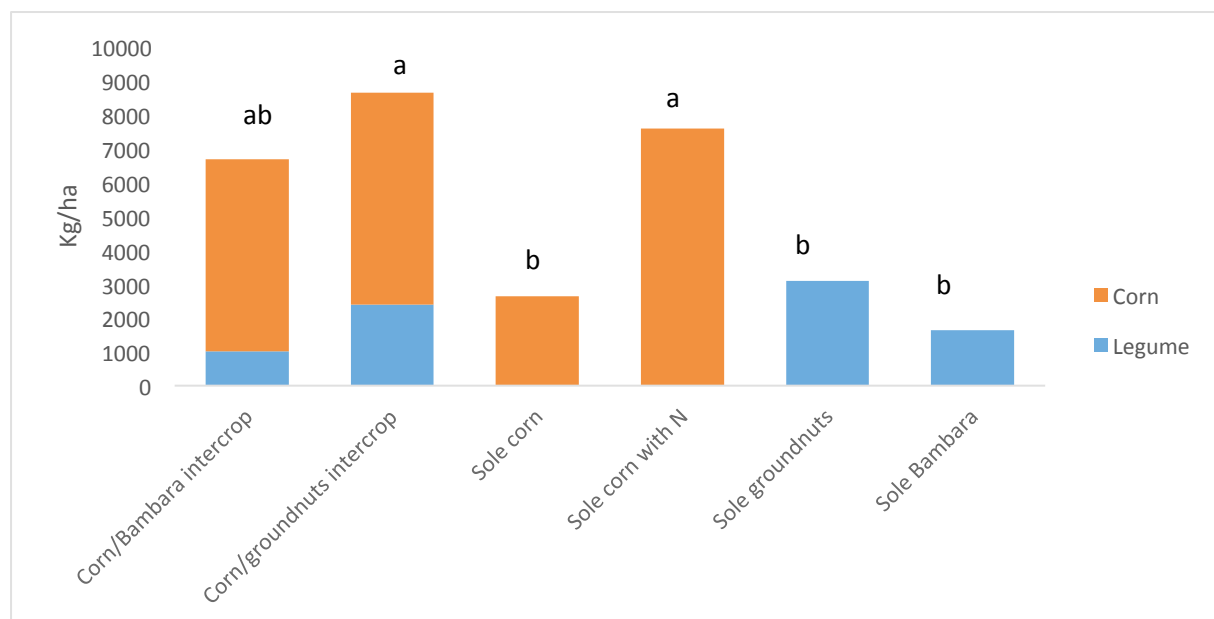


Figure 1: Total Dry matter yield of corn and legume during intercropping and mono-cropping

Table 1 shows the physiological characteristics of intercropping legumes and corn. Crop growth rate (CGR) was significantly higher ($P < 0.05$) in SG as compared to SB and CB. The difference could be due to crop growth habit and stature of the groundnut. The CGR for corn differs significantly ($P < 0.05$) among treatments with SCN having the highest CGR of $178.9 \text{ g m}^{-2} \text{ d}^{-1}$ and the SC with the least of $123.4 \text{ g m}^{-2} \text{ d}^{-1}$. The higher CGR could also be attributed to the availability of plant nutrients and its resulting effect on the growth of the plant. Corn responds rapidly to nitrogen availability as nitrogen from fertiliser and fixed nitrogen from legumes thus contributing to its higher CGR in either case. Leaf chlorophyll differs significantly between sole grown legumes and legumes grown in association with corn. The SPAD readings for corn reveals a significantly higher SPAD in SCN as compared to SC and CB. Corn grown without nitrogen fertilization performed poorly due to poor nourishment from the soil. This result agrees with the findings of Ahmad *et al.*, (2012), that leaf chlorophyll content is positively correlated with soil fertility. Photosynthesis for the legumes was higher in SG, although it did not significantly differ ($P > 0.05$) from the other treatments except with CB ($P < 0.05$) that recorded lowest rate of $10.16 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Shading from the tall corn plant may have interfered with the light interception of lower canopy legume thus reducing its photosynthesis rate. Leaf area index (LAI) was not significantly different among all the legume treatments ($P > 0.05$) but LAI for corn was significantly higher in SCN ($P < 0.05$) compared to SC and CB. The LAI

obtained for corn at 6 weeks after sowing in this study is in agreement with the result of Yao *et al.* (2008), who obtained a similar value at 7 weeks after sowing in forage corn varieties.

Table 1: Crop growth rate, leaf chlorophyll (C_L), and photosynthesis (Pn) of corn and legume for intercropping and mono-cropping

Treatment	Legume Crop growth rate ($g\ m^{-2}d^{-1}$)	C_L (SPAD)	LAI	Pn ($\mu mol\ m^{-2}s^{-1}$)	Corn				
					Crop growth rate ($g\ m^{-2}d^{-1}$)	C_L (SPAD)	LAI	Pn ($\mu mol\ m^{-2}s^{-1}$)	
Corn/ intercrop (CB)	Bambara	27.00c	32.53b	0.87	10.16b	137.48c	40.50b	1.94b	27.44
Corn/ intercrop (CG)	groundnuts	52.83ab	34.30b	1.16	18.10a	166.75b	45.43a	3.07a	21.69
Sole SG)	groundnuts	72.75a	44.03a	1.03	22.24a	-	-	-	-
Sole Bambara (SB)		46.75bc	42.87a	1.04	17.80a	-	-	-	-
Sole corn (SC)		-	-	-	-	123.43d	30.03c	1.39c	25.71
Sole corn with N (SCN)		-	-	-	-	178.91a	46.63a	3.24a	21.23
LSD _{0.05}		22.57	4.52	-	7.09	7.09	4.68	0.45	-
CV (%)		22.67	5.89	32.08	20.78	2.34	5.77	9.41	30.53
SE		5.24	1.209	0.260	2.007	2.027	1.829	0.129	4.080
P > F		0.014	0.0016	0.774	0.030	<.0001	0.0005	0.0002	0.687

N.B.: Means within columns with similar letters are not significantly different ($P>0.05$), LC= Leaf chlorophyll, LAI = Leaf area index

Nutritive values of the forage are presented in Table 2. The fibre characteristics (NDF, ADF and ADL) did not significantly differ ($P>0.05$) among treatments. The NDF content in forage ranged from 62.89% in SG to 69.35% in CB. Similarly, the ADF and ADL content of forage ranged from 36.58% in CB to 48.73% in SC and 3.73% in CB to 7.05% in SB respectively. Thus, the addition of legumes to forage maize did not influence the fibre concentration, indicating no effects on the anti-nutritive characteristics of the forage.

On the other hand crude protein (CP) differed significantly among treatments ($P<0.05$). The application of N-fertilizer significantly ($P<0.01$) increased the crude protein content of sole corn from 8.21% to 10.06%. Intercropping of corn with both legumes increased crude protein ($P<0.05$) in total forage of sole corn without N to 12.98% and 10.82% when intercropped with groundnut and bambara nut respectively. Corn intercropped with groundnut showed a higher ($P<0.05$) CP than nitrogen fertilized corn but corn intercropped with bambara nut did not differ ($P>0.05$) in CP with nitrogen fertilized corn. This implied that intercropping corn with groundnut provided forage with higher protein than the application of nitrogen fertilizer in sole corn. This result is similar to the findings of Hamdollah (2012) who showed a significant increase in CP of corn/legume mixtures compared to sole corn as a result of nitrogen contribution by the legumes.

The in-vitro dry matter digestibility (DMD) was also influenced by the treatments. Intercropping with either bambara nut or groundnut gave a forage with significantly higher ($P<0.05$) DMD compared to sole corn with nitrogen. Sole corn without nitrogen (SC) showed similar DMD ($P>0.05$) with corn/legume intercrop probably because its poor growth rate may have delayed its maturity. The conclusion is that forage digestibility was increased by inclusion of legumes to nitrogen fertilized corn. A similar result was obtained by Dahmardeh *et al.* (2010) who reported higher total forage dry matter digestibility in maize-cowpea intercropping than in maize or cowpea sole crops.

Table 2: Forage nutritive quality and digestibility in sole and in mixture

Treatments	NDF (%)	ADF (%)	ADL (%)	CP (%)	DMD (%)
Corn/Bambara Intercrop (CB)	69.35	36.58	3.73	10.82b	57.52a
Corn /Groundnut Intercrop (CG)	66.99	45.77	4.45	12.98a	56.35ab
Sole Bambara (SB)	65.02	36.60	7.05	11.23b	53.11bc
Sole Corn (SC)	68.97	48.73	4.98	8.21c	54.56abc
Sole Corn with N (SCN)	65.18	38.93	5.03	10.06b	51.80c
Sole Groundnut (SG)	62.89	42.79	6.99	13.74a	55.08abc
LSD _{0.05}	-	-	-	1.20	3.86
CV (%)	7.13	17.08	34.88	5.92	3.87
SE	3.34	3.74	0.82	0.373	1.366
P > F	0.551	0.270	0.251	<.0001	0.041

N.B.: Means within columns with similar letters are not significantly different ($P>0.05$), ADF= acid detergent fibre, NDF = Neutral detergent fibre, ADL = acid detergent lignin, DMD = Dry matter digestibility.

Conclusions

In conclusion, groundnut was a better legume for intercropping with corn than bambara in terms of yield, protein and digestibility improvement although bambara contributed equally to improved forage digestibility.

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Influence of Ascorbic Acid Addition on Betalain Production

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Introduction

Betalain, a group of nitrogen-containing compounds, is a potential food colorant since the use of synthetic colorants demonstrated negative health effects (El-Wahab and El-DeenMoram, 2012). There are two main types of betalain: red-violet betacyanin and yellow-orange betaxanthin (Wybraniec et al., 2001). They are present in most plants belonging to the order Caryophyllales. Betalain has been reported to have anti-aging, anti-inflammatory, anti-toxin, reducing risk of blood clots and anti-cancer properties (Moreno et al., 2008). Initially beetroot was used as the main source of betalain for production of food colorant. Later, alternative source was preferred because betalain from beetroot possessed an earthy-smell and contained high pyrazines as well as poor colour variability (Lu et al., 2003). *Hylocereus polyrhizus*, also known as red pitaya is an ideal alternative source of betalain (Stintzing and Carle, 2007). Betalain can be easily degraded by several factors such as temperature, pH, and light intensity. For this reason, extraction of betalain is not economically feasible and easily degraded during processing and storage. Callus system was introduced to enhance production of secondary metabolites. The used of ascorbic acid in callus culture system can effectively prevent browning since it can lead to necrosis (Jain et al., 2008). In betalain biosynthesis, ascorbic acid acts as a reducing agent to prevent oxidation of L-DOPA (Gandía-Herrero and García-Carmona, 2013). Studies on ascorbic acid revealed that it maintained the stability of betalain extracted from red pitaya (Herbach et al., 2006; Woo et al., 2011). Therefore in this study, ascorbic acid was tested to determine its effect on callus induction using red pitaya flesh and in enhancing stability of the pigment produced.

Materials and Methods

Sample Collection

Red pitaya (*Hylocereus polyrhizus*) was used in the study. The fruits were purchased from Multi-Rich Pitaya Sdn Bhd in Sepang, Selangor (Longitude-101.74197; Latitude-2.68803), Malaysia. Red pitaya fruit was washed with detergent and wiped with alcohol prior to placing into the laminar flow hood. Fruit flesh was used as the explant for callus induction. The flesh was cut into approximately 1cm² in size and all seeds were removed.

Callus Induction and Multiplication

Callus was induced using the red pitaya flesh, cultured on callus induction medium consisting of full-strength Murashige and Skoog (Murashige and Skoog, 1962), 3% (w/v) sucrose, plant growth regulators (PGRs) 2 mg/L naphthaleneacetic acid (NAA), 4 mg/L thidiazuron (TDZ) and 0.3% (w/v) phytigel. The cultures were incubated in the dark at 25 ± 2°C for a month. Ascorbic acid at different concentrations (0, 0.1, 0.5, 1.0, 1.5, 2.0 g/L) was added, respectively, into the callus induction medium to evaluate its effect on pigment production.

Data Collection and Analysis

The morphology of the calli produced was observed to determine the intensity of the pigmented calli produced for each treatment. Betalain was extracted by soaking the pigmented callus in distilled water for 5-10 minutes. The betalain content in the pigmented calli produced was quantified using spectrophotometry at wavelength 480 nm (betaxanthin) and 537 nm (betacyanin). The absorbance readings obtained were used to calculate the betalain concentration for each sample (Stintzing et al., 2003; Ravichandran et al., 2013). Reverse-phase HPLC was further used to evaluate the content of the betalain extracted from the pigmented calli.

Results and Discussion

The optimal concentrations and types of PGRs for callus induction were based on previous study (unpublished data) where the best combination was reported as 2 mg/L NAA and 4 mg/L TDZ. The calli produced after one month was red and friable, and the colour was consistent after five subcultures with two weeks sub-culturing intervals. The addition of ascorbic acid in the MS medium with 2 mg/L NAA and 4 mg/L TDZ produced calli with bigger diameter, ranging between 2.80±0.02 to 3.50±0.02 cm. Calli supplemented with ascorbic acid were able to maintain their colours even after seven subcultures. Figure 1 shows the red pitaya calli grown on media supplemented with 2 mg/L NAA, 4 mg/L TDZ and ascorbic acid.

In general, betacyanin production was higher (up to 2.9-fold) than betaxanthin. Fresh fruit had the highest content of betalain, 3.9-fold, compared to the calli cultured on MS basal medium without PGR or ascorbic acid (MSO). Callus culture is only a partial system involving undifferentiated cells for production of secondary metabolites. In a complete plant system, secondary metabolites were said to be produced following long biosynthesis pathways that involved dozens of enzymes (Bourgaud et al., 2001). This probably accounts for the lower betalain production in calli cultures compared to the amount of betalain extracted directly from the fruit. The addition of ascorbic acid (0.5 g/L) enhanced betalain production in the pigmented calli, a 3.1-fold higher than the control calli grown on MSO and 1.8-fold higher than calli grown on MS basal medium supplemented with only PGRs (Figure 2). HPLC analysis detected four compounds, betacyanin, betaxanthin, phenolic acid and flavonoid. Betacyanin showed 2.5-fold increment and betaxanthin 2.0-fold in calli grown on MS media supplemented with 0.5 g/L ascorbic acid when compared to the control (Figure 3).

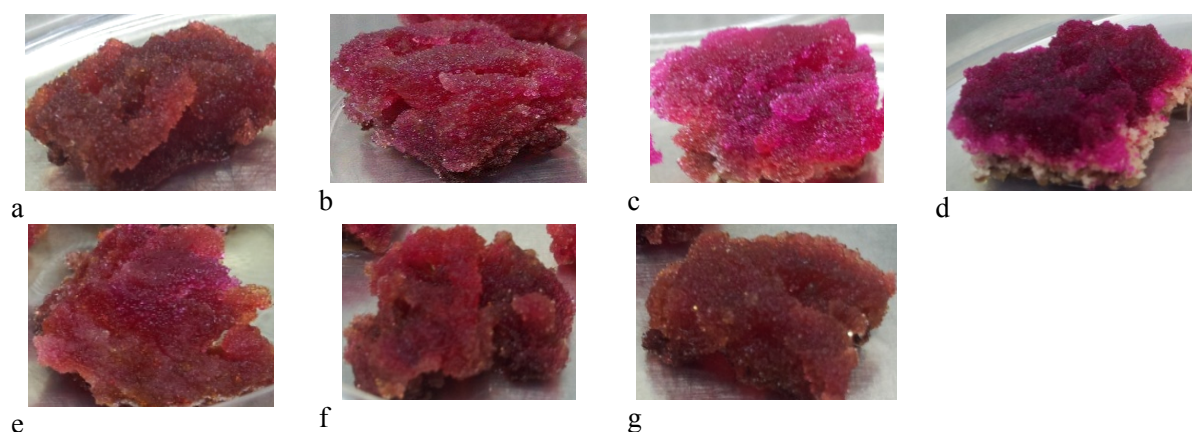


Figure 1 Morphological appearance of pigmented calli. Different callus morphologies were observed, (a) callus induced on MS basal medium without phytohormone or elicitor. The calli were dull in colour and appeared watery (soft). The calli induced in 2 mg/L NAA and 4 mg/L TDZ with (b) 0 g/L, (c) 0.1 g/L, (d) 0.5 g/L, (e) 1.0 g/L, (f) 1.5 g/L, and (g) 2.0 g/L ascorbic acid produced different red colour intensities.

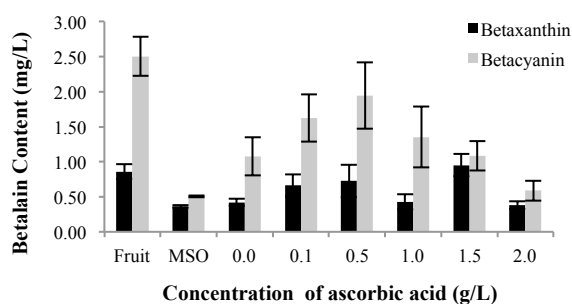


Figure 2 Betalain quantification. Betalain content in the pigmented calli was calculated. Values plotted are the means of five replicates and error bars indicate standard deviations.

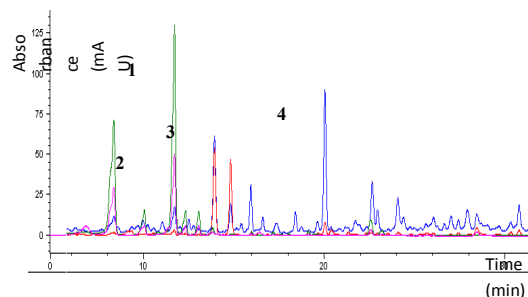


Figure 3 HPLC chromatogram. Calli were cultured on MS media supplemented with 2 mg/L NAA, 4 mg/L TDZ and 0.5 g/L ascorbic acid. The following peaks were identified: betacyanin [1], betaxanthin [2], flavonoid [3] and phenolic acid [4].

Conclusions

Callus culture of *Hylocereus polyrhizus* can be a potential tool for betalain production and other secondary metabolites. The addition of 0.5 g/L ascorbic acid was able to produced betalain 3.1-fold higher than control. RP-HPLC was able to detect four compounds namely betacyanin, betaxanthin, flavonoid and phenolic acid.

Acknowledgments

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Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Yield and Yield Components of Rice Varieties

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Introduction

In year 2010, two isolates of PGPR from rice field were identified as potential PGPR by Malaysian Agricultural Research and Development Institute (MARDI) (Saad et al., 2010). These PGPR were successfully identified using Biolog GP2 MicroPlate as *Burkholderia glumea* (BCA29) and *Burkholderia vietnamiensis* (2A1).

The genus of *Burkholderia* has just been known for only the last fourteen years. Its species composition, distribution and ecological importance are still largely unknown. All *Burkholderia* species have heterotrophic style of metabolism making them capable of thriving on plant exudates. They can be found in most rhizospheres (Balandreau and Mavingui, 2007) and in all reported cases *Burkholderia*, the population density is much higher (up to 4000 folds) in the rhizosphere than in bulk soil (Hebbar et al., 1994).

In rice, β -proteobacteria of the genus *Burkholderia*, for example, *Burkholderia brasilensis*, *B. vietnamiensis* (Gillis et al., 1995) and *Burkholderia* spp. (Muthukumarasamy et al., 2007) have been reported in high numbers.

Recently, public health and safety concerns about the environmental impact of chemical fertilizers and pesticides have led to PGPR being considered as a natural approach to maintaining crop health and yield enhancement. Biofertilizers are becoming increasingly popular in many countries and for many crops. They are defined as products containing active or latent strains of soil microorganisms, either bacteria alone or in combination with algae or fungi that increase the plant availability and uptake of mineral nutrients.

