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APPROACHES TO SUSTAINABLE PLANT PRODUCTIVITY AND SAFETY IN A CHALLENGING ENVIRONMENT

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22nd Malaysian Society of Plant Physiology Conference (MSPPC 2011)

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CHAPTER 1

PLANT GROWTH, DEVELOPMENT AND PRODUCTION

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Fruit Production under Department of Agriculture Selangor, Malaysia

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Introduction

The agriculture sector plays an increasingly important role in the national economy through its contribution to the national income and export earnings and creation of employment. The sector is also a major supplier of food as well as raw materials to resource based industries. In accordance with Ninth Malaysian Plan (2006-2010), Department of Agriculture (DOA) inquired the farmers to increase the production of main crops such as paddy, fruits, vegetables, plantation crops as well as coconut from 2.6% in Eight Malaysian Plan (2001-2005) to be 7.5% in Ninth Malaysian Plan (Department of Agriculture Malaysia, 2010; Economic Planning Unit, 2010). This step was taken in view of the significant increase in the needs and demand for food following the increase of population in Malaysia. Selangor is one of the major fruit production states in Peninsular Malaysia. DOA Selangor has taken further steps to boost the fruit production to fulfil the demand for food, especially in Selangor itself, by implementing several programs and projects. Among the projects undertaken include group projects, entrepreneurs, and Permanent Food Park Programme (TKPM) that consist of seven crop categories namely floriculture, herbs and spices, fruits, vegetables, industrial crops, short term crops and paddy (Department of Agriculture Selangor, 2010). These projects were implemented in selected districts according to soil suitability, size and condition of the farmers' land. Besides, DOA Selangor also provides various incentives to registered farmers, e.g. fertilizers, seeds, plant hormones, pesticides etc.

This study presents the status and the distribution of fruit production projects of DOA Selangor. The data were imperative for more structured monitoring and improvement of these fruit production projects. In addition, positive pictures reflected by this study would also encourage more farmers to be involved in the fruit production projects.

Methodology

This study involved the analysis of fruit production under DOA Selangor. In general, Selangor is divided into nine administrative districts, namely Gombak, Hulu Langat, Hulu Selangor, Klang, Kuala Langat, Kuala Selangor, Petaling, Sabak Bernam and Sepang (Jabatan Perancangan Bandar dan Desa Selangor, 2007). The data concerning the population of farmers, acreage, the fruit crop type, and the production on the agricultural lands under DOA Selangor in the year 2009 were obtained from the Headquarter of DOA Selangor. Data were classified and subjected to descriptive analysis. Graphical statistics were used to describe the fruit production in quantitative terms.

Results and Discussion

Fruit cultivation under DOA Selangor was mainly distributed in Hulu Langat, Hulu Selangor, Kuala Langat and Sepang (Figure 1). It covered a total area of 579 ha involving a total of 360 farmers. Hulu Selangor had the highest number of farmers with 113.56 ha involved in fruit tree cultivation.

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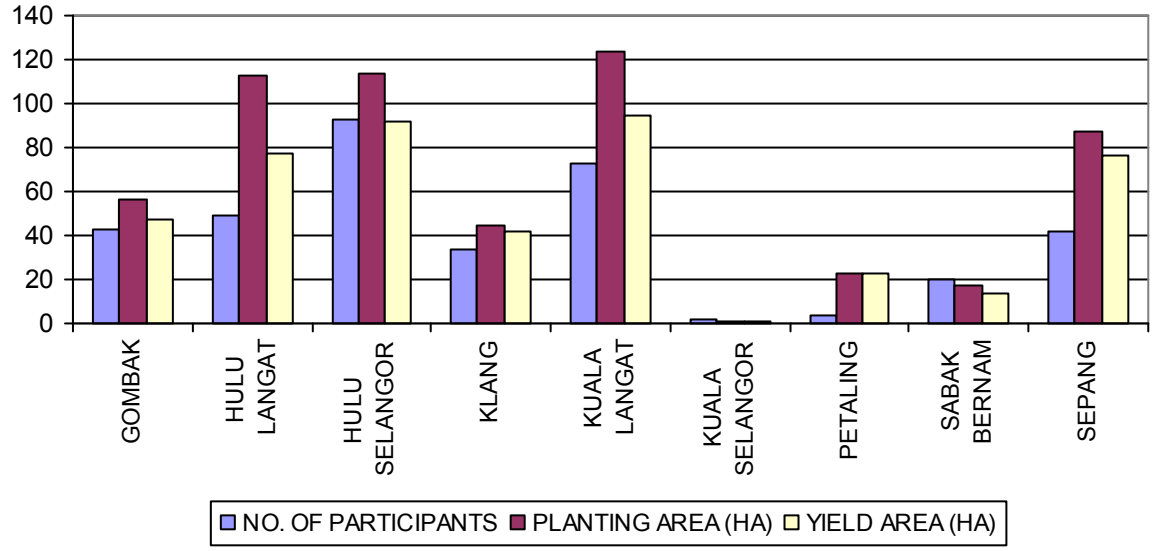

Figure 1. Number of farmers, planting area and yield area

Musa acuminata was the most popular fruit tree adopted by farmers in Hulu Selangor (Table 1). This species covered a planting area of approximately 170 ha. Fruit tree cultivation was dominated by *M. acuminata* probably due to easy husbandry of this crop.