In general, they contain free-living organisms associated with root surfaces but they may also include endophytes, microorganisms that are able to colonize the intercellular or even intracellular spaces of plant tissues without causing apparent damage to the host plant. The concept of biofertilizers was developed based on the observation that these microorganisms can have a beneficial effect on plant and crop growth (Davidson, 1988). Consequently, a range of plant growth-promoting rhizobacteria (PGPR) has been identified and well characterized. Direct beneficial effects can occur when the microorganisms provide the plants with useful products. Thus, the main objectives of these experiments are to assess and determine the effect of PGPR on yield and yield components of rice varieties.

Materials and Methods

This experiment was conducted in farmers field, namely Parit 3 ½ Timur and Parit 12 Timur, Sungai Besar, Barat Laut Selangor, Malaysia. Both locations were selected based on high soil fertility. Two PGPRs were used in this experiment in combination with subsidized fertilizer levels manipulated to 0, 25, 50, 75 and 100% (Table 1). Two rice varieties, MR283 as a new potential variety and MR263 as a

control variety were used in this experiment for assessment. The experimental design was factorial Randomized Complete Block Design (RCBD) with four replicates.

Table 1. Treatments and description of treatments

Treatment	Description
T1	Subsidized fertilizer package (NPK ratio = 104.3:41.7:54.0)
T2	PGPR 1 (BCA29)
T3	PGPR 2 (2A1)
T4	25% T1 + PGPR 1 (BCA29)
T5	25% T1 + PGPR 2 (2A1)
T6	50% T1 + PGPR 1 (BCA29)
T7	50% T1 + PGPR 2 (2A1)
T8	75% T1 + PGPR 1 (BCA29)
T9	75% T1 + PGPR 2 (2A1)
T10	100% T1 + PGPR 1 (BCA29)
T11	100% T1 + PGPR 2 (2A1)

A total of 88 plots were constructed with the size of 5 x 5 m each. Five seedlings were transplanted into one point at the age of 16 days after seeding (DAS) with planting distance of 25 x 25 cm. About 5 cm of standing water was maintained in each plot until 90 days after transplanting (DAT). PGPRs were applied at four different growth stages on 4, 24, 44 and 64 DAT. Meanwhile, the fertilizer were applied one day after PGPR application at 5, 25, 45 and 65 DAT. Crop management practices including fertilizer application were based on MARDI's Sustainable Rice Production Manual.

Effect of PGPR on yield and yield components were assessed simultaneously. The recorded parameters included yield at 14% moisture (from 4 x 4 m per plot), panicle per meter square, spikelet per panicle, panicle length, percent of filled grain (%) and 1000 grain weight (g). All the parameters were collected at maturity stage on 107 DAT. Data from the study were combined over locations and analyzed using Statistical Analysis Software (SAS 9.3, 2007), where location was designated as the main plot and the other treatments as sub-plots. Mean comparisons were done utilizing single df orthogonal contrast or Duncan's Multiple Range Test (DMRT) where appropriate.

Results and Discussion

Yield

The average yield for this study was 7.2 t/ha. The yield was significantly affected by the main effects of treatment and variety (Table 2). The result showed that 100% fertilizer + BCA29 resulted in the highest yield of 8.2 t/ha followed by 100% fertilizer + 2A1 with 8.1 t/ha. The yield performance for 100% fertilizer (control) was only 7.9 t/ha (Figure 1). The results showed that BCA29 and 2A1 increased the yield of rice under full recommended rate of subsidized fertilizer, with enhanced yield by 5% and 3% as compared to the control. The use of BCA29 and 2A1 may be more beneficial to promote the higher percent of rice yield in low fertility soil. Some reports from groups promoting the use of biofertilizers indicated considerable yield increases upon their use. *Trichoderma harzianum* used as a coating agent for rice seed was reported to result in a 15–20% yield increase as compared with rice plants receiving full inorganic fertilizer rates (Cuevas, 1991).

Both BCA29 and 2A1 also had significant linear positive response on rice yield with increasing percent of subsidized fertilizer level. The pattern between yield and treatment showed that BCA29 have better yield performance than 2A1 for every percent of subsidized fertilizer level. At 75%

fertilizer, BCA29 achieved yield that was not significantly different as compared to 100% fertilizer (control) and had potential to reduce the amount of subsidized fertilizer by 25%.

According to Tran Van *et al.* (2000), rice varieties grown in low fertility soil in Vietnam inoculated with *B. vietnamiensis* TVV75 strain gave yield increases of 13 to 22%. Elcoka *et al.* (2008) also hypothesized that microbial inoculants can replace mineral fertilizer. Adesemoye *et al.* (2009) have shown that microbial inoculants are good and reliable supplements to fertilizer. The tested PGPR did increase yield significantly, especially BCA29, and this result was similar to the rice grain yield increases reported by Nino *et al.* (2012).

Yield was also significantly affected by variety (Table 2). Yield for MR263 was 300 kg higher than MR283.

Table 2. Mean Square and ANOVA for the Study on Effect of PGPR on Yield and Yield Component of Rice Varieties

Factors	Parameters					
	Yield (t/ha)	Panicle number per square meter	Panicle length (cm)	Spikelet per panicle	Percent filled grain (%)	1000 grain weight (g)
Loc	77700.0	1.45	8.38**	3801.84**	71.53*	19.11**
Error(a)	1660393.2	1475.39	2.49	339.17	76.25	0.85
Trt	10797004.5**	6320.30**	2.72**	181.48	9.28	0.93
Var	2232452.8*	13.09	14.89**	18409.09**	1325.51**	298.48**
Var*Trt	316351.2	669.09	2.37	574.02	8.78	0.99
Loc*Var	59719.1	15431.27	3.67*	7488.09**	127.84**	11.30**
Loc*Trt	622683.1	433.45	0.48	162.39	24.10	1.01
Loc*Var*Trt	360575.4	676.07	0.64	116.39	11.09	0.80
CV%	9.9	12.10	3.81	10.90	4.27	3.03
Grand Mean	7152.0	238.27	22.68	109.38	84.88	27.78

Mean squares followed by * denotes significant difference at $p < 0.05$.

Mean squares followed by ** denotes significant difference at $p < 0.01$.

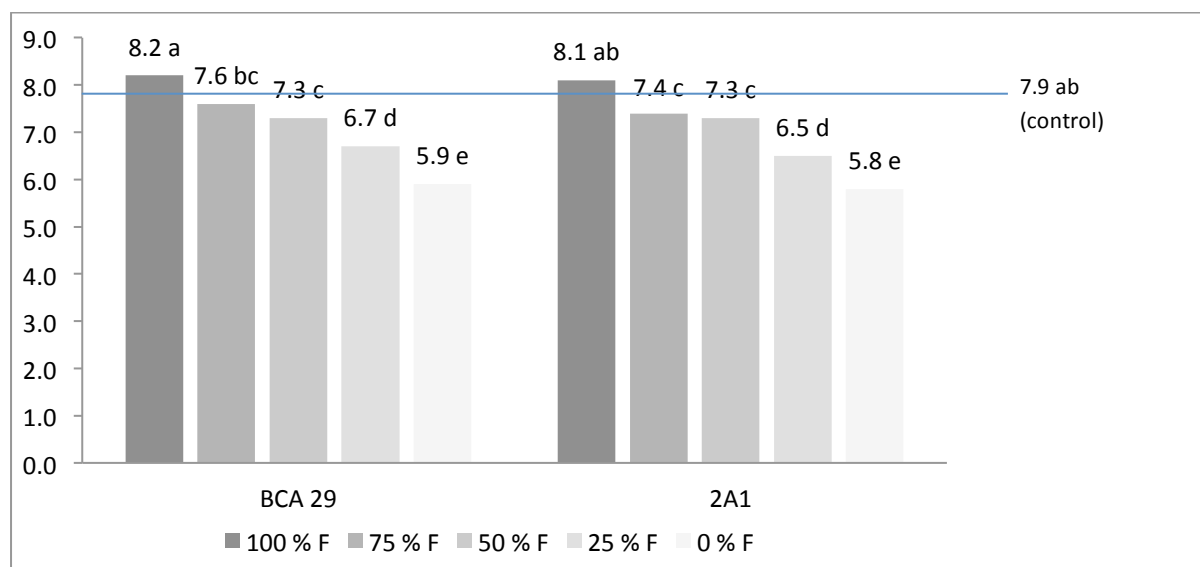


Figure 1. Effect of PGPR on yield according to treatment

Yield Components

Panicle number per meter square was significantly affected by treatment (Table 2). Increased in panicle number per meter square was observed with the increased amount of subsidized fertilizer level with additional PGPR application. The highest number of panicle per meter square was observed in treatment with 100% fertilizer + BCA29 followed by 100% fertilizer + 2A1 as compared to 100% fertilizer (control). Panicle number per meter square also increased with application of both BCA29 and 2A1 started at the level of 25% to 100% subsidized fertilizer consequently (Figure 2). BCA29 and 2A1 significantly increased the panicle number per meter square under full recommended rate of subsidized fertilizer by 11% and 5% as compared to the control.

According to Baldani *et al.* (2000), PGPR can affect plant growth directly by the synthesis of phytohormones and vitamins, inhibition of ethylene synthesis, improving nutrient uptake, enhancing stress resistance, solubilization of inorganic phosphate and mineralization of organic phosphate. Biswajit *et al.* (2013) also found out that *Burkholderia* strain SDSA-I10/1 significantly increased the number of tillers by 29%.

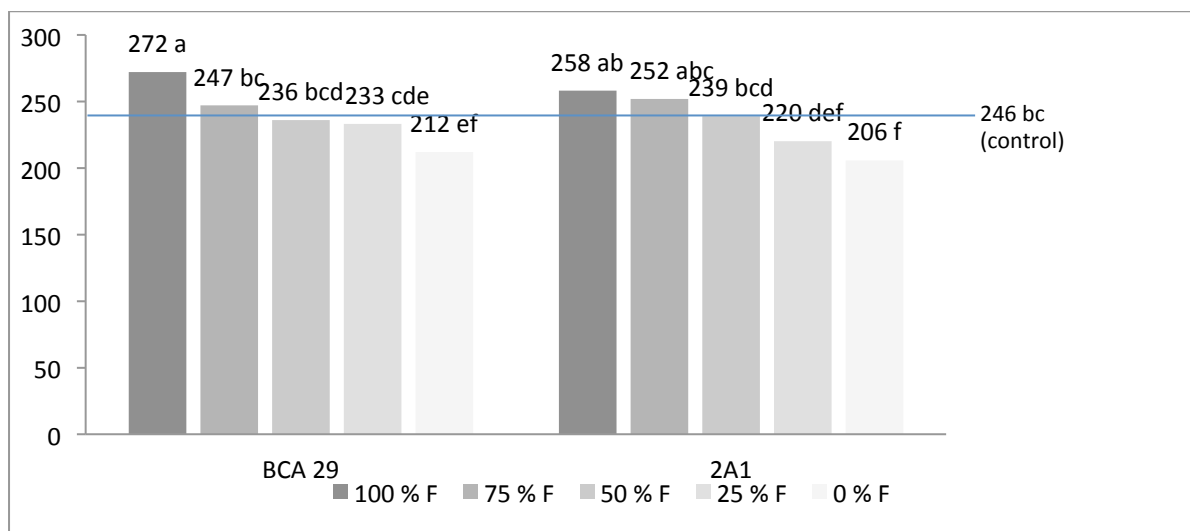


Figure 2. Effect of PGPR on panicle number per meter square according to treatment

Panicle length, spikelet per panicle, percent of filled grain and 1000 grain weight were significantly affected by interaction between location and variety (Table 2). Panicle length and spikelet per panicle for MR263 were higher than MR283 in Parit 3 ½ Timur, Sungai Besar. Meanwhile, percent of filled grain and 1000 grain weight for MR263 were higher than MR283 in Parit 12 Timur, Sungai Besar. It may indicate that the rice variety performed differently in different locations.

Conclusions

Both the BCA29 and 2A1 had significant linear positive response on rice yield and panicle number per meter square with increasing percent of subsidized fertilizer level. This indicated that sufficient fertilizer levels were more important than using any growth promoting substances. The results also indicated that BCA29 and 2A1 had the ability to increase the yield of rice under full recommended rate of subsidized fertilizer by 5% and 3% as compared to the control. Hence, these two PGPRs could be used as potential efficient microbial biofertilizers in rice growing, to increase the yield and had the potential to reduce the amount of subsidized fertilizer by 25%.

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Potential of Roselle (*Hibiscus Sabdariffa* L.) Calyces Quality as Affected by Pruning Intensity Method Attributes in Controlled Environment Structure

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Introduction

Roselle or scientifically named *Hibiscus sabdariffa* L. was identified to be one of the 10 herbs that need to be commercialized in Malaysia. This is due to the increasing world population and their standard of living in most countries including Malaysia. It has created high demand on food, beverages, cosmetic and also in herbal industry with high quality product. In view of high demand on herbal product, especially in traditional medicine nowadays, *H. sabdariffa* has been chosen for this study. Due to its global demand, cultivation of *H. sabdariffa* is increasing. This valuable herb is getting high demand in industry due to its known properties as remedy for various serious ailments like blood pressure, cancer, diabetes, cough, fever and other illness (Orwa et al., 2009). Conventionally, *H. sabdariffa* is small sized but the stem is erect and can grow up to 3.5 m tall (Yadong et al., 2005). This may easily damage by strong wind and produces uncontrolled matured fruit. Pruning is one of method that can reduce and control the height and canopy of plant. Pruning also serves several other functions like training of the plant, maintaining plant health, restricting growth, reducing the risk of branch failure and also improving the quality of flowers, fruits, foliage and stems of plant (Wheeler, n/a). Nowadays, there are many researchers studying pruning in order to improve yield and quality of fruits such as carambola (*Averrhoa carambola* L.), plum cultivar, kiwifruit (*Actinidia deliciosa*), cherry tomato (*Lycopersicon esculentum*), rose (*Rosa spp.*) etc. According to Anber (2010), light pruning on rose gave the best pruning intensity in order to increase period, quantity and quality of flowers compared to medium and heavy pruning. Manoj et al. (2010) also stated that heavily pruned peach decreased the number of yield while medium pruning gave the best yield. In this study, pruning intensity method was applied on *H. sabdariffa* under controlled environment via aggregate system in order to determine the best pruning intensity method for high yield and quality of *H. sabdariffa*.

Materials and Methods

Seeds of *H. sabdariffa* from variety UMKL1 were obtained from Herbs Unit, Department of Agriculture Serdang. Seedlings were prepared by using 100% peat moss as growing media. Seedling trays were used to sow the seeds and seedlings were irrigated manually with water. Seedlings were placed under covered shelter at 60%-70% light intensity for two weeks at Ladang 15, Faculty of Agriculture, UPM, Selangor. After two weeks, uniform seedlings were selected and transplanted into 16 x 16 inches white polibags containing coconut coir dust (CCD) and Peat Gro (PG) as an additive at 3:1 ratio. In this experiment, there were four treatments applied, i.e. non pruning or control (T0), light pruning (T1), medium pruning (T2) and heavy pruning (T3) with four replications and eight plants per replication, arranged in randomized complete block design (RCBD) under 60 x 30 feet of fully covered rain shelter. The rain shelter structure had average daily temperature of 27.9 °C and relative humidity of 69.6%. For nutrient supply, Cooper's formulation (Cooper, 1979) with some modifications was prepared and irrigated directly to rooting area via drip irrigation system. Each plant was irrigated two times daily for 15 minutes per fertigation cycle at an interval of 12 hours per cycle. Electrical conductivity (EC) was adjusted based on age of plant starting from 0.5 dS/m and was increased to 3.5 dS/m with pH of 5.5-6.0. EC and pH were monitored by using hand held pH meter (model IQ150, Spectrum Technologies Inc., USA). Quality parameters of fresh calyces weight per plant (FCW, g), total phenolic content (TPC, mg GAE/g), ascorbic acid (AA, mg/l) and soluble solid

content (SSC, Brix %) were measured at 12 weeks after transplant (WAT) in laboratory. FCW were recorded by using electric analytical balance while TPC, AA and SSC were measured by scanning spectrophotometer model UV3101 PC. Data were analyzed using Statistical Analysis System (SAS) software program. Analysis of Variance (ANOVA) was carried out to determine significant differences among data. Means among the treatments were compared based on Duncan's Multiple Range Test (DMRT) at 0.05 probability level.

Results and Discussion

Table 1 indicates that FCW, AA and SSC, respectively, were significantly different among four treatments while TPC and TA were not significantly different with DMRT at 5% probability level. T2 gave the highest value of FCW (1014.17 g) followed by T0 (842.25 g), T1 (708.08 g) and T3 (463.12 g). For AA, T3 resulted in the highest mean value of 46.42 mg/100 g of AA followed by T2 (44.79 mg/100 g), T0 (44.74 mg/100 g) and T1 (44.56 mg/100 g) while SSC was the highest with T2 (15.95%) followed by T1 (14.68%), T3 (11.88%) and T0 (11.68%). Statistical analysis indicated no significant difference among the treatments. T3 resulted in the highest TPC with mean of 25.96 mg GAE/100 g followed by T2 (25.34 mg GAE/100 g), T0 (24.35 mg GAE/100 g) and T1 (23.0 mg GAE/100 g). T2, i.e. medium pruning, also gave the highest content of TA with mean of 1.33% (% citric acid) followed by T3, T1 and T0 with means of 1.30%, 1.20% and 1.09% (% citric acid), respectively. Other than training and restricting the growth of plant, pruning was also meant for improving the quality of flowers, fruits, foliage and stem (Wheeler, n/a).

Table 1. Quality parameters of *H. sabdariffa* in relation to four pruning treatments

Treatments	Fresh Calyces Weight per Plant (g)	Total Phenolic Content (mg GAE/g)	Titrateable Acidity (% citric acid)	Ascorbic Acid (mg/100g)	Soluble Solid Content (Brix %)
T0	842.25ab	24.35	1.09	44.74b	11.68b
T1	708.08b	23.00	1.20	44.56b	14.68ab
T2	1014.17a	25.34	1.33	44.79b	15.95a
T3	463.12c	25.96	1.30	46.42a	11.88b
Pr > F	***	ns	Ns	*	*

Within each column, same letter indicates no significant difference among treatments ($p > 0.05$). T0: non-pruning (control); T1: light pruning; T2: medium pruning; T3: heavy pruning; * $P < 0.05$, *** $P < 0.001$, ns not significant

Based on results in Table 1, we can conclude that pruning gave significant effect on yield and quality of *H. sabdariffa*, especially with medium and heavy pruning. This result strongly agreed with Manoj et al. (2010) in their study on peach where heavy pruning decreased fruit yield but medium pruning gave the best results where yield increased. For *H. sabdariffa*, calyces are the main yield or product part. Pruning produces vigorous shoot growth and there has been a reduction in top growth but there is a full complement of roots producing a flow of sap capable of supplying more tissue than is now available. According to Kumar et al. (2010), increase in size of leaves increases the amount of plant food manufactured and thus nourishes the developing fruits. Pruning also affects the total photosynthetic site due to the size of leaves and its number. T3 gave the highest significant content of AA due to good light interception between the leaf canopy. T2 was medium pruning where secondary branches were removed from main stem. Medium pruning led to more open canopies and resulted in a change in canopy architecture, so that fruit yield and SSC were improved. Saeed et al. (2005) in their study on Kinnow fruit indicated no significant difference in SSC but Sites and Reitz (1949) reported that pruning increased SSC and improved colour was correlated with light intensity of plant. In this case, T2 gave more open space by the plant canopy. Fruit in main stem might have been exposed to higher light levels throughout the growing season due to the constant removal of vegetative shoots

along the center of the stem. In order to increase fruit quality, leaves and fruits need to be exposed to light directly (Biasi et al., 1993). From this finding, we can conclude that medium pruning is sufficient for optimum yield production and high quality of *H. sabdariffa*. Continuous study was recommended on pruning with specific branching system in order to increase growth performance and other phytochemical content of *H. sabdariffa*.

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CHAPTER 8

PEST AND DISEASE MANAGEMENT

Sequence Analysis of Endo- β -1,3-1,4- Glucanase Gene (β glu) Isolated from *Bacillus* SP 289, an Antagonist Bacteria Against Rice Sheath Blight Pathogen

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Introduction

Glucanases are enzymes that function to break down a glucan, a polysaccharide made of several glucose subunits. As they perform hydrolysis of the glucosidic bond, they are hydrolases and classified as glycoside hydrolases EC.3.2 in the EC number classification of enzymes. Glycoside hydrolases are a well-known group of enzymes. Their functions are to hydrolyse the glycosidic bond between a carbohydrate and non-carbohydrate moiety or between 2 or more carbohydrates. Naturally, β -glucanases are involved in catalyzing β -glucan degradation. These β -glucanases are divided into 4 type according to their glycosidic linkage that they cleave; i) β -1,3-1,4-glucanases (lichenases, EC 3.2.1.73), ii) β -1,3-glucanases (laminarinases, EC 3.2.1.39), iii) β -1,4-glucanases (cellulases, EC 3.2.1.4) and iv) β -1,3(4)-glucanases (EC 3.2.1.6) (Yang et al., 2008).

Since β -glucan is the main component in fungal cell wall, β -glucanase plays an important role as antifungal protein. Extensive hydrolysis of β -glucan polymer by the enzyme has lead to fungal cell disrupt by weakening the mechanical strength of the cell walls. In the literature, antifungal activity was mainly observed in β -1,3-glucanases. The enzymes have been isolated from various sources including plants, fungi and bacteria. Previous studies also reported several number of β -1,3-1,4-glucanases which have been isolated and purified especially from bacteria. However, it was due to their importance enzymatic functions in industrial application especially in animal feeds production and brewing industry (Luo *et al.*, 2010, Qiao *et al.*, 2009, Beckmann *et al.*, 2006). Little is known about the antimicrobial activity of β -1,3-1,4-glucanase since there are not many researchers who made reports regarding the antifungal activity of the enzyme (Britto *et al.*, 2013, Luo *et al.*, 2010).

This study is aimed to isolate potential antifungal protein gene of endo- β -1,3-1,4-glucanase (β glu) from an antagonist bacteria against Rice Sheath Blight pathogen, *Bacillus* SP 289. The bacterium was isolated from rhizosphere soil paddy field in our previous study. We believed that β glu enzyme has involved as one of the antifungal compound which responsible in suppressing the growth of sheath blight pathogen, *Rhizoctonia solani*. Therefore, β glu sequence has to be analyzed to gain prediction of important information.

Materials and methods

PCR amplification of Bacillus SP 289 β glu

Primers for PCR were designed based on the complete sequence of endo- β -1,3-1,4- glucanase gene from two *Bacillus subtilis* registered in the GenBank database with accession number BSU60830 and EU082110 respectively. *Bacillus* SP 289 Genomic DNA was isolated using GenElute Bacterial Genomic DNA Extraction Kit (Sigma-Aldrich, USA) according to protocol provided by the

manufacturer. PCR amplification was carried out in 25 µl reaction for 30 cycles using thermostable DyNAzyme™ EXT DNA polymerase (Finnzymes, Finland) in PTC-200 thermal cycler (MJ Research, USA). The reaction mixture consisted of 1x PCR buffer; 2.0 mM of MgCl₂, 0.2 mM of each dNTPs, 2 µM of forward and reverse primers, 100 ng of *Bacillus* SP 289 genomic DNA as a template and 2.5 U of the enzyme mix. PCR product was resolved using 1% of agarose gel and purified using QIA quick gel extraction kit (QIAGEN, Germany). The purified PCR product was cloned directly into a vector using TOPO TA Cloning® Kit (Invitrogen, USA). Plasmid DNA of recombinant clones were isolated using QIAprep Spin miniprep kit (QIAGEN, Germany) and sent for automated DNA sequencing service (Research Biolabs Technologies, Singapore).

Sequence analysis

Sequence analysis was conducted using several publicly available web servers and integrated software. The web servers which has been used for the analysis of βglu gene are as listed below:

1. <http://www.ncbi.nlm.nih.gov/BLAST/>
2. <http://web.expasy.org/protparam/>
3. <http://www.biogem.org/tool/chou-fasman/>
4. <http://www.sbg.bio.ic.ac.uk/phyre>

For the phylogenetic tree construction, the amino acid sequence of βglu and other endo-β-1,3-1,4-glucanase genes obtained from the GenBank database were aligned by T-coffee and analysed by the MEGA5 program.

Results and discussion

The βglu gene showed as expected size of open reading frame of 720 bp in length (Figure 1) which codes for 239 amino acids. A database search performed with BLAST software shows that the βglu gene sequence indicated 100% similarity with Endo-beta-1,3-1,4 glucanase from *Bacillus amyloliquefaciens* subsp. Plantarum CAU B946 (YP 005132370.1) followed by 99% similarity with beta-1,3-1,4-glucanase from *Bacillus amyloliquefaciens* (ACX55805.1). Conserved motif EIDIEF of glycoside hydrolase family 16 was found in the acid amino sequence of the βglu based on the sequence alignment analysis. The conserved motif is located from amino acids 130 to 135. Aspartic acid residue (D) and two of the glutamic acid residues (E) act as the catalytic residues.

βglu from *Bacillus* sp. 289 is classed as a stable protein because its instability index is 16.80, according to the Expasy ProtParam tool. βglu from *Bacillus* sp. 289 is made up of 3668 atoms of which 1208 are carbon, 1778 are hydrogen, 312 are nitrogen, 361 are oxygen and 9 sulphur. This protein is thus represented by the formula C₁₂₀₈H₁₇₇₈N₃₁₂O₃₆₁S₉. βglu is composed of 239 amino acids including 20 amino acids excluded Pyl (O) and Sec (U). βglu has molecular weight of 26.7 kDa and pI 6.41. The total number of positively charged residues (Arg + Lys) is 19 while the total number of negatively charged residues (Asp + Glu) is 20.

Secondary structure prediction of βglu from *Bacillus* sp 289 using Chou & Fasman Prediction Server showed that the βglu structure consists of 113 (47.3 %) of α helices, 101 (42.3 %) of β sheets and 24 (10 %) of turns (Figure 2). The theoretical three dimensional structure of the glucanase was modelled using Phyre2, based on homology recognition techniques based on the template structure of endo-β-1,3-1,4- glucanase (Figure 3). Further evaluation of the model generated was considered using RAMPAGE for the reliability of predicted structure. Rampage showed the 95.6% residues in favoured regions and 4.4% in the allowing region whereas no residues were in the outlier region.

```
1
ATGAAACGAGTGTGCTAATTCTTGCTACTGGATTGTTTTTGAGTTTGTGTGCG
ATCACTTCTGCTGCAT 70
      M K R V L L I L V T G L F L S L C
A I T S A A
71
CAGCTCAAACAGGCGGATCGTTTTTTGAACCTTTTAAACAGCTATAACTCCGGTT
TCTGGCAAAAAGCAAA 140
      S A Q T G G S F F E P F N S Y N S
G F W Q K A N
141
TGGTTACTCCAATGGAGATATGTTCAACTGCACTTGGCGTGCAAATAATGTATC
CGTGACGTCATCAGGT 210
      G Y S N G D M F N C T W R A N N
V S V T S S G
211
GAAATGCGTTTGGCGCTGACAAGCCCGTCTTATAACAAGTTTGGACTGCGGGGAG
AACCGCTCCGCTCAAA 280
      E M R L A L T S P S Y N K F D C G
E N R S A Q
281
CCTATGGCTATGGACTTTATGAAGTCAGAATGAAACCGGCTAAAAACACGGGGA
TCGTTTCATCGTTCTT 350
      T Y G Y G L Y E V R M K P A K N T
G I V S S F F
351
CACTTATACAGGTCCAACGGATGGTACCCCTTGGGATGAGATTGATATCGAATT
TTTAGGAAAAGACACG 420
      T Y T G P T D G T P W D E I D I
E F L G K D T
421
ACAAAGGTTCAATTTAATTATTATACAAATGGCGCGGAAACCATGAGAAGGTT
GCGGATCTCGGATTTG 490
      T K V Q F N Y Y T N G A G N H E K
V A D L G F
491
ACGCGCCAATGCCTATCATACTGCGTTCGATTGGCAGCCGAACCTCTATAA
AATGGTATGTCGATGG 560
      D A A N A Y H T Y A F D W Q P N S
I K W Y V D G
561
CGAATTTTAAACATTTCTCCGACAAACCGCAATTCGGCGCAACGACCCCAAAACATGAT
```

Figure 1. Nucleotide sequence of β glu gene from *Bacillus* sp. 289 and deduced amino acid sequence. Underlined is the conserved motif of glycoside hydrolase -16.

Query 1

MKRVLILVTGLFSLCAITSAASAQTGGSEFFEPFNSYNSGFWQKANGYSNGDMFNCTW
R 60

Helix 1 HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH HH HH 60
 Sheet 1 EEE 60
 Turns 1 T T T T T T 60

* * * * *

Query 61

ANNVSVTSSGEMRLALTSPSYNKFDCGENRSAQTYGYGLYEVRMKPAKNTGIVSSFFTY
T 120

Helix 61 HHHHHHHHHH HHHHHHHHHH HHHHHH 120
 Sheet 61 EEEEE EEEEEEEEEEE EEEEEEEEE 120
 Turns 61 T TT TT T T 120

* * * * *

Query 121

GPTDGTWPWEIDIEFLGKDTTKVQFNYYTNGAGNHEKVADLGFDAANAYHTYAFDWQ
PNS 180

Helix 121 HHHHHHHHHHHHHHHHHHHH HHHHHHHHHHHHHHHH HH 180
 Sheet 121 EEEEE EEEEEEEEEEE EEEEEEEEE 180
 Turns 121 T T T T TT 180

* * * * *

Query 181

IKWYVDGQLKHTATSQIPTTPGKIMMNLWNGIGVDDWLGSYNGVNPLYAHYDWVRYT
KK 239

Helix 181 HHHHHHHHHHHHHHHHHH HHHHHH HHHHHH HH 239
 Sheet 181 EEEEEEEEE EEEEEEEEE EEE 239
 Turns 181 T T T T T 239

Total Residues: H: 113 E: 101 T: 24
 Percent: H: 47.3 E: 42.3 T: 10.0

Figure 2. Secondary structure prediction using Chou & Fasman Prediction Server. H: α helices, E: β sheets, T: turns

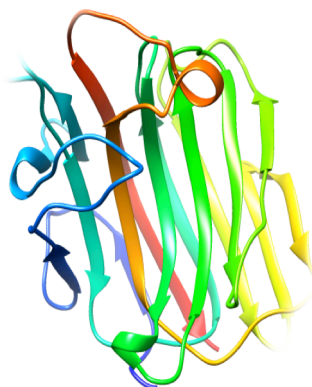


Figure 3. Prediction of three dimensional structure of the β glu peptide using Phyre 2 constructed with 100% confidence

Conclusions

Based on the gene sequence analysis, β glu protein is classed as stable protein with formula of $C_{1208}H_{1778}N_{312}O_{361}S_9$. β glu enzyme will be produce through recombinant protein to prove that either it significantly involve in suppressing the sheath blight pathogen.

Acknowledgements

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Foliar and Root Responses of the Oil Palm (*Elaeis guineensis*) to Placement of *Ganoderma boninense* Inoculum

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Introduction

In *Ganoderma* pathogenicity studies, the fungus is often perceived as a weak competitor against other microbes naturally present in soil and frond debris (Rees et al., 2007). Previously, various inoculation methods have been tried (Ariffin and Idris, 1990; Kok et al., 2013) but all are strongly reliant on large inoculum to enable infection in the nursery. Rees et al. (2007) demonstrated that infection is possible with small inocula if they are in close contact with the roots. The role of multiple small inocula compared to a single large inoculum in increasing disease severity or increasing persistence of *Ganoderma* has not been demonstrated experimentally and is addressed in this study. Based on current knowledge on the importance of a food base to *Ganoderma* infection and that oil palm roots likely travel to the inocula, we hypothesize that a degraded food base results in reduced infection compared to a less-degraded substrate. To investigate this, we utilized rubber wood block (RWB) inocula colonized by two isolates of different aggressiveness, and placed at various depths in soil. Various parameters were measured to obtain a more complete understanding into the development of disease symptoms in response to the different treatments.

Materials and Methods

The two different *Ganoderma boninense* isolates were used in this study were previously described in Kok et al. (2013). Isolate Fraser G8 (denoted 'T4' here) was the least aggressive and Batu Lintang G10 ('T10') was the most aggressive. Culture maintenance and RWB inoculum preparation followed Kok et al. (2013), except RWBs were in different sizes. Commercial Tenera (Dura x Pisifera) pre-germinated oil palm seeds were planted in Hyplug trays (G-Planter[®] Sdn Bhd) in a mixture of burnt red soil: cocopeat: compost at a ratio of 2:2:1. Nursery maintenance also followed Kok et al. (2013). After 2 months, seedlings were transplanted into large polybags containing sandy clay loam topsoil. Seedlings were acclimatized for another 2 weeks before *Ganoderma* inoculum was inoculated. Treatments A-D comprised T4 inoculum whereas E-H comprised T10 inoculum. For A and E, four colonized RWBs (3 x 3 x 3cm) were buried just slightly below the soil surface at four equidistant positions at the edge of the polybag. For B and F, four wedge-shaped slits were made in the sides of the polybag at 10cm depth and one RWB were pushed into each slit. Treatments C and G were similarly conducted but at 20cm depth. Treatments D and H were inoculated at 10cm depth but with a single larger RWB (6 x 6 x 3cm). Control plants did not receive any *Ganoderma boninense* inoculum. There were 10 replications per treatment. The treatments were arranged in a completely randomized design.

Fruiting body emergence was recorded almost bi-weekly. At each census, the length of frond number 2 and chlorophyll level were measured as well as the number of green leaves counted. At 26 weeks post-inoculation (WPI), the entire root systems of all seedlings were extracted as carefully as possible and *Ganoderma* RWB inocula were also removed. The roots and boles were washed and air-dried for 2 days. Assessments of root parameters were performed and boles were dissected longitudinally to determine extent of internal rot, if any. Similarly, the *Ganoderma* RWBs were counted, washed thoroughly and air-dried for 2 days. All 10 replicates of root samples for each treatment were then bulked, placed into paper bags and dried at 105°C overnight to constant weight. The same procedure was carried out for bole and RWB samples. The dried mass of samples was then recorded. Data was

analysed using SPSS software (IBM SPSS Statistics) version 20.0. Fruiting body emergence was analysed via Chi square test of independence whereas factorial ANOVA was used for the other foliar parameters at each census.

Results and Discussion

Over the 26-week period, *Ganoderma* fruiting bodies formed in most treatments appeared normal but smaller. In this experiment, the presence of fruiting bodies from the substrate merely denotes that the *Ganoderma* is viable and does not indicate infection of the host. Significant differences between isolate x burial depth treatments were observed for 6, 8 and 10WPI (p-values were 0.030, 0.005 and 0.046 respectively) and this corresponded with the period of most rapid fruiting body emergence (Figure 1). Presumably, the drier conditions close to the soil surface were less conducive for fruiting body development, evident in treatments A and E. Similarly, significant differences between isolate x inoculum size treatments were detected for 4, 6, 8 and 10WPI (p-values were 0.045, 0.025, <0.001 and 0.017 respectively). Using a single larger block for isolate resulted in faster emergence of fruiting bodies compared to four smaller blocks and this was more obvious for T4 (Figure 1). A larger block would contain more energy utilizable for the fungus when it enters the reproductive phase.

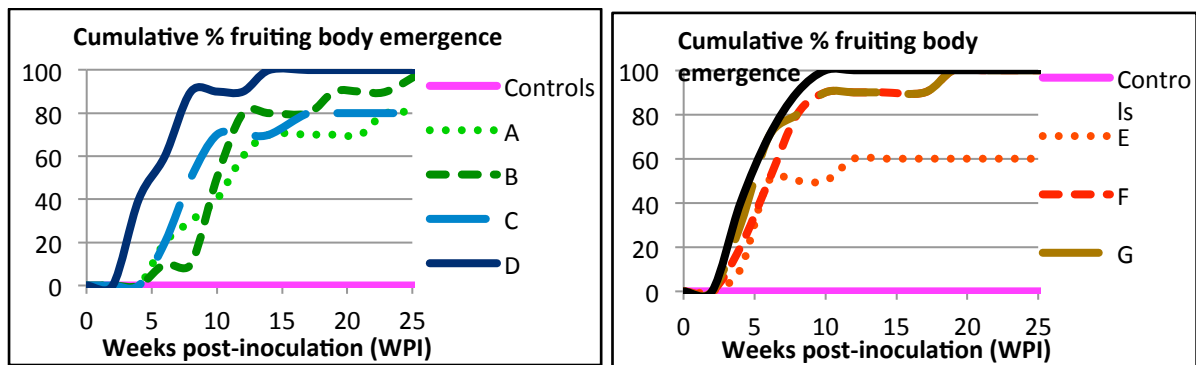


Figure 1. Percentage emergence of *Ganoderma* fruiting bodies for different burial depth and inoculum size for T4 (left) and T10 (right)

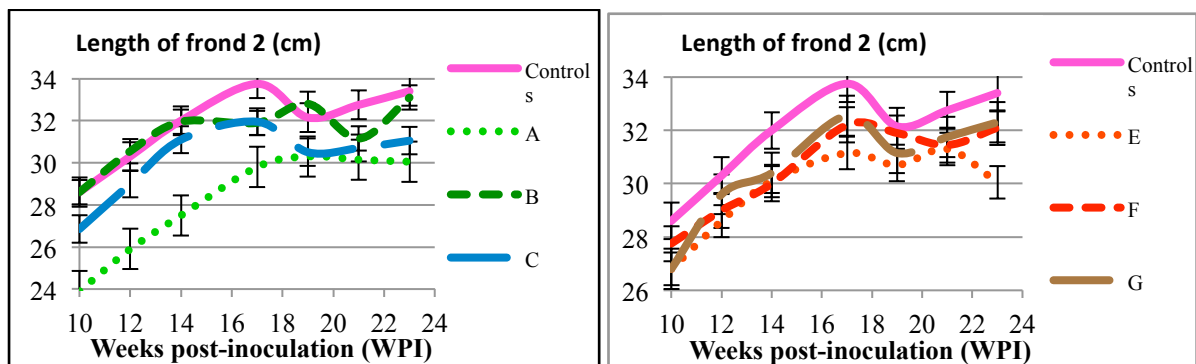


Figure 2. Average length of frond 2 in response to placement of *Ganoderma* RWB inoculum of isolate T4 (left) and T10 (right) at different depths. Error bars represent standard error.

Frond 2 lengths generally increased over the census duration (Figure 2). Control plants were not significantly different from inoculated plants. The decline in growth between 18-21WPI coincided with a heavy rain period in December 2014. Significant differences (p-value < 0.04) between 0cm vs. 10 and 20cm depths were detected between 10-14 WPI, but these were not isolate-specific.

Treatments A and E in which the RWB inoculum was buried closest to the soil surface affected frond 2 length the most and this was consistent over the census period (Figure 2). There was no significant effect of RWB size.

No significant differences were detected between control and inoculated plants for number of green fronds. Significant differences (p -value < 0.03) between 0cm vs. 10 and 20cm depths were detected between 12-19 WPI, and these were not isolate-specific. Inocula buried closest to the soil surface (data not shown) had fewest leaves. From 21-26 WPI, significant differences (p -value < 0.05) were detected for chlorophyll levels between 0cm vs. 10 and 20cm depths. RWB size did not significantly impact these two parameters.

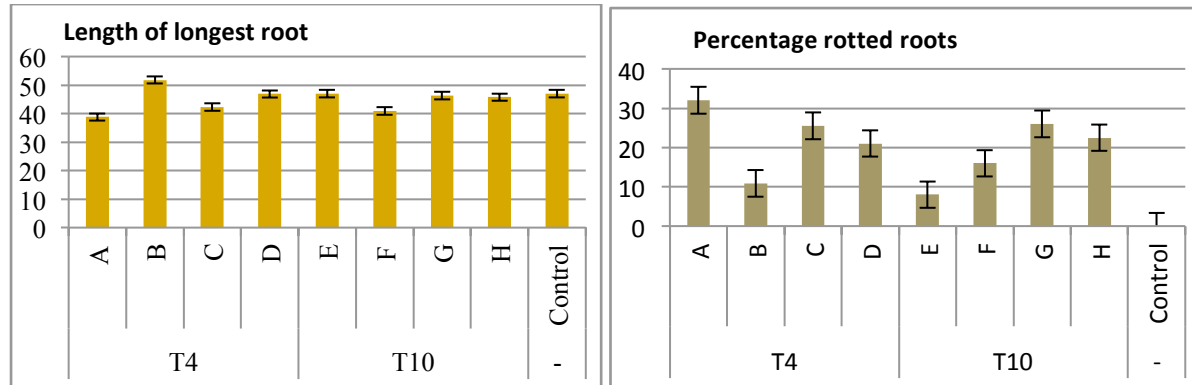


Figure 3. Left: Length of longest root (cm) and right: percentage of root rot in response to different placement of *Ganoderma* inoculum. Error bars represent standard error.

Inoculated plants did not differ significantly from the controls for length of the longest root (Figure 3), but a significant interaction ($p = 0.011$) occurred between the *Ganoderma* isolate and burial depth, which was highest in treatment B and shortest in treatment A. Similarly for percentage of rotted roots, a highly significant interaction between the *Ganoderma* isolate and burial depth ($p = 0.008$) occurred, which was highest in treatment A and lowest in E (Figure 3). Increase in burial depth decreased the root rot by T4 and conversely for T10. Rotting of the roots was more easily discernible visually in treatments A and C and the rot observed was diagnostic of damage caused by *Ganoderma*; primarily the cortex of the root was decayed (hollow), leaving the shrivelled outer epidermis and inner stele intact. Rot in bole was also observed only in treatment B, although incidence was very low.

The dry mass of roots was highest in controls but not for the boles (data not shown). Among all inoculated treatments, treatment B has the highest dry mass for all parameters whereas treatment A has the lowest dry mass. All T4 treatments, except B, caused reduction in total root dry mass by approximately 20% compared to controls and this was also lower than in T10 treatments. For both isolates, a single larger block (B vs. D, F vs. H) resulted in lower dry mass for all measured parameters compared to four smaller inocula. Control plants had a total root: bole dry mass ratio of 2.7 whereas treatment A had the highest ratio at 3.29, followed by H at 2.85. When RWB size was increased, this ratio increased substantially from 2.28 to 2.43 for T4 and from 2.38 to 2.69 for T10.

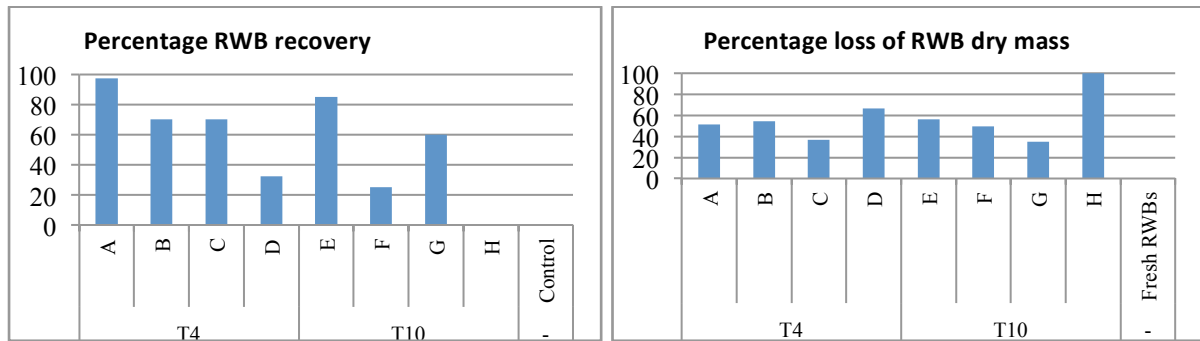


Figure 4. Percentage recovery of *Ganoderma* RWB inoculum (left) and % loss of dry mass

Percentage recovery of RWBs was highest in treatment A and lowest in H (Figure 4) and decreases with increased depth for both isolates. Overall, percentage recovery for isolate T10 was lower compared to T4. Although more RWB pieces were recoverable at 0cm for both isolates, the loss of dry weight was also much higher than at 20cm depths, possibly indicating greater microbial competition in the root-zone or topsoil. Generally, percentage recovery decreased when a single large RWB was used compared to multiple smaller RWBs. The larger surface area to volume ratio of tough, melanised sclerotia-like structures (SLS) on the surfaces of smaller blocks have likely conferred this persistence.

When we view collectively the fungal data with the responses of the host, conditions which encourage rapid *Ganoderma* growth (either as mycelia or fruiting bodies) caused increased degradation of the substrate and consequently, less food source is available for subsequent infection. We also find that this interaction could be isolate-specific and other factors such as light and oxygenation may also play a part. For example, in T4, multiple smaller pieces close to the surface (treatment A) resulted in slowest fruiting body growth and highest percentage root rot. Although many RWB pieces were recovered, they were also more degraded. For T10, both treatments G (multiple small RWBs at 20cm depth) and H (single large block at 10cm depth) resulted in most rapid fruiting body formation and highest root rot, which utilized almost all of the substrate. Therefore, *Ganoderma* may have only a narrow window from growing from a substrate to successfully infecting its host. Once it switches to producing brackets, the original substrate is likely decreased in its capacity to infect available host(s). Greater soil depth is less conducive for wood degradation, but this may be due to other factors such as temperature, oxygenation, moisture and microbial activity. These aspects need to be further explored to better understand implications in the field. Although complete removal of woody material from the field is ideal, planters are restricted by the current labour situation, high expense of mechanical removal and terrain limitations. Nevertheless, the recommended practice of removing infected palms soonest, deboling and excavating holes of 1.5 x 1.5 x 1.5m dimensions should be practiced whenever possible, so that the scourge of *Ganoderma* does not threaten the livelihood of planters.

Conclusions

In conclusion, a combination of suitable above-ground and below-ground measurement parameters enable a more holistic understanding of the disease cycle, which may be isolate-specific. The switch to the reproductive phase in *Ganoderma boninense* depletes the substrate, reducing the risk of subsequent infection of susceptible hosts.

Acknowledgements

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Compatibility Mixtures of Bacterial Antagonists of Durian Canker, *Phytophthora palmivora*

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Introduction

Durian, known scientifically as *Durio zibethinus* is one of the important economic crops for local farmers with a planted area of 75,713 ha throughout Malaysia in 2013. The production rate of durian is 373,084 MT, whereby durian has the largest hectareage out of all the other fruits in Malaysia (Department of Agriculture, Malaysia, 2015).

The durian cultivation has been compromised because of durian canker. Durian canker is caused by *Phytophthora palmivora*, a natural soil-borne pathogen capable of infecting all parts of the durian tree at all stages of its development. In affected plantations, the normal control used is by application of chemical fungicides. The use of fungicides has been becoming increasingly more restricted because of the accumulation of toxic compounds potentially hazardous to humans and the environment. It is therefore necessary to find an alternative method to control the pathogen.

As an alternative approach, biocontrol agents are being used for the management of various diseases (Kavino et al., 2008; Harish et al., 2009). In a study done by Akila, R. et al. (2011), combined application of botanical formulations and biocontrol agents was found to be effective in the management of fusarium wilt of banana. Mixtures of biological control agents can be superior to individual agents in suppressing plant diseases, providing enhanced efficacy and reliability relative to a single biocontrol strain (Stockwell, V.O. et al, 2011). Previously, we evaluated biological control agents designated as B68, CD7, 8C and CA21, through dual culture and *in vivo* screenings as individual strains against the pathogen (Nor Dalila et al., 2012 and Nor Dalila et al., 2014). In this study, four isolates were tested individually and in combination for the management of *P. palmivora* *in vitro*.

Materials and Methods

Bacterial strains

Biological control agents tested by Nor Dalila et al in 2014, designated B68 was isolated in durian fields of Mardi Sintok. Bacterial strains designated CD7, 8C and CA21 were obtained from Strategic Resource Research Centre, MARDI Headquarters, Serdang, Selangor.

Pathogen

P. palmivora was isolated from infected durian tree bark in MARDI Sintok, Kedah. It was isolated on V8 juice agar and then maintained at room temperature. For this experiment, 14 days old V8 plate of *P. palmivora* was used.

Treatments

Control plates comprised only PDA with *P. palmivora*. Antagonistic bacterial strains B68, CD7, 8C and CA21 were tested individually and as a combination. Five plates were prepared for each observation and incubated at room temperature ($\pm 25^{\circ}\text{C}$). Inoculation of pathogen was done by placing

a 0.8 mm diameter plug of *P. palmivora* facing down, 1.5 cm from the centre of the PDA petri dish. Bacterial strains were grown individually in nutrient broth for two days to be used individually or mixed by treatments. A filter paper was dipped in solution of bacteria and thawed on sterile filter paper. It was then placed 3 cm away from the *P. palmivora* isolate. Growth of *P. palmivora* was taken by measuring the diameter of the mycelium growth. Data of the percentage growth inhibition (PGI) was calculated using the formula of Zivkovic *et al.* (2010).

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

Where,

R1 = Growth of pathogen alone without antagonist (control)

R2 = Growth of pathogen along with the antagonist

Experimental design

The laboratory experiment consisted of sixteen treatments including the control treatment. The experiment was conducted using the completely randomized design (CRD) with five replications. All data were subjected to variance analysis (ANOVA) using SAS 9.3 TS Level 1M1. Differences between the means were compared by using Duncan's multiple range test.

Results and Discussion

The mean diameter growth of *P. palmivora* of control treatments are shown in Table 1. There was no significant difference in the antagonistic effects between T1 (B68) and T3 (8C) compared with the control treatment. The results showed that T11 (B68 + CD7 + 8C) had the significantly lowest diameter growth of *P. palmivora* compared to the other treatments.

PGI was taken into account to test the efficacy of the treatments. Here, most of combination treatments application showed better percentage of disease suppression with the usage of CD7 and CA21. Results of PGI showed that from a total of 16 treatments, T11 (B68 + CD7 + 8C) mixture controlled the pathogen significantly. Combination treatment of all four biological control agents, T15 (B68+CD7+8C+CA21) gave only a 53.7% percentage of PGI. This experiment demonstrates that certain biological control agents are mechanistically incompatible, in that one strain interferes with the mechanism by which a second strain suppresses a plant disease. In a similar case, Stockwell, V.O. *et al* (2011) tested a bacterial strain, A506, which diminishes the biological control activity of C9-1 and Eh252, thereby reducing the efficacy of biocontrol mixtures. Hence, the right combination of biological control agents gives the best result.

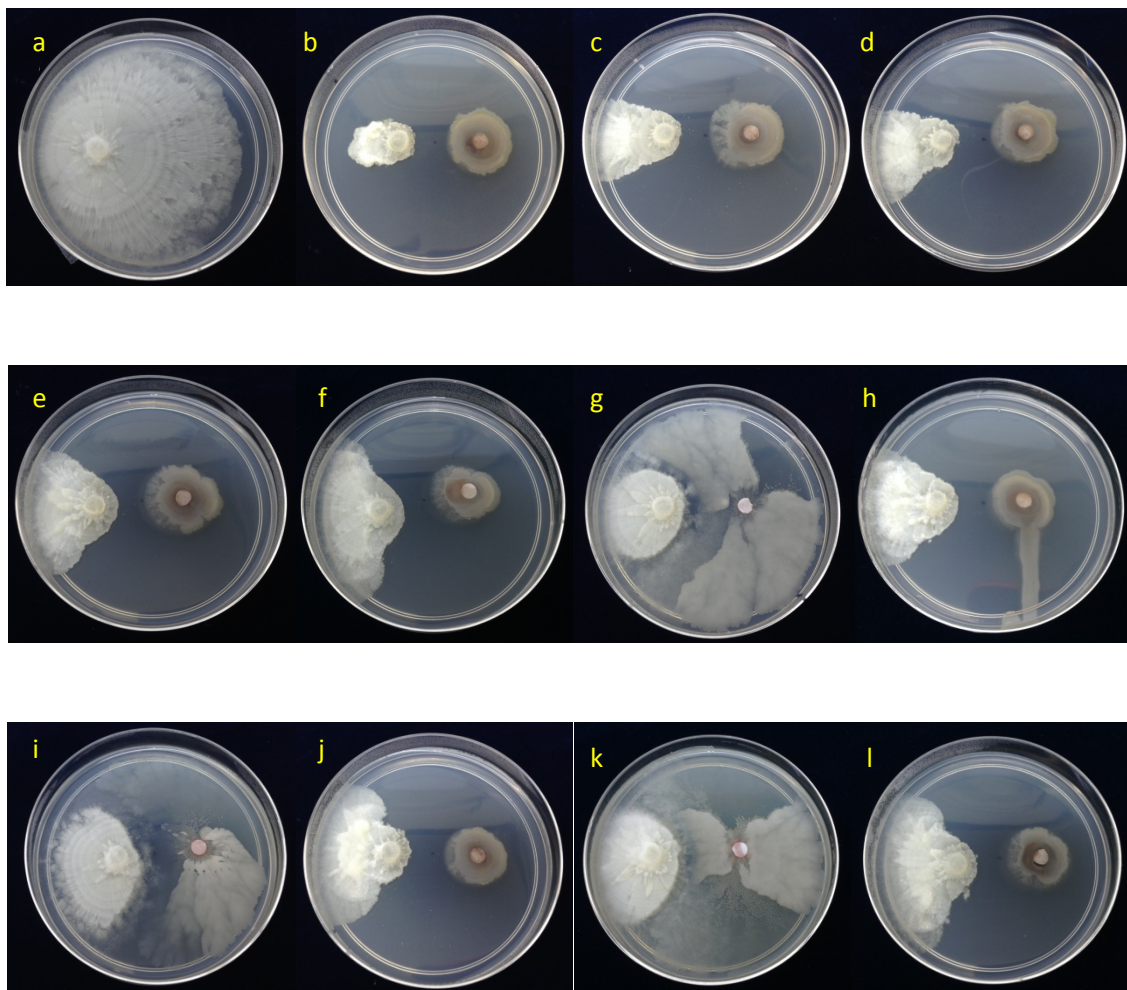
Table 1. Comparisons in diameter growth and percentage of growth inhibition among 16 treatments of *Phytophthora palmivora* and antagonistic bacterial strains applied individually or in combination to control durian canker.

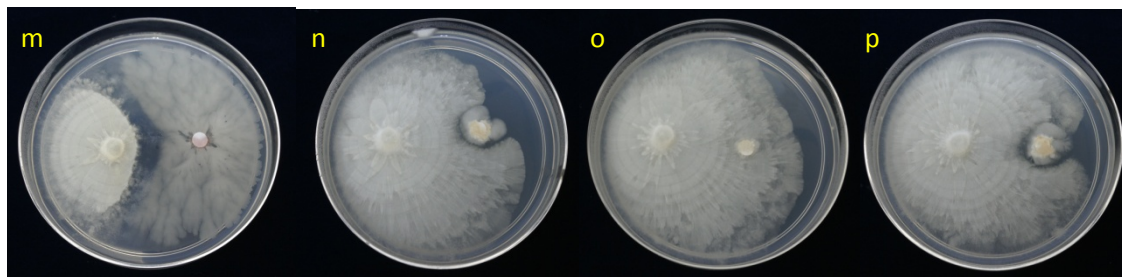
Treatment	Bacterial Strains	Diameter growth (cm)	Percentage of Growth Inhibition
Control	N/A	7.00 ab	N/A
T1	B68	7.35 a	-5.00 f
T2	CD7	3.48 cde	50.36 bcd
T3	8C	7.08 ab	-1.14 ef
T4	CA21	3.45 cde	50.71 bcd

T5	B68 + CD7	3.26 de	53.43 bc
T6	B68 + 8C	6.66 b	4.86 e
T7	B68 + CA21	3.64 cd	48.00 cd
T8	CD7 + 8C	3.38 cde	51.79 bcd
T9	CD7 + CA21	3.66 cd	47.71 cd
T10	8C + CA21	3.80 c	45.71 d
T11	B68 + CD7 + 8C	2.64 f	62.29 a
T12	B68 + CD7 + CA21	3.56 cde	49.14 bcd
T13	CD7 + 8C + CA21	3.10 e	55.71 b
T14	B68 + 8C + CA21	3.54 cde	49.43 bcd
T15	B68+CD7+8C+CA21	3.24 de	53.71 bc

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

Plate 1. Interaction between *P. palmivora* and antagonistic bacterial strains 15 days after inoculation. Each treatment was treated with *P. palmivora* and bacterial strain: Control (a); T11 (b); T13 (c); T15 (d); T5 (e); T8 (f); T4 (g); T2 (h); T14 (i); T12 (j); T7 (k); T9 (l); T10 (m); T6 (n); T3 (o) and T1 (p).





Conclusions

Mixtures of bacterial antagonists designed to be mechanistically compatible afforded significantly better control, with less variation in efficacy, than single-strain inoculants or mechanistically incompatible mixtures. Thus, the use of mixtures (B68 + CD7 + 8C) in treatment T11 may lead to more consistent management of durian canker.

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Effect of Seed-borne Pathogens on Germination of Chilli (*Capsicum annuum*) Seeds

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Introduction

Seed-borne pathogens have some detrimental effects on seed such as reducing seed viability, vigour, germination capability, shortening longevity of storage, and causing physiological changes. Some seed-borne pathogens are also seed-transmitted, which can cause severe diseases in the field that may usually result in yield losses (Neergard, 1979). Healthy or pathogen free seeds are considered as the key factor for desired plant population, good harvest, a successful crop establishment and production. Health of seeds can be affected by direct infection by pathogens or through contamination (Rashid and Fakir, 2000). For a good crop, the seed should be pure, viable and healthy which can contribute towards increased germination as well as yield. The most effective disease management strategy is accomplished by using seed testing or seed detection assays to screen and eliminate infested seed lots before planting. Moreover, early identification and listing of plant pathogens in the intended planting area may be part of control development and management strategies in order to avoid crop losses and to prevent the spread of plant diseases to new areas (Ekhuemelo and Ebenezer, 2013). Seeds of vegetables are more vulnerable to be attacked by pathogens and quickly deteriorate during storage as compared to other seeds (Hamim et al., 2014). Chilli is one of the most significantly important vegetable crops in the world and the lack of high quality chilli seeds and the prevalence of seed-borne diseases are among the main constraints in maintaining the sustainability of chilli crop production. Considering the above facts, this study was carried out to assess seeds of a potential inbred line of chilli, Line 5, for seed-borne pathogens and their effect on germination.

Materials and Methods

Preparation of seed samples

Chilli seeds (Line 5) were obtained from Horticultural Research Centre, Malaysian Agricultural Research and Development Institute (MARDI). A total of 600 plants were grown according to the standard agronomic practices and seed samples were extracted from their freshly harvested mature chilli fruits. Samples were then sun-dried until they reached 10% moisture content. Samples were kept in aluminium foil bags and were stored in the cold room (11°C, 56% RH) until they were used for subsequent studies.

Detection of seed-borne fungi

The presence of the major seed-borne fungi associated with the selected seed samples was detected using agar plate method. A total of 400 seeds were tested in eight replications. The experiment was repeated twice. Surface disinfected seeds (0.1% mercuric chloride) were plated onto PDA medium and the plated seeds were incubated for seven days at ambient conditions. At the end of the incubation period, fungi growing out from the seeds on the agar medium were examined and identified based on morphological characteristics of colony and sporulation structures of pathogen under a compound microscope using reference manuals by Watanabe (2010) and Mathur and Kongsdal (2003).

Effect of seed-borne fungi on germination

The effect of seed-borne fungi on germination and seedling infections were determined by germination test where 400 seeds were randomly taken from the samples with eight replications. Fifty seeds were placed directly on top of moist paper towels in each plastic container. The containers were placed inside the germination room. After 14 days of incubation, observations were recorded pertaining to percentages of germination (normal seedlings), non-germinated seed (rotten seed and dead seed), abnormal seedlings, diseased seedlings, shoot length, root length and vigour index. For determination of seedling vigour, 10 seedlings (normal/abnormal) were randomly selected from each paper and their individual shoot and root lengths were measured. Shoot length was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, root length was measured from the starting point of the root to the largest available lateral root apex. Vigour of the seedling was determined by the following formula:

$$\text{Vigour Index} = (\text{Mean of root length} + \text{Mean of shoot length}) \\ \times \text{Percentage of seed germination}$$

Experimental design

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

Results and Discussion

A total of 400 chilli seeds were tested and 11 fungi were detected whereby *Aspergillus niger*, *A. flavus*, *A. Parasiticus*, *Colletotrichum* sp., *Phoma* sp., *Pythium* sp. and *Rhizopus* sp. were the most predominant with percentage of incidence as shown in Table 1. They were considered as predominant because each of them constituted more than 5% of the total seed-borne fungi infection. The results revealed that seed-borne fungi were present in chilli seeds and most samples infected by these fungi showed damage of seeds to varying degrees, causing seed shrinkage or colour change.

The isolated fungi indicated the possible diseases that could affect chilli seeds and seedlings emerging from such infected seeds. These fungi have been reported to be pathogenic to seeds causing diseases such as damping off, root rot and fruit rot (Al-kassam and Monawar, 2000). Fungi from the genus *Aspergillus* produced aflatoxin which could make changes in the chemical ingredients inside the seeds, reduce nutritive value and viability of seeds and even cause seed death (Duan et al., 2007). *Pythium* sp. could cause damping off or root rot disease while *Rhizopus stolonifer* and *Phoma* sp. could lead to soft rot and seedling rot diseases. Seeds attacked by *Pythium* sp. usually fail to germinate resulting in poor stand development (Goldberg, 2011). *Colletotrichum* sp. has been found to be a serious causal agent of Anthracnose or ripe fruit rot of chillies worldwide. The use of *Colletotrichum* sp. infected seeds can give rise to weak seedlings which then become the primary inoculum source in chilli growing areas.

Table 1. Infection percentages and diseases caused by major seed-borne fungi isolated from chilli

Fungi	Common disease name	Effect on crops	Incidence (%)
<i>Aspergillus flavus</i>	Yellow mold	Reduced seed viability	18.75
<i>Pythium</i> sp.	Water mold	Damping off/root rot	12.5
<i>Phoma</i> sp.	Seedling rot	Reduced seed quality	12.5
<i>Aspergillus niger</i>	Crown rot/Collar rot	Damping off/root rot	12.5
<i>Colletotrichum</i> sp.	Anthraxnose	Ripe rot	6.25
<i>Rhizopus stolonifer</i>	Rhizopus rot/bread mold	Soft rot	6.25
<i>Aspergillus parasiticus</i>	Aflatoxin mold	Reduced seed quality	6.25

A low percentage of germination with 49.5% normal seedlings, 43.5% rotten and dead seeds, 5% abnormal seedlings and 2% diseased seedlings at 14 days after sowing (Figure 1). Seedling vigour index in chilli was 239.09 at 14 days after sowing with a mean root length of 3.06 cm and a mean shoot length of 1.77 cm. The findings showed that the percentage of seed germination and vigour index were decreased due to dead and rotten seed which was directly associated with seed-borne pathogen infection (Figure 2). Seed-borne fungi decreased seed germination by causing seedling death (Al-kassam and Monawar, 2000).

Furthermore, seed-borne fungi could be present on the seed surface and also inside the seed as the causal agents of diseases invading the roots, stems and leaves. Seed moisture content, temperature and degree of invasion of seeds by pathogens were among the factors that influenced the development of seed-borne pathogens (Anjorin and Mohammed, 2009). Besides, fungi could spread from seed to placenta of the fruit, and then penetrate the developing ovules or young seed with unignified testa at any point on their surface. Infection of seeds could also occur directly from the mother plant and could also be mechanically attached to the surface of the testa, and then remaining dormant until the seed germinated (Sariah and Zainun, 1988).

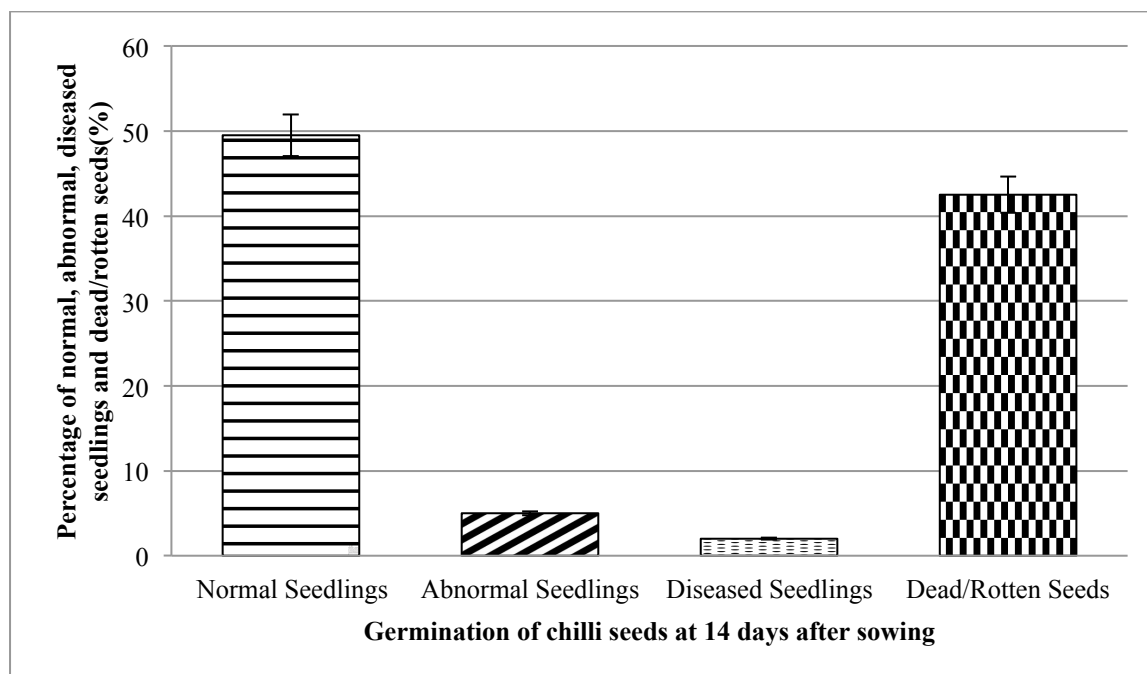


Figure 1. Percentage of normal seedlings, abnormal seedlings, diseased seedlings and dead/rotten seeds of chilli seed germination test at 14 days after sowing



Figure 2. Germination of chilli seeds; (A) Normal seedlings, (B) Abnormal and diseased seedlings, and (C) Dead/rotten seed at 14 days after sowing

Conclusions

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

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Conjugation of Antibody with Activated Peroxidase (Horseradish Peroxidase) in the Development of Sandwich Assay Immunosensor for Rice Tungro Disease Detection

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Introduction

More than 90% of the world's rice is produced and consumed in Asia (Hossain & Pingali, 1998). Of all viral diseases on rice, Tungro disease is the most economically devastating in South and Southeast Asia and has been recognised as a serious constraint to rice production (Bunawan et al., 2014). Epidemics of the disease have occurred since mid-1960s (Azzam and Chancellor, 2002). Rice Tungro Disease (RTD) is reported to be responsible for 5–10% annual losses of rice yield in Asia and about 2% in India.

This disease is caused by two types of viruses, *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV). Both viruses are transmitted by the green leafhopper (GLH), *Nephotettix virescens*. The symptoms and severity of this disease depends on these two types of viral agents. Severe typical symptoms of yellow-orange leaf discoloration, plant stunting and reduced yield will show if rice is co-infected by both viruses. On the other hand, if rice is infected only with RTBV, it shows milder symptoms while rice plants will show no disease symptom if they are only infected by RTSV (Tangkananod et al., 2005).

Since rice is a staple food for Asians and world's important cereals, early detection is important for a successful disease control which can reduce disease spreading and yield loss. Although symptoms of Tungro infected plants can be seen by visual or occasionally by insect transmission of the viruses to assay plants, but diagnosis of the disease by the symptoms alone is not reliable. This is because other disease and non-pathogenic disorders such as excess water after drought, insect injury or nutritional deficiencies also can show similar symptoms (Nath et al., 2000, Boltovets et al., 2004). PCR (polymerase chain reaction) is a reliable and accurate technique for viral detection but it is a destructive technique. DNA or RNA of the viruses needs to be extract before the PCR can be carried out. Therefore a rapid, simple, efficient and reliable technique should be develop for Tungro Disease detection. In this study, we described the conjugation of antibody with activated peroxidase (Horseradish Peroxidase) as part of the component in the Development of Sandwich Assay Immunosensor for Rice Tungro Disease Detection.

Materials and Methods

Conjugation of antibody with activated peroxidase

The conjugation process was done using EZ-Linked Plus Activated Peroxidase (Horseradish Peroxidase) which commercially provided by Pierce Ltd., UK. 1 mg of lyophilized Horseradish Peroxidase was dissolved in 100 ml of ultrapure water. Antibodies were then added into the solution and incubated for 1 hour at room temperature. A 10 μL of sodium cyanoborohydride solution was added and incubated for 15 min. Then, a 20 μL of quenching buffer was added and incubated again for 15 min. The mix solution was then dialyzed in 0.01 M PBS buffer.

Purification of conjugated antibody-HRP

Antibody-HRP conjugate need to be purify through gel filtration column using AktaPrime machine. Samples were injected into the column and eluted peak fractions were collected and stored at 4 $^{\circ}\text{C}$. Phosphate buffer saline pH 7.4 was used as buffer.

Confirmation test of purified antibody-HRP conjugate

Purified antibody-HRP conjugate was confirmed using ELISA method. Antibodies (RTBV and RTSV) were coated on the microtiter plate and incubated for 2 hours at 37 $^{\circ}\text{C}$. Plate was then washed with PBS-Tween buffer for 3 times. Next, microtiters well were blocked with 1% of Bovine Serum Albumin (BSA) at 37 $^{\circ}\text{C}$. After 30 min incubation, plate was washed for 3 times. Samples (purified viruses) were coated into the well and incubated overnight at 4 $^{\circ}\text{C}$. Plate was washed again for 3 times. Purified antibody-HRP conjugate was then added and incubated for 30 min. Lastly, 50 μL of TMB (3,3',5,5'-Tetramethylbenzidine) was added and the reaction was measured at 405 nm.

Results and Discussion

Conjugation of RTBV and RTSV antibodies with activated peroxidase is one of the important components in the development of sandwich assay immunosensor for Rice Tungro disease detection. Figure 1 shows the theoretical concept of the developed assay which involved 2 components. In this assay, virus samples will bind between antibody –nanogold conjugate (component 1) and antibody-HRP conjugate (Component 2).

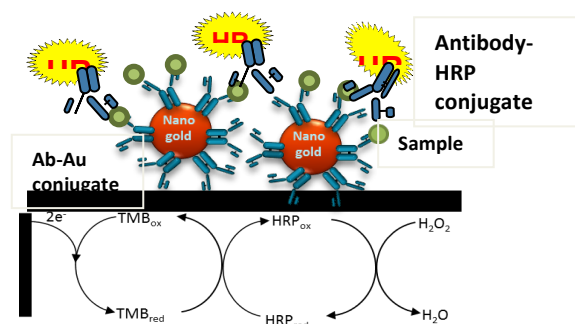


Figure 1. The theoretical concept of sandwich assay immunosensor

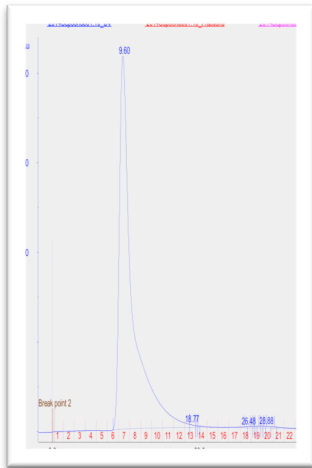


Figure 2. Peak obtained during purification of RTBV antibody-HRP conjugate

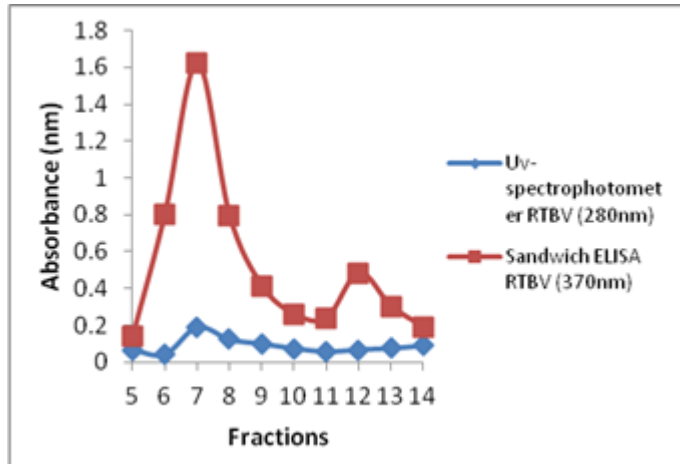


Figure 3. Plotted graph of confirmation test results using sandwich ELISA for RTBV antibody-HRP conjugate

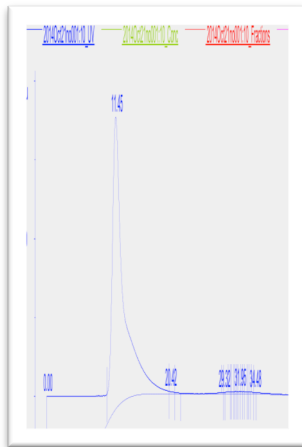


Figure 4. Peak obtained during purification of RTSV antibody-HRP conjugate

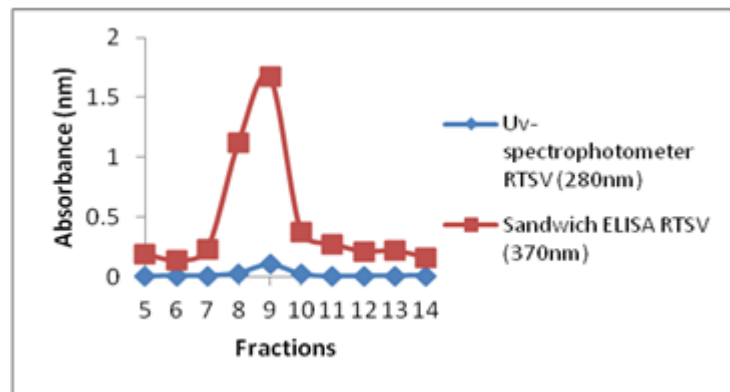


Figure 5. Plotted graph of confirmation test results using sandwich ELISA for RTSV antibody-HRP conjugate

Horseradish peroxidase has been used for conjugated with RTBV and RTSV antibodies. Antibody-HRP conjugate was then purified using gel filtration column. One peak was obtained for both RTBV (Figure 2) and RTSV (Figure 4) antibodies at fraction 7 and fraction 9, respectively, after run through the gel filtration column. Confirmation tests using sandwich ELISA was done for all fractions in order to confirm the successful of the conjugation process. Based on the graph plotted, the resulting peak showed at the same fraction. Antibody-HRP conjugate for RTBV was at fraction 7 (Figure 3) while antibody-HRP conjugate for RTSV was at fraction 9 (Figure 5). These results confirm that the conjugation process were successful.

Conclusions

Conjugation of RTBV and RTSV antibodies with HRP were successful. The antibody-HRP conjugate will be used for the next experiment in order to complete the development of Immunosensor for Rice Tungro Disease Detection.

Acknowledgements

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CHAPTER 9

STRESS BIOLOGY

Effects of Supplemented Calcium on Growth and Physiological Changes of Tomato Grown Under Salt Stress

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Introduction

Salt stress is one of environmental stress problems which can seriously threatened—agriculture practices. It was reported to have inhibited crops growth and significantly reduced yields (Shiam et al., 2015; Tuna et al., 2007). The low osmotic potential (osmotic stress), specific ion effects, nutritional imbalance and/or combination of these effects are some of the contributing factors leading to unfavorable growth conditions of the plants. (Parida and Das, 2005). Na^+ which is the general constituent of salt may not be essential elements for most plants, but under low Na concentrations, growth of plants can be stimulated or reduced (Marschner, 1995). For instant, under salt stress, Na concentrations increased in the rhizosphere and alter nutritional requirement by plants in order to alleviate salt stress effects that inhibited the growth of plants (Cerdeira and Martinez, 1988). Coincidentally, most crops symptoms associated with nutrient deficiency symptoms are always due to salt stress such as calcium, one of essential elements for plants growth. The abnormal regulation of cellular Ca^{2+} partitioning and distribution in the fruits, leaves and roots are the sequence from the competition of Na^+ - Ca^{2+} uptake in the root zone when Na^+ is highly accumulated. To date, the interactions of Na-Ca are very well documented (Cramer, 2002), and any external calcium supply in the solution form can ameliorate salt stress effects on plants. Calcium which is responsible for maintaining membrane integrity, controls selectivity of ion uptake and transport (Marschner, 1995) can reduce the permeability of plasma membranes to Na^+ when present in high concentrations due to passive influx (Cramer et al., 1985). Hence, by using this theory, it is suggested that supply of calcium can ameliorate salt stress effects and improved growth of plant. In Malaysia, lowland tomato is also cultivated in the glasshouse apart from growing on the highlands. The risk of having salt stress in the soilless culture system is also possible from a wide source such as the tap water itself, or non-flush media that leads to high accumulation of salt in the root zone. Thus, the main objective of this study is to determine whether Ca^{2+} would correct calcium deficiency at certain level in the presence of medium level of salinity and improve growth of tomato plants at a physiological level.

Materials and Methods

This study was conducted in the Glasshouse Centre, Faculty of Agriculture, UPM. The tomato seeds are from the Pearl cultivar, obtained from a local seed company. It was then sown and transplanted in white polybags containing 100% coconut coir dust (CCD) for two weeks and each plant consists of 4-5 true leaves. Basic nutrients solution used in this study was based on Cooper formulation (Cooper, 1979), and applied via drip irrigation. Treatments used in this study were: (1) nutrient solution plus 66 mM NaCl (control); (2) nutrient solution and 66 mM NaCl plus 2.5 mM CaSO_4 ; (3) nutrient solution and 66 mM NaCl plus 10 mM CaSO_4 . When the flowers fully bloomed in the first truss, basic nutrient solution was increased up to EC= 2.5 mS/cm. The pH was adjusted periodically up to 5.6 with a minimum volume of 1.0 mM KOH. The electrical conductivity for all treatments was maintained throughout the experiment at: (1) C: EC 9.3 mS/cm; (2) EC 10.1 mS/cm; (3) EC 10.6 mS/cm. Each treatment was replicated five times and consists of six plants per replication which were arranged in a nested design. At week eight, data collected were include plant height, total leaf area, root and shoot dry weight, and root to shoot ratio. The gas exchange measurements on photosynthetic rate and stomatal conductance in the upper fully expanded leaves were measured at 9 am in the glasshouse using portable photosynthesis system (Licor Model 6400-XT).

Results and Discussion

The results obtained after eight weeks showed that supplementation of calcium sulphate can significantly influenced and improved the plant growth in terms of height, total leaf area, shoot dry weight and root dry weight due to salt stress and have constant effects on them. However, supply of 2.5 mM CaSO₄ showed slightly higher plant height, shoot dry weight and root dry weight compared to 10 mM CaSO₄ (Table 1). The root shoot ratio however, decreased as calcium level increased. This indicated that root was more affected than shoot by the salt treatment. A growth similar growth effects of calcium in ameliorating salt stress has also been reported on other crops (Kaya et al., 2002; Hao and Papadopoulos, 2004; Tuna et al., 2007; Soualem et al., 2014). However, there are some other species and cultivars that responded differently to supplemental calcium when salinized (Cramer, 2002). Kenaf (*Hibiscus cannabinus*) and wheatgrass (*Thinopyrum ponticum*) are examples of genotypes which responded negatively to supplementation of calcium on growth by showing chlorosis in the lower leaves (Cramer, 2002). The amelioration effect of supplemental Ca²⁺ in salt stressed plants has been related to a direct apoplastic role of divalent cations, such as Ca²⁺ and Mg²⁺, which reduce Na⁺ uptake by inhibiting nonselective cation channels (Tester and Davenport, 2003). Contrast to growth, the presence of external calcium had significantly lowered the photosynthetic rate of tomato Pearl by about 53% at both 2.5 and 10 mM CaSO₄. (Figure 1a) On the other hand, supplemental of calcium significantly increased stomatal conductance at 2.5 mM CaSO₄ by about 40% but there was no significant difference for both control and 10 mM CaSO₄. (Figure 1b).

From this study, it was observed that calcium treatment was not consistent with the physiological variables above, contrary to the findings in the previous shown in different crops (Vieira-Silva et al., 2003). There was no Na-Ca interaction observed in photosynthesis of the tomato and both levels of calcium were not able to ameliorate medium salt stress which effect on photosynthesis rate. The limited ability of Ca²⁺ to ameliorate salt stress could be due to salinity-induced osmotic stress that cannot be overcome by external calcium (Reid and Smith, 2000). This showed that salinity significantly inhibited the gas exchange for certain crops by causing stomatal closure and decreased CO₂ assimilation (Cabot et al., 2009). Stomatal closure with supplementation Ca²⁺ was related to a decrease in root hydraulic conductivity and xylem-sap flow due to mono/divalent cation ratio on water uptake and transport. Similarly, photosynthesis rate, stomatal conductance are also been inhibited at high calcium level as shown in this study.

Nutrient concentrations in leaves and roots were determined in this study. Based on the results in Table 2, supplementation of calcium at both levels was significantly improved by the nutrient uptake for K⁺ and Ca²⁺, and at the same time lower the uptake of Na⁺ and Cl⁻. K⁺ which was improved by 32% and 49% as compared to control at 2.5 and 10 mM CaSO₄, respectively. However, K⁺ in the roots was reduced at highest level of calcium sulphate. Concentrations of Ca²⁺ in the leaves increased about 15% and 20% at both 2.5 and 10 mM CaSO₄. In this study, supplementary of calcium shown there is a corrected nutritional imbalance in the plants due to the presence of high Na⁺ concentration in the root zone that inhibit the uptake and transport of the other ions (Perez-Alfocea et al., 1996). The obvious response was shown on K⁺ and Ca²⁺ concentrations in both leaves and roots of tomato where the plants shifting the uptake in favor of K⁺ and Ca²⁺ at the expense of Na⁺ (Tuna et al., 2007). According to Tyerman and Skerrett (1999), Na⁺ also able to enter the cells through the ion channels which are more selective to Na⁺ than K⁺. The increase in external Ca²⁺ concentration reduces Na⁺ conductance through these channels, and regulates the other ions through Ca²⁺ signaling pathway (White and Broadley, 2001), mineral balance, and K⁺/Na⁺ selectivity of the plant (Liu and Zhu, 1998).

Table 1. Effects of supplementary calcium sulphate on growth of tomato (*Lycopersicon esculentum* Mill. cv. Pearl) under saline condition (66 mM NaCl) at flowering stage.

CaSO ₄ (mM)	Plant height (cm)	Total leaf area (cm ²)	Shoot dry weight (g)	Root dry weight (g)	Root to shoot ratio
0	96.2 ^b	1362.6 ^b	16.46 ^b	1.41 ^b	0.088 ^a
2.5	111.2 ^a	1973.6 ^a	24.37 ^a	1.85 ^a	0.076 ^b
10	107.2 ^a	2237.5 ^a	23.94 ^a	1.37 ^b	0.056 ^c
Pr > F	*	**	**	**	**

Mean separation within columns and factors followed by the different letters are significant by DMRT
 *P<0.05, **P<0.01

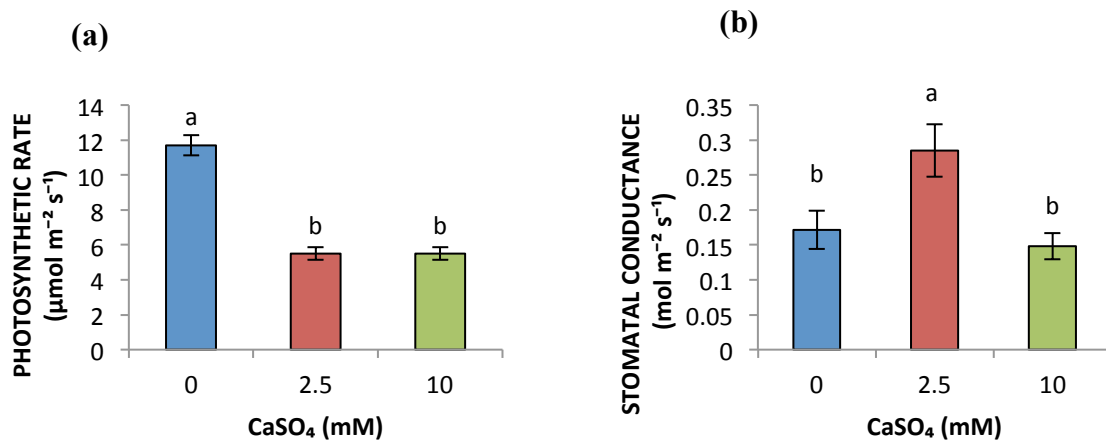


Figure 1 label the figure (a, b). Photosynthetic rate and stomatal conductance of tomato (*Lycopersicon esculentum* Mill. cv. Pearl) at flowering stage supplemented with calcium sulphate under saline condition (66 mM NaCl). Bar indicates the standard error of mean (n=5). Means with different letters are significantly different at p<0.05 according to Duncan multiple range test analysis.

Table 2. Effects of supplementary calcium sulphate on K, Ca, Na and Cl concentrations (% dry matter) in leaves and roots of tomato (*Lycopersicon esculentum* Mill. cv. Pearl) under saline condition (66 mM NaCl) at flowering stage.

CaSO ₄ (mM)	Leaf				Root			
	K	Ca	Na	Cl	K	Ca	Na	Cl
0	1.72 ^b	2.97 ^b	1.44 ^a	64.20 ^a	2.29 ^a	3.19 ^{ab}	1.29 ^a	13.23 ^a
2.5	2.53 ^{ab}	3.50 ^a	0.97 ^a	48.33 ^a	2.44 ^a	1.84 ^b	0.95 ^a	16.66 ^a
10	3.40 ^a	3.73 ^a	1.10 ^a	61.50 ^a	1.75 ^b	3.42 ^a	1.05 ^a	11.60 ^a

Pr > F	*	*	ns	ns	**	ns	ns	Ns
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Mean separation within columns and factors followed by the different letters are significant by DMRT

**P<0.05, **P<0.01, ns not significant*

Conclusion

The supplementation of calcium sulphate to salt stress may offer a simple solution to plant growth and overcome physiological process caused by salinity. However, in this study it was suggested that medium level of salinity could be corrected by using low level of calcium sulphate at 2.5 mM. This will result in lower uptake of Na⁺ and increase Ca²⁺ concentrations, whereby there will be ~~and~~ partial preservation of membrane integrity damage caused by NaCl

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Efficiency of *Bambusa vulgaris* to Clean up Heavy Metal Contaminants

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Introduction

Bamboo is known as a highly useful plant; providing poles and timber for scaffolding, fencing, roofs, furniture, and many craft items. However, it also play valuable environmental role that important for ecological protection in water conservation and erosion control. Bamboo is a water loving plant and it has complex root system which is good for water filtration, removing nutrients such as nitrogen, phosphorous and dangerous contaminants such as heavy metals (Ndzana, 2008). Because of that, bamboo could have many offers in slum areas that being used to clean up waste water. Bamboo also has the capacity for high level of nitrogen uptake. Hence, it is suitable for disposal of effluents and reduce of waste water pollution. Planted alongside rivers, creeks and ditches and holding dams, bamboo can catch the excess nutrients in the runoff water, thus, preventing from entering nearby streams. Some perennials bamboo species are known to have several merits, including high stress tolerance, high growth and biomass production (Gratani et al., 2008). Recently, some researchers reported on the heavy metal tolerance and accumulation in some bamboo species (Truyens et al., 2012).

Metals are naturally present in the pedo-geochemical background of soils at various levels. They are essential to plants but some of them may be toxic at higher concentrations. Heavy metals ions, when present at an elevated level in the environment, are excessively absorbed by roots and translocated to shoot which can lead to impairing metabolism and reducing the growth. Metals such as Cu, Zn, Cd, Cr, Ni and Pb are known to be serious environmental pollutants that can pose a major environment and health problems. Besides that, excessive metal would result in a reduction in microbial activity, soil fertility, and yield loss. Metals accumulate in soil is due to anthropogenic contamination through fertilizer, organic manure applications, irrigation, industrial and municipal wastes, and wet and or dry deposits (Doelsch et al., 2010).

Materials and Methods

The study was conducted in the nursery of Faculty of Forestry, Universiti Putra Malaysia, Serdang, Selangor from May 2014 until August 2014. The range of average daily temperature was 27-30°C with the relative humidity of 60-71%. In this current project, a total of 32 saplings were obtained from a nursery, Forest Research Institute Malaysia (FRIM), Selangor, Malaysia. After acclimatization for one month, uniform bamboo plant species that were grown on the same substrate were carefully transplanted into a lysimeter, a plastic pot that was filled with soil and sand (ratio of 3:2). The pots were arranged in Completely Randomized Design (CRD) and were then covered with plastic to control the transpiration via soil medium. This study was designed with three treatments and control in eight replications. These treatments consist of different heavy metals copper, zinc and iron mix with the water to reach certain level concentration 100 ppm, 200 ppm and 300 ppm, respectively. The concentration of heavy metal contents were evaluated as an initial data and the samples of water leachates were be collected every two weeks before and after being infiltrated by bamboo. The leachates were then gone through a screening process using solar Thermo Atomic Absorption Spectrophotometer (AAS) in order to determine the contents of heavy metal. Morphological attributes of bamboo growth such as height and diameter of bamboo culm, number of shoots and branches as

well as their physiological attributes such as chlorophyll content, chlorophyll fluorescence and gaseous exchange were identified to determine their tolerance towards heavy metals. Statistical analysis was carried out using the statistical software SPSS22.0 by one way ANOVA with *post hoc test* by Tukey's HSD at $P \leq 0.05$ significance level to elucidate further the significance between treatments.

Results and Discussion

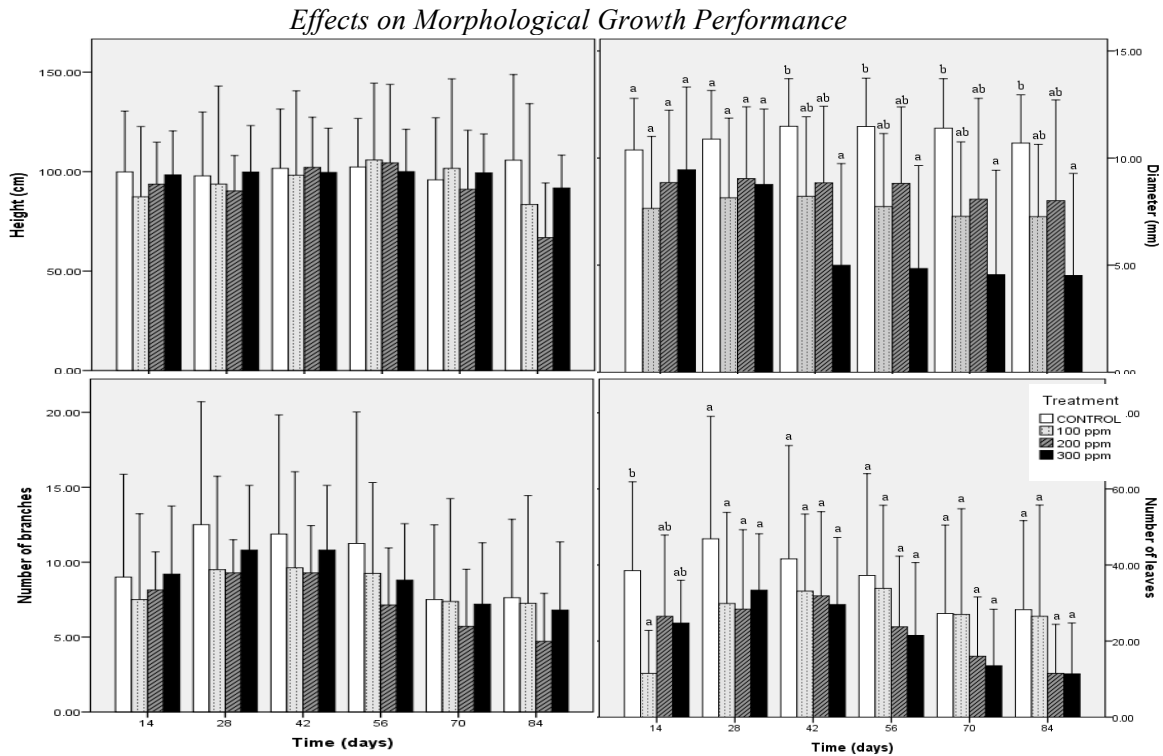


Figure 1: Height, diameter, number of branches and leaves of *B. vulgaris*. Values with different letters indicate significant differences between treatments by Tukey's HSD at $P \leq 0.05$

The highest total height and culm diameter of the *B. vulgaris* plants were recorded in control. *B. vulgaris* plants in control also produced most number of branches and leaves. This showed that *B. vulgaris* exhibited the best growth in terms of height, number of leaves and basal diameter for control. However, the *B. vulgaris* plants in treatment 300 ppm exhibited the lowest height and culm diameter. Furthermore, *B. vulgaris* plants in 300 ppm treatment also recorded the lowest number of branches and leaves. This is a clear indication that the *B. vulgaris* is unable to grow optimally in soil with very high amounts of heavy metal treatment. The growth or increase in the culm height and diameter of *B. vulgaris* was found in the 2 – 8 weeks of observation, which likely occurred due to the plants attempting to acclimatize themselves to their new growing medium. Improvement in the growth parameters of *B. vulgaris* is due to the micronutrient contribution from heavy metal (Majid et al., 2011; Parisa et al., 2010). These results prove that *B. vulgaris* has the ability to accumulate metals. After 8 weeks, *B. vulgaris* planted in 200 and 300 ppm heavy metal treatment exhibited the worst growth performance, indicating that the highest concentration of heavy metal would be the least ideal growth media for the plant, maybe due to higher toxicity from heavy metal content and soil acidity (Mangkoedihardjo and Surahmida, 2008). High metal concentrations in the growth media of plants would normally restrict germination and negatively affect the roots, shoots and leaf growth of the plants (Parisa et al., 2010).

Physiological Tolerant Rates in Leaves

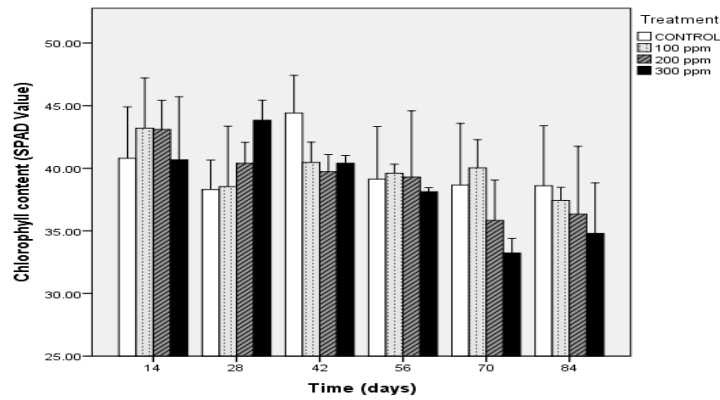


Figure 2: Mean chlorophyll content of *B. vulgaris*

The content of chlorophyll was reduced by the presence of Cu, Zn and Fe in the growth medium especially at the highest concentration. The decline in chlorophyll content is believed to be a consequence of the substitution of the heavy metal in chlorophyll. This prevents photosynthetic light harvesting in the affected chlorophyll molecules and results in breakdown of photosynthesis. From the observation throughout the experiment period, the plants which being exposed to heavy metals have changed its colours from bright green to light green, yellow or brown while the control remained the colour of green. This shows that the heavy metals affected the chloroplast negatively. The reduction in chlorophylls is in parallel with the toxicity symptoms observed in *B. vulgaris*.

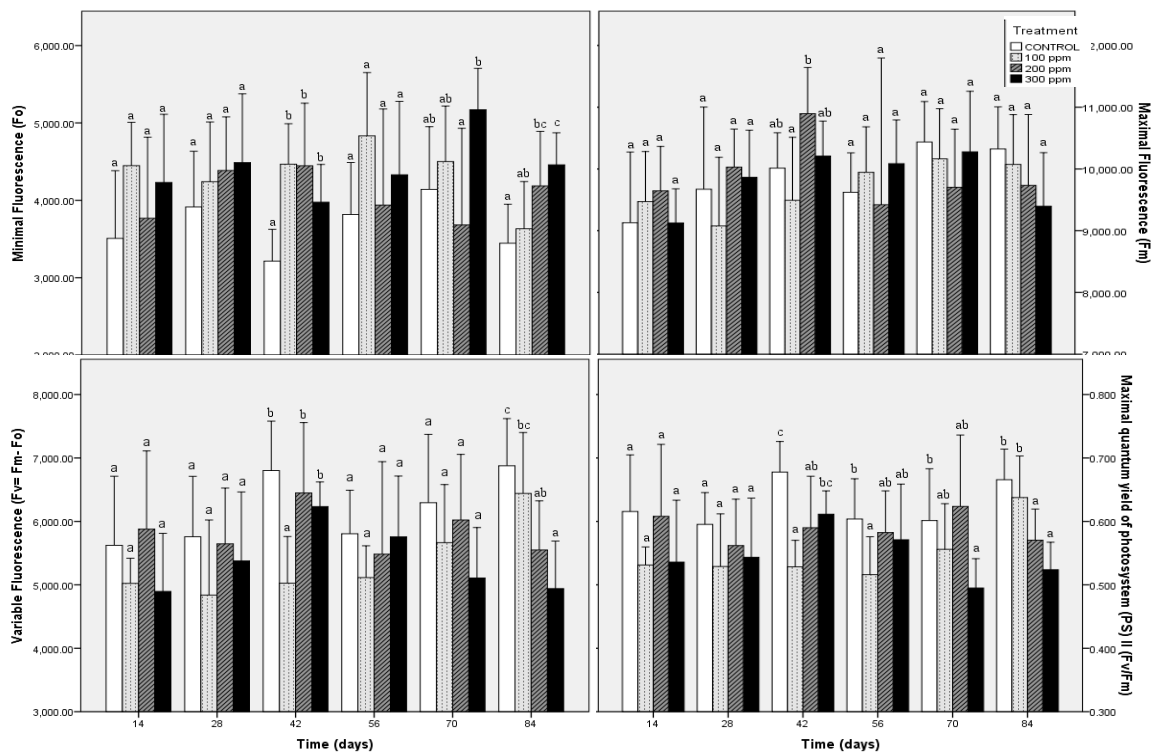


Figure 3: Mean values over time of chlorophyll fluorescence parameters F_o , F_m , F_v and F_v/F_m of *B. vulgaris*. Values with different letters indicate significant differences between treatments by Tukey's HSD at $P \leq 0.05$

Chlorophyll fluorescence is very useful to study various fundamental aspects of photosynthesis. It is an indicative of the photosynthetic activity and status of the device of photosynthesis. When plants previously adapted to darkness are illuminated, the intensity of chlorophyll fluorescence kinetics shows highly dependent photochemical reactions of photosynthesis (Juneau, 1999). Measurement of chlorophyll fluorescence is a potential indicator of photosynthetic efficiency in plants and proved as a rapid, non-invasive, and reliable method to assess photosynthetic performance under environmental stress (Krause and Weis, 1991; Schreiber et al., 1994) and allows the location of primary site of damage induced by environmental stress. There was a significant change of F_o , F_m , F_v and F_v/F_m under different stresses which decreased gradually levelled off with increasing concentration, indicating that heavy metal had inhibitory effect on the photochemical activity of *B. vulgaris*. It usually be explained as decrease of number of the closed PSII reaction centres, which do not participate in electron transport (Toth et al., 2005).

Gaseous Exchanges

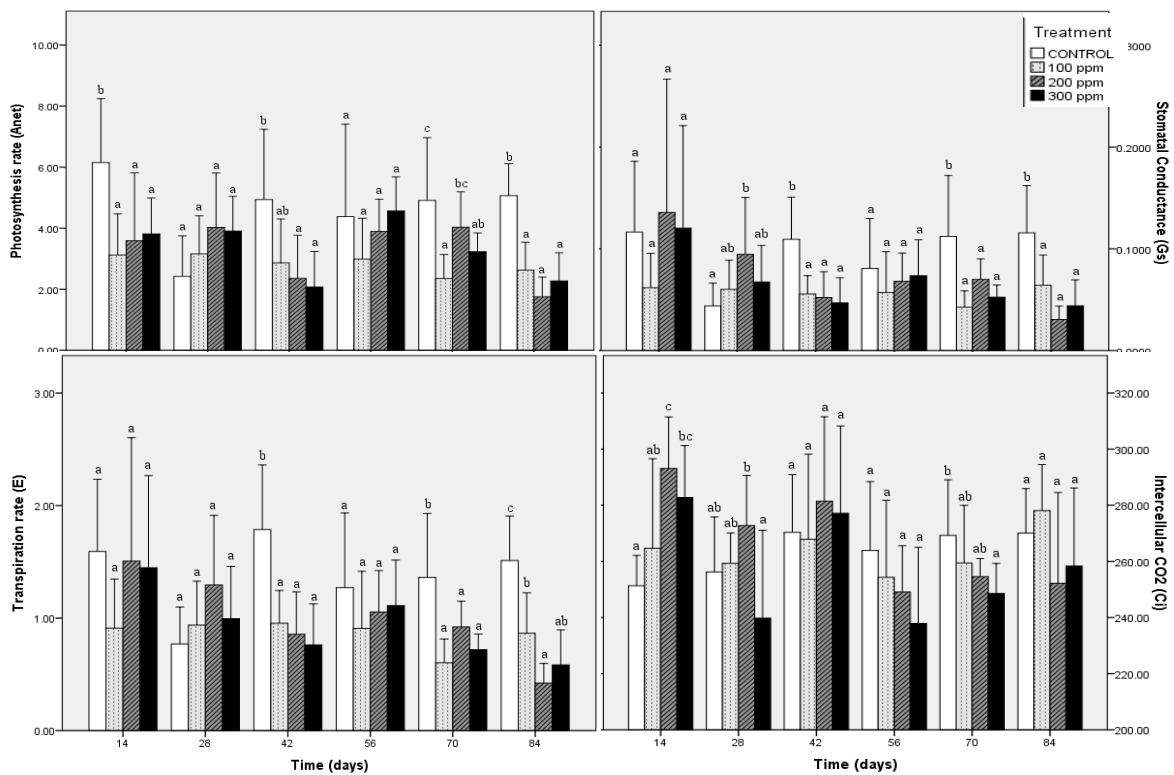


Figure 4: Mean values over time of gaseous exchange parameters A_{net} , G_s , E and C_i of *B. vulgaris*. Values with different letters indicate significant differences between treatments by Tukey's HSD at $P \leq 0.05$

Generally, gas exchange results strongly support the decline in morphological growth. The morphological growth decline trends were observed for each and every parameter taken in gas exchange, chlorophyll content as well as in transpiration and photosynthetic rates. Rates of gas exchange in plant differ amongst species due to some limiting factors such as light, carbon dioxide, temperature, oxygen and water (Robert et al., 1971). The parallel change of A_{net} (photosynthesis rate), G_s (stomatal conductance) and C_i (intercellular CO_2 concentration) in this study provided evidence that the photosynthetic responses of *B. Vulgaris* to excess heavy metal might be mainly due to the alteration of the pigment contents and stomatal conductance under heavy metal stress. Photosynthesis is a highly sensitive process significantly affected by heavy metals in several of plant species. The degree of heavy metals effect on photosynthesis depends on the growth stage, plant conditions as well as on the duration of stress. Heavy metal application was shown to affect photosynthetic functions directly or indirectly. There was a clear decreasing tendency for photosynthesis rate and stomatal

conductance. Reduction in net photosynthesis rate was strongly correlated with depressed growth. This implies that inhibition of plant growth can be partially attributed to the reduction of carbon assimilation under stress (Lovelock and Ball, 2002). The reduction of photosynthesis may be the consequences of stomatal closure (Downton et al., 1985; Youssef, 2007) and/or non-stomatal inhibition of photosynthesis (Jeranyama and DeMoranville, 2008). Stomatal closure is likely the first defense of heavy metal stress. According to Farquhar et al. (1989), lower photosynthesis rate (A_{net}), accompanied by lower stomatal conductance (G_s) and lower intercellular CO_2 (C_i) at control and low concentration of heavy metal (100 ppm) might be mainly ascribed to stomatal closure, which restricts CO_2 entry into leaves. Whereas, lower A_{net} accompanied by lower G_s and higher C_i at high concentration of heavy metal (200,300 ppm) may be attributed to non-stomatal limitation, including changes in leaf biochemistry that results in inhibition or down-regulation of photosynthesis.

Conclusions

Heavy metals copper zinc and iron are considered to be essential for plant growth due to the positive response in morphological and physiological at low concentration however brings phytotoxicity effects at high level of concentration and time. Heavy metal toxicity in plants depends on plant species, specific metal, concentration, chemical form and soil composition (Nagajyoti et al., 2010). Thus we can conclude that *B. vulgaris* is excellent to clean up heavy metal but weak to tolerate with high concentration of heavy metal contaminants. It has a potential to be a phytoremediator plant and also can be grown on moderately contaminated soil when put up with a growth reduction but it still needs to be further evaluated to optimize its potential.

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Antioxidative Defense Mechanism of Coconut Stem (*Cocos nucifera*) Against the Invasive Coconut Pest, Red Palm Weevil (*Rhynchophorus ferrugineus* Olivier)

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Introduction

Coconut is known as a popular industrial crop worldwide since every part of it has its own commercial value. There are 12 different varieties of coconut that can be found and one of them that is commonly planted in Malaysia is Malaya Yellow Dwarf/Malayan Red Dwarf X Tagnanan Tall (MATAG) (Mohd. Taufik and Md. Akhir, 2014).

Coconut nowadays has been attacked by the invasive red palm weevil (RPW), *Rhynchophorus ferrugineus* from the family of Curculionidae, which originates from Southeast East. The RPW-coconut interaction contributed to great loss in coconut industry. El-Mergawy and Al Ajlan (2011) reported that *R. ferrugineus* were spread slowly and attacking many palm species, especially in the Middle East and several countries of the Mediterranean Basin in three ways (Wahizatul et al., 2013); through the shoot and straight to the cabbage of the coconut, through the trunk and lastly through the root system.

Infestation of *R. ferrugineus* is believed to trigger the oxidative stress response in coconut plants resulting in the overproduction of highly reactive oxygen species (ROS) (Gill and Tuteja, 2010). In response to the pathogen attack, coconut plants activate both enzymatic and non-enzymatic antioxidants. This research was conducted to elucidate the defense mechanism of coconut plant in coconut-RPW infestation by measuring the enzymatic (catalase: CAT, ascorbate peroxidase: APX and guaiacol peroxidase: g-POD specific activities) and non-enzymatic antioxidants (ascorbic acid, α -tocopherol and carotenoids contents) in two different parts of MATAG cultivar stem.

Methodology

CAT specific activity was extracted according to the method of Clairbone (1985). The rates of changes in absorbance of the reaction mixture were monitored at 240 nm for 3 minutes. APX specific activity was analyzed following the method of Sairam et al. (1998) and Nakano and Asada (1981). The changes in absorbance were monitored at 290 nm at 3 minutes and were expressed as moles ascorbate oxidized per hour per mg protein. g-POD specific activity was estimated based on the method of Agrawal and Patwardhan (1993) and the changes in absorbance was monitored at 470 nm for 3 minutes. Protein content was measured according to the method of Bradford (1976). The absorbance of the enzyme extract was analyzed at 595 nm and the protein content was calculated according to a standard curve prepared at various concentrations of Bovine Serum Albumin (BSA) (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml). Ascorbic acid was extracted according to the procedure of Jagota and Dani (1982). Absorbance of the mixture was measured at 760 nm. A standard curve was prepared using ascorbic acid at various concentrations (0-60 μ g/ml) and the amount of ascorbic acid was calculated based on the standard curve. α -tocopherol was extracted based on the method by Hodges et al. (1996) while the assay mixture was prepared as described by Kanno and Yamauchi (1977). The amount of α -tocopherol in stem samples were calculated based on the standard curve prepared using α -tocopherol at various concentrations (0-1.4 μ g/ml). Carotenoid content was analyzed based on the method proposed by Lichtenthaler (1987). The supernatant was measured at 663.2 nm, 646.8 nm and 470 nm while 80% acetone was used as a blank.

Results and Discussion

Based on Figure 1A, CAT specific activity in infested upper stem increased significantly ($p < 0.05$) on 7 days of infestation. This indicated that CAT enzyme may involve in defense response and probably decreased the toxicity of ROS (Khorshidi and Sherafatmandjour, 2013). However, both CAT specific activities in control and infested plant dropped at day 14 due to the inactivation and degradation of CAT (Feireabend et al., 2012). Figure 1B showed that CAT specific activity of both non-infested and infested lower stem decreased, especially from day 14 to 28.

Non-infested upper stem showed significantly ($p \leq 0.05$) highest specific activity of APX in response to RPW infestation at day 21 (Figure 1C) and this same pattern went to the infested lower stem at 14 days of infestation. The findings of this APX specific activity accretion probably decreased the toxicity of ROS as APX plays a secondary role in H_2O_2 scavenging (Gondim et al., 2012; Khorshidi and Sherafatmandjour, 2013). On the other hand, infested upper stem seemed increasing from day 14 to 28 (Figure 1D). This described that continuous increases in level of antioxidant enzyme had the function to help the preservation of the cell structure much longer by scavenging the activated oxygen species produced (Ahn et al., 2005).

Infested upper stem significantly enhanced ($p < 0.05$) the g-POD specific activity and showed maximum activity compared to non-infested stem at day 7 due to RPW-coconut infestation (Figure 1E). When plant grew in stressed environment, toxic oxygen free radicals were either directly or indirectly generated (Dandan et al., 2011). Thus, the increment in g-POD specific activity helps to reduce the oxidative stress in plant (Weckx and Clijsters, 1996). As in lower part of infested MATAG stem, g-POD specific activity drastically decreased ($p < 0.05$) from day 7 to 28 (Figure 1F).

Based on Figure 1G, ascorbic acid amount in infested upper part of stem of MATAG decreased at day 21. However, infested lower stem had significantly ($p < 0.05$) higher amount of ascorbic acid at day 14 of infestation compared to non-infested stem as shown in Figure 1H. This indicated that ascorbate as the hydrogen donor had eliminated the generation of H_2O_2 in plant peroxidase (Asada, 1994).

The level of α -tocopherol was recorded to be significantly ($p < 0.05$) the lowest in non-infested upper (Figure 1I) and lower (Figure 1J) MATAG stem at 7 days of infestation compared to infested stem. However, no significant difference was generally observed in both infested upper and lower stem throughout the period. The α -tocopherol levels showed changes during plant growth, development and in response to oxidative stress as a result of the altered expression of pathway related genes, degradation and recycling (Munne-Bosch, 2005). This also represents that α -tocopherol level and its composition vary during cell development and in response to biotic stress (Collakova and Dellapenna, 2003).

Generally, the level of carotenoid content fluctuated in both control and infested upper stem of MATAG cultivar throughout the experiment (Figure 1K). Nevertheless, significantly higher ($p < 0.05$) carotenoid content was produced in infested stem at day 7 of the infestation (Figure 1K). This increment helps the cell to encounter the overproduction of ROS resulting in damage to the photosynthetic apparatus that can cause photoinhibition (Breusegem et al., 2001).

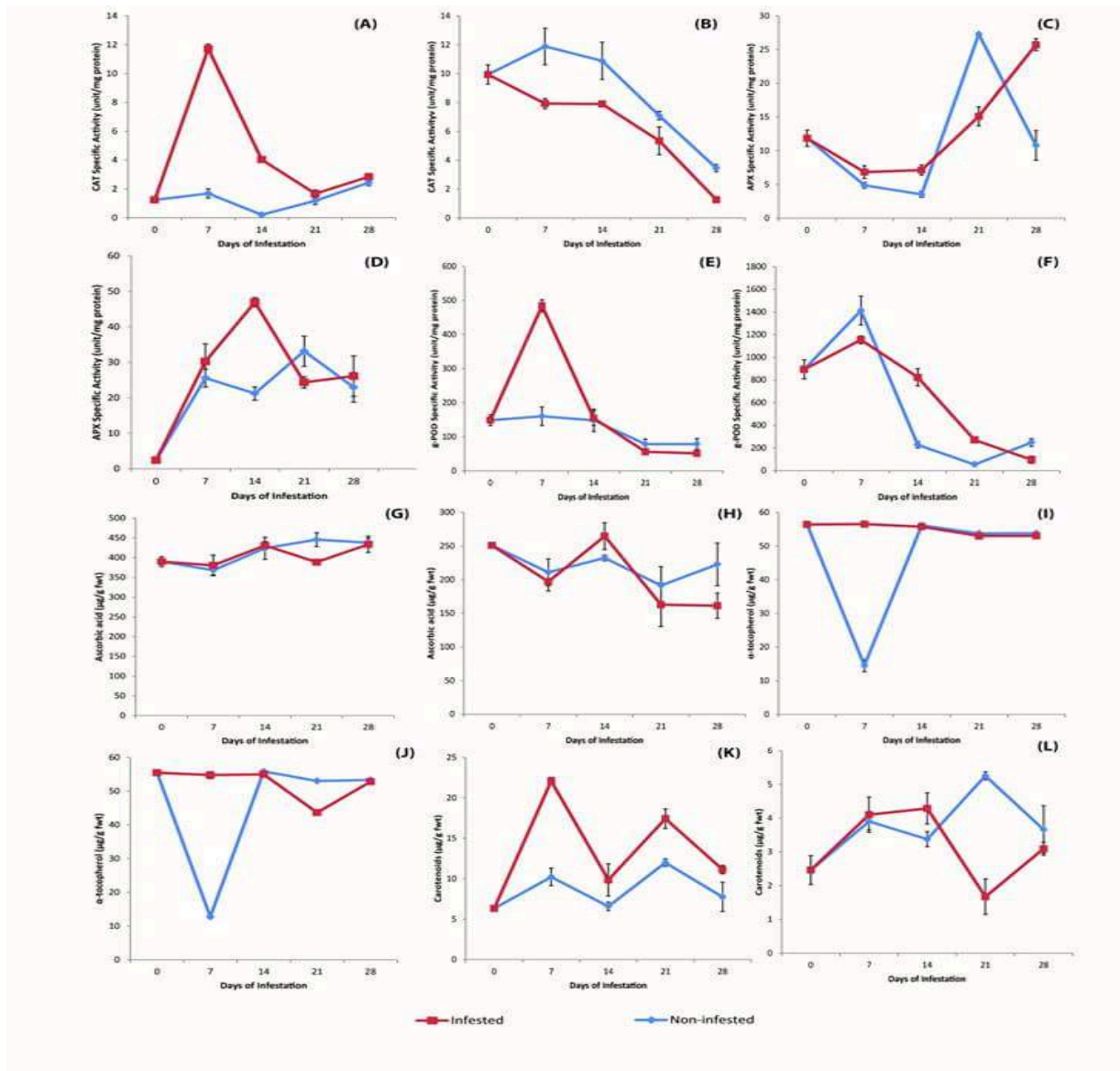


Figure 1. The effect of coconut-RPW infestation on enzymatic and non-enzymatic antioxidants in different parts of MATAG stem at 0, 7, 14, 21 and 28 days of infestation; (A) CAT specific activity (upper part), (B) CAT specific activity (lower part), (C) APX specific activity (upper part), (D) APX specific activity (lower part), (E) g-POD specific activity (upper part), (F) g-POD specific activity (lower part), (G) ascorbic acid amount (upper part), (H) ascorbic acid amount (lower part), (I) α -tocopherol amount (upper part), (J) α -tocopherol amount (lower part), (K) carotenoids content (upper part), (L) carotenoids content (lower part). Data are mean \pm standard error (n=3).

Conclusion

Results indicated that the CAT, APX and g-POD specific activities as well as ascorbic acid, α -tocopherol and carotenoid content give different responses toward the oxidative stress due to the infestation of RPW. From the results obtained, both non-infested and infested MATAG lower stem were generally likely to had stable specific activities of CAT and g-POD along with ascorbic acid amount compared to the upper stem. After all, most of the antioxidant activities appear to be influenced by the RPW-coconut infestation which may affect the expression of the enzymes. However, the activation of antioxidant enzymes may enhance the resistance of MATAG cultivar

toward RPW infestation by better preserving the cellular organelles, membrane integrity and compartmentalization. Further studies need to be done to better understand the tolerance level of MATAG plant against RPW infestation.

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Influence of Mycorrhizal Fungi on Plant Growth Performance and Physiological Changes of Nursery Oil Palm

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Introduction

Arbuscular mycorrhizae (AM) fungi are considered vital components of nearly all terrestrial ecosystems, forming mutually beneficial symbioses with the roots of plants. Most studies on various crops have documented that AM improves plant growth and increases plant nutrient uptake (macro and micro-nutrients) with a predominant increase in phosphate (P) uptake.

Several past reports have documented positive interactions *Elaeis guineensis* (oil palm) with mycorrhizae (Sundram, 2010; Shazril and Tey, 2011; Galindo-Castaneda and Romero, 2013). Field grown oil palms are reported to be frequently colonised by mycorrhizas and hence reinforcing notions that oil palm forms strong mycorrhizal relationships (Nadarajah, 1980; Phosri *et al.*, 2010). From an agriculture perspective, the benefits conferred from mutualistic mycorrhizae relationships are also associated with concomitant reductions in inorganic fertilizers due to their suppressive effects on AM formation. High soil phosphorus levels and prolonged nitrogen fertilization are reported retardants to AM formation and their abundances in agricultural soils (Baath and Spokes, 1989; Rillig *et al.*, 2002). While this effect is widely known, concerns are often made on whether fertilizer reductions will affect optimal oil palm/crop growth. In this paper, we report plant growth benefits from AM inoculation of nursery oil palms and furthermore observed variable yet incremental growth responses with different soils.

Materials and Methods

Two nursery trials were carried out to evaluate mycorrhizal-oil palm growth responses with commercially available mycorrhizal inoculum (identified as Myco-A, -B and -C). Myco-A and Myco-B are produced locally in Malaysia while Myco-C is an imported product from United States of America. Trials was carried out with 3 month old nursery palms following a two-stage nursery planting technique.

Experimental design: For Trial 1, the treatments were arranged in a randomized complete block design (RCBD) comprising four mycorrhizal treatments (Myco-A, -B, -C and without AM inoculation). The plants were fertilized at 50% of control fertilizer rate (consisted of 12% N, 12% P₂O₅, 17% K₂O and 2% MgO) throughout the 9-month trial duration (Table 1). The media used in this study was Bungor soil series, *Typic Kandiudults*. For Trial 2, 2 x 3 x 3 factorial treatments were arranged following an RCBD design comprising two soil series (Bungor soil series, *Typic Kandiudults* and Briaah/Selangor soil series, *Typic Endoaquepts*), three mycorrhizal treatments (Myco-B, -C and without AM inoculation) and three fertilizer rates (0%, 75% and 100% fertilizer rates applied throughout the 9-month trial duration. The 100% fertilizer is the standard rate and serves as a control (Table 1). Soil types used in Trial 1 and 2 were classified based on Malaysian Soil Taxonomy description (Paramanathan, 1998) and confirmed based on physico-chemical analysis (total N, total P, available P, exchangeable K, Ca, Mg, CEC, pH, organic C, content of clay, silt, fine sand and coarse sand) (data not shown). Thus, there were 4 and 18 treatment combinations laid out for Trial 1 and 2 respectively, and each trial experiment was replicated 12 times.

Host plant preparation: For both trials, plants were initially raised from oil palm semi-clonal D x P seeds from a single cross (AA Hybrida IS) to minimise variability in plant growth responses. Seedlings were grown in polybags consisted of pre-mixed soil with rock phosphate fertilizer (10 g per seedling) and placed under pre-nursery conditions (with gradual shade reduction from 70% to 0% in the first two months). Foliar fertilizer (11% N, 8% P₂O₅, 6% K₂O diluted to 10 ml L⁻¹) were applied at a rate of 40 ml per seeding at weekly intervals after the 1st leaf emerged and subsequently application increased to twice a week. Prior to transplanting, seedlings were selected based on uniformity of growth (height, number of leaves, colour and vigour). Three month old pre-nursery plants were transplanted into larger polybags (size 38 x 45 cm) and transferred into the main nursery stage. In the main nursery, seedlings were spaced approximately 3 feet apart (3 ft. x 3 ft. triangular spacing) to give each seedling the optimum growth space. Soils for main nursery polybags were pre-mixed with Rock Phosphate (100 g per palm) and fertilized monthly followed treatment given in Table 1. Watering was carried out twice a day, supplied via an overhead sprinkler system for 30 minutes and watering ceased on rainy days.

Inoculation treatments: The respective commercial AM inoculum treatments were placed into each planting hole before transplanting (Myco-A and -B) or on the soil surface after transplanting (Myco-C). Inoculum (50 g of Myco-A and -B) was placed as a thin layer to a depth of 10 cm to ensure better infection. While 25 g of Myco-C inoculum was applied on the soil surface immediately after transplanting. After treatment application, oil palm kernel shells were applied onto the polybag soil surface.

Table 1. Summary of fertilizer and AM inoculant rates tested in Trial 1 and 2.

Fertilizer rate	Rates applied (g/palm)			
	Trial	Myco-A	Myco-B	Myco-C
50%	#1	50	50	50
0%	#2		50	25
75%	#2		50	25
100% (standard rate)	#2		50	25

Measurements: Monthly vegetative growth measurements were carried out for all palms and comprised of the following parameters: (i) number of green fronds, (ii) diameter of the bole and Frond 1 measurements (plant height, frond length, number of pinnae, length and width of pinnae sampled from the mid-region of Frond 1, petiole width and depth). Pinnae measurements were only recorded once leaflets pinnated.

After 12 months of growth observations, plant tissues were collected for estimating plant biomass, (includes shoot that representing oil palm pinnae, rachis and bole, and oil palm roots) and nutrient content. Plant biomass was measured for all palms. Plant tissues were analysed for macronutrients content (N, P, K, Ca and Mg). Prior to analysis, plant tissues from the 12 individual palms per treatment were pooled into 4 replicates randomly. Briefly, N concentration was estimated via the Kjeldahl method following wet digestion in sulphuric acid. For measuring P, K and Mg concentrations, samples were dry ashed, digested in nitric acid, and analysed via spectrophotometric flow detection of vanado-molybdophosphate complex (P), flame photometry (K) and atomic absorption spectrophotometry (Mg). The uptake efficiency of fertilizers, i.e. the apparent nutrient recovery (REC) is estimated on the basis of nutrient uptake of the unfertilized control (Greenwood *et al.*, 1989), based on the following formula:

$$REC = f_F = \frac{(U_F - U_O)}{N_F}$$

Where N_F – individual nutrients applied (g per seedling), U_F = nutrient uptake in treatments with fertilizers, U_o = nutrient uptake in nonfertilized treatments. U_o and U_F were estimated based on nutrient content in total plant dry matter (shoot and roots), i.e. % nutrient content in shoots/roots X dry weight of shoots/roots. Nutrient uptake for U_o and U_F were determined based on the following formula:

$$\text{Nutrient uptake } (U_o \text{ and } U_F) = \text{Nutrient concentration} * \text{x Total plant dry weight (g)}$$

Where Nutrient concentration represents the concentration of N, P, K and Mg. Total plant dry weight was determined by subtracting the difference in weight of fresh and dried plant tissue samples.

Tertiary roots were sub-sampled and washed under running water. Samples of washed roots were cut into approximately 1 cm-segments for *in-situ* root staining microscopy to detect for acid phosphatase (AP) and succinate dehydrogenase (SD) activities, based on published protocols (Saito *et al.*, 1993; Amaya-Carpio *et al.*, 2009). The number of hyphae and arbuscules in roots exhibiting positive AP- and SD-activities were counted, determined by grid intersection (1-mm squares) based on 100 hyphae intersections.

Results and Discussion

For identification of dominant AM species colonising oil palm roots, PCR-DGGE analysis was carried out using total DNA extracts obtained from plants with and without AM inoculation. PCR primers and conditions were based on published protocols (Cornejo *et al.*, 2004; Liang *et al.*, 2008).

Data were statistically analysed by Analysis of Variance (Minitab® Statistical Software 16). When a significant ($p < 0.05$) treatment effect was found, the mean values were compared using Tukey's multiple range test ($p < 0.05$).

In Trial 1, a 50% fertilizer rate was purposely selected on the basis that plant nutrient demand was not optimal so to be able to account for incremental/beneficial effects of mycorrhizae-plant growth beneficial responses. By the 12th month, mycorrhizae inoculated treatments exhibited incremental differences in plant dry matter albeit not significantly different ($p > 0.05$).

From the trends obtained (although not significant), treatments with Myco-C produced the maximum growth response measuring an average of 12.5% increase in total plant dry matter compared to the control treatment without inoculation (Table 2). Myco-A and Myco-B produced very similar growth trends and both equally exerted incremental growth effects on palm growth.

Table 2. Trial 1 shows the dry weight (g seedling⁻¹) of 12 month old plants.

Fertilizer rate	Soil	Treatment	Shoot		Root		Total	
50%	Bungor (Inland soil)	Nil (control)	338.8	a	112.6	a	451.4	a
		+ Myco-A	383.4	a	130.4	a	513.8 (↑ 13.8%)	a
		+ Myco-B	386.1	a	143.7	a	529.8 (↑ 17.4%)	a
		+ Myco-C	427.2	a	179.8	a	607 (↑ 34.5%)	a

Values in each column with the same letters are not significantly different to each other. The percentage values within parentheses represent total difference in plant dry matter between individual AM treatments vs. the control treatment.

From the growth responses obtained from Trial 1, Myco-B and Myco-C were shortlisted for further evaluation in Trial 2. The objectives of Trial 2 is to (1) address whether the growth enhancement observed in Trial 1 can account for a reduction of inorganic fertilizers, and (2) whether the growth responses would vary in different soil types. To address our first objective, different fertilizer dosages were selected. From Trial 1, the growth responses obtained with a 50% reduction of inorganic fertilizers (90 g/palm) with AM inoculation, though better than the control treatment, did not achieve the optimal growth obtained with a 0% fertilizer reduction (i.e. 100% fertilizer dosage treatment at 180 g/palm) (standard rate) (data not shown). On this basis, we assessed growth responses in Trial 2 with a 75% and 100% fertilizer dosage. A 0% fertilizer dosage (i.e. without fertilizers) was included as a control for subsequent estimation of nutrient uptake efficiencies.

In our second trial, growth response of nursery oil palms inoculated with Myco-B and Myco-C were assessed with different soils and fertilizer dosages (0%, 75% and 100%) (Table 3). Different soils were selected based on their inherent soil fertility status with the Briah/Selangor soil, classified as a coastal soil (and inherently more fertile) compared to Bungor soil (an inland soil) (Goh *et al.*, 1994), also confirmed via soil physico-chemical analysis (data not shown). At the 0% fertilizer dosage rate, no significant differences were observed though mycorrhizae inoculated plants exhibited a marginal increase in plant growth ($p>0.05$). The largest growth responses were however obtained with all treatments comprising 75% fertilizer dosages (or 25% reduction compared to standard rates i.e. 100% dosage). With our control treatments without AM inoculation, we observed a reduction in plant dry matter with treatments receiving the full fertilizer dosage rate (i.e. 100% fertilizers). The control treatments with a 25% reduction in fertilizer rates (i.e. 75% fertilizers only) exhibited a 17% (Bungor soil treatment) and 22% (Briah/Selangor soil treatment) increase in total plant dry matter in comparison to the 100% fertilized treatments (Table 3). Though the differences in plant dry matter is not significantly different, we postulate that the higher fertilizer dosage rate (100% fertilizer dosage) are likely to exert an inhibitory effect on growth, possibly due to root scorching based on the reduction in nutrient uptake efficiencies (refer to control treatments in Table 4).

With the 75% fertilizer dosage treatments, AM inoculated plants produced better growth responses (in comparison with their control treatments without AM inoculation). Growth responses in AM inoculated plants however varied with both soil types, ranging from 8% and 15% (Myco-B and –C, respectively) in Briah/Selangor series soil, and from 13% and 22% (Myco-B and –C, respectively) in Bungor series soil. The increase in growth was also correlated to the increase in nutrient uptake efficiencies (Table 4). The growth responses that we have obtained are in agreement with studies on oil palm-mycorrhizae interactions and their reported growth enhancement effects (Sundram, 2010; Shazril and Tey, 2011; Galindo-Castaneda and Romero, 2013).

To account for mycorrhizae-oil palm interactions conferring to the better palm growth observed, *in-vitro* microscopy of oil palm roots (with and without AM inoculation) was investigated. Metabolically active mycorrhizae hyphae and arbuscules will reportedly express succinate dehydrogenase and phosphatase, enabling detection via *in-vitro* staining of enzyme activities (Saito *et al.*, 1993; Amaya-Carpio *et al.*, 2009). With Trial 2, succinate dehydrogenase-active sites and phosphatase activity were detected in mycorrhizae inoculated plants (Table 5). Control treatment (without inoculant) also exhibited differential staining although very low levels detectable which we postulate is attributed to the presence of naturally occurring mycorrhizae in soils (non-sterile). Only hyphae were detected with no arbuscules observed with the control treatments. The presence of metabolic active mycorrhizae hyphae in all inoculated treatments concurs with studies with other crops documenting enhanced growth of the plant in the presence of mycorrhizae. The detection of metabolic active hyphae based on SDH-active sites in inoculated plants declined with increased fertilizer dosages. Succinate dehydrogenase and acid phosphatase activity was confined to the vacuolar compartments of arbuscules and also intraradical hyphae.

Table 3. Dry weight (g/seedling) of oil palm seedlings 9 months after treatment with different fertilizer dosages and soil types (Trial 2).

Soil	Fertilizer dosage	Treatment	Shoot	Root	Total
Bungor (Inland soil)	0%	Control	113.1 a	105.8 a	218.9 a
		+ Myco-B	125.2 a	114.1 a	239.6 a
		+ Myco-C	145.5 a	138.1 a	283.6 a
	75%	Control	417.9 b	144.6 a	562.5 b
		+ Myco-B	462.5 bc	173.2 a	635.7 b
		+ Myco-C	498.4 c	190.2 a	688.6 b
	100%	Control	394.9 b	130.4 a	525.29 b
		+ Myco-B	429.5 b	129.8 a	559.3 b
		+ Myco-C	434.1 b	136.4 a	572.41 b
Briah/Selangor (Coastal soil)	0%	Control	137.6 a	132.5 ab	270.1 a
		+ Myco-B	148.1 a	127.4 a	275.5 a
		+ Myco-C	168.9 a	152.2 ab	320.9 ab
	75%	Control	606.9 bc	173.6 ab	780.5 bc
		+ Myco-B	643.4 bc	201.7 ab	845.1 bc
		+ Myco-C	668.4 c	229.9 b	898.3 c
	100%	Control	517.2 b	122.8 a	640.0 b
		+ Myco-B	510.8 b	159.6 ab	670.4 b
		+ Myco-C	562.81 bc	173.36 ab	700.17 bc

Values in each column with the same letters are not significantly different to each other. Statistical analyses were carried out separately according to soil type.

Table 4. Nutrient uptake efficiency of oil palm seedlings at 12 months with different fertilizer dosages and soil types (Trial 2)

Fertilizer dosage	Soil	Treatment	Nutrient Uptake Efficiency (%)							
			N		P		K		Mg	
75%	Bungor (Inland soil / Typic Kandiudults)	Control	28.9	a	15.1	a	22.5	a	43.0	a
		+ Myco-B	36.5	ab	37.5	ab	33.4	a	62.8	ab
		+ Myco-C	54.6	b	42.0	b	45.2	a	79.7	b
100%	Bungor (Inland soil / Typic Kandiudults)	Control	23.7	a	12.3	a	14.7	a	14.6	a
		+ Myco-B	28.2	a	15.5	a	15.4	a	29.8	a
		+ Myco-C	26.8	a	17.4	a	18.6	a	38.3	a
75%	Briah/Selangor (Coastal soil / Typic Endoaquepts)	Control	46	a	22.3	a	31.2	a	82.7	a
		+ Myco-B	41.5	a	24.5	a	35.5	a	98.3	ab
		+ Myco-C	43.8	a	29.6	a	40.7	a	127.5	B
100%	Briah/Selangor (Coastal soil / Typic Endoaquepts)	Control	36	a	19.2	a	20.6	a	59.2	A
		+ Myco-B	45.4	a	24.1	a	28.6	a	65.1	A
		+ Myco-C	42	a	20.2	a	29.3	a	75.9	A

Values in each column with the same letters are not significantly different to each other.

AM are reported to be non-host specific, capable of colonizing different plants yet several reports revealed that the capacity of different fungi to promote growth of the same species of plant is variable (Gaur and Adholeya, 2002; Smith *et al.*, 2004). Sundram (2010) reported differences in nursery oil palm growth responses toward different AM species. Hence, we postulated that the differences in growth between Myco-B and Myco-C could be attributed to differences in species of AM colonising oil palm roots. Both products share a few similar and dissimilar AM species in their product

formulation. To identify the dominant species, we carried out a metagenomic biodiversity study with oil palm roots (comparing inoculated vs. control plants). 18S rRNA PCR-DGGE analysis of inoculated treatments with Myco-B and Myco-C revealed that a *Glomus* sp. was the dominant species colonising oil palm roots. Different primers were tested to account for the possible biases in primer binding specificity with mycorrhizae DNA (Figure 1 and Table 6). From our evaluation, the nested PCR approach with primer pairs ITS2/NS31 and Glo1/GC-NS31 was able to detect differences between inoculated and control treatments. It is likely that more than 1 species is colonising oil palm roots but we are unable to account for it due to the sensitivity of the methodology.

Table 5. Proportion (%) of succinate dehydrogenase and acid phosphatase-active hyphae and arbuscules in tertiary roots in treatments (Trial 2).

AMF-product	Myco-B				Myco-C			
Fertilizer dosage	100%	75%	0%	C	100%	75%	0%	C
<i>Succinate dehydrogenase</i>								
IRM-Hyphae	17 b	39 bc	51 c	1-4 a	13 a	49 b	64 c	1-3 a
IRM-Arbs	11 a	37 b	58 b	0 a	22 b	44 c	76 d	0 a
<i>Acid phosphatase</i>								
IRM-Hyphae	13 a	39 b	42 b	2-5 a	11 a	45 b	51 b	1-3 a
IRM-Arbs	15 a	36 b	39 b	0 a	19 b	39 bc	44 c	0 a

Controls (C) comprise of fertilizer dosage treatments without AMF inoculation. Values for control treatments represent the range detected. Abbreviations: Arbs – arbuscules, IRM – intraradical hyphae/mycelium, nd – not detected. Values represent the average. Values in each row with the same letters are not significantly different to each other.

Table 6. Number of ribotypes detected in DGGE profiles of oil palm roots with different primer pairs. H' index represents Shannon diversity index of dominant ribotypes.

DGGE Lane*	PCR amplification	Samples	Number of ribotypes	H' index
1		1 kb DNA ladder		
A	2 AM1/NS31→	+ Myco C	9	0.77
	3 AM1/GC-NS31	Control	9	0.73
B	4 AM1/NS31→	+ Myco-C	5	0.46
	5 Glo1/GC-NS31	Control	9	0.84
C	6 ITS2/NS31→	+ Myco-C	13	0.97
	7 Glo1/GC-NS31	Control	11	0.86

*DGGE lane numbering is referenced to Figure 1.

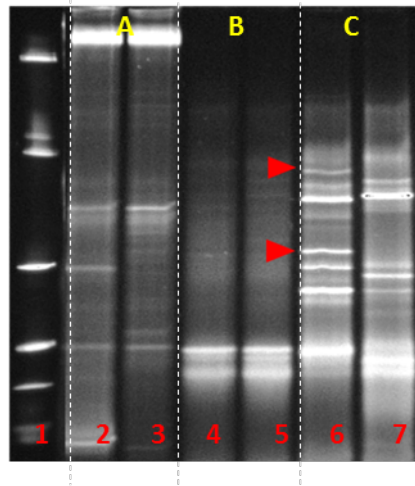


Figure 1. PCR-DGGE analyses on 18S rRNA gene fragments on 5% polyacrylamide gel with 40-60% urea denaturant. Distinct unique ribotypes (indicated by red arrows) were excised and cloned for subsequent taxonomic identification.

Conclusion

AM technology to increase agricultural productivity has long been acknowledged as an effective method. The benefits they confer range from plant growth enhancement to reduction in plant stress. However, their full benefits as reported in other crops have yet been fully investigated on oil palm. Identifying factors conferring mutualistic benefits with the plant and their environmental variables such as soil type will enable growers to make sound decisions on their application and integration into oil palm best management practices.

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