Ananas comosus and *Hylocereus undatuss* were also major fruit trees planted in Selangor. *Ananas comosus* thrives on peat soil and was mainly found in Kuala Langat. It is one of the highly dominated fruits around urban areas with high population like Kuala Langat. Popularity of fresh-market *A. comosus* outranked processed *A. comosus* goods (including juice and canned varieties) (Timco Worldwide, 2009). Malaysia is among the world's top 20 best producers for *A. comosus*. On the other hand, the largest *Averrhoa carambola* orchard in Selangor was located in Hulu Langat. It covered an area of approximately 40 ha.

Figure 2 presented the average production of fruits under DOA Selangor according to districts. Fruit production was highest in Kuala Langat with approximately 24,900 kg/ha followed by Sepang with the average production of about 13,800 kg/ha. Kuala Langat that was mainly planted with *A. comosus* and *H. undatus* recorded the highest production per area basis. High production of fruits in Sepang, on the other hand, was also probably attributed to the cultivation of *A. comosus*.

With the production of high value *A. comosus* and *H. undatus* in Kuala Langat, the farmers in this district also recorded the highest average monthly revenue. The farmer in Kuala Langat earned almost RM2,500 per month per ha (Figure 3). The high revenue gained by the farmers in Kuala Langat was also probably related to the higher prices of the produce marketed in urban areas at vicinity.

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Table 1. Fruit species and planting area

District	Fruit species	Planting area (ha)	Planting area (%)
Gombak	*Averrhoa carambola*	8.00	1.38
	Carica papaya	0.80	0.14
	Artocarpus integer	8.56	1.48
	Lansium domesticum (dokong)	1.00	0.17
	Durio zibethinus	8.88	1.53
	Lansium domesticum (langsat)	0.25	0.04
	Mangifera indica	2.00	0.35
	Garcinia mangostana	0.92	0.16
	Artocarpus hetrophyllus	1.00	0.17
	Hylocereus undatus	12.00	2.07
	Nephelium lappaceum	3.25	0.56
	Artocarpus altilis	8.00	1.38
	Cucumis melo (honey dew)	1.30	0.22
	Cucumis melo (muskmelon)	0.03	0.01
Hulu Langat	*Averrhoa carambola*	40.60	7.01
	Carica papaya	15.00	2.59
	Annona muricata	6.40	1.11
	Psidium guajava	6.00	1.04
	Citrus maxima	0.80	0.14
	Musa acuminate	25.20	4.35
	Hylocereus undatus	7.00	1.21
	Nephelium ramboutan-ake	11.50	1.99
Hulu Selangor	*Averrhoa carambola*	0.60	0.10
	Carica papaya	1.00	0.17
	Artocarpus integer	9.75	1.68
	Durio zibethinus	23.20	4.01
	Syzygium aquem	3.00	0.52
	Psidium guajava	7.90	1.36
	Artocarpus hetrophyllus	1.00	0.17
	Musa acuminate	41.86	7.23
	Hylocereus undatus	8.00	1.38
	Nephelium lappaceum	17.25	2.98
Klang	*Ananas comosus*	30.00	5.18
	Musa acuminate	13.40	2.31
	Cucumis melo (honey dew)	0.80	0.14
Kuala Langat	*Syzygium jambos*	4.30	0.74
	Citrus maxima	1.20	0.21
	Ananas comosus	47.60	8.22
	Musa acuminate	27.30	4.72
	Hylocereus undatus	40.00	6.91
	Cucumis melo (watermelon)	1.00	0.17
	Cucumis melo (honey dew)	1.40	0.24
	Cucumis melo (muskmelon)	1.00	0.17
Kuala Selangor	*Citrus aurantifolia*	1.00	0.17
	Cucumis melo (watermelon)	0.26	0.04
Petaling	*Artocarpus integer*	0.20	0.03
	Syzygium jambos	18.00	3.11
	Musa acuminate	3.90	0.67
	Nephelium lappaceum	0.20	0.03
Sabak Bernam	*Mangifera indica*	9.80	1.69
	Musa acuminate	7.86	1.36
Sepang	*Durio zibethinus*	2.51	0.43
	Ananas comosus	21.10	3.64
	Musa acuminate	50.81	8.78
	Hylocereus undatus	13.19	2.28
	Cucumis melo (muskmelon)	0.10	0.02
Total		578.98	100.00

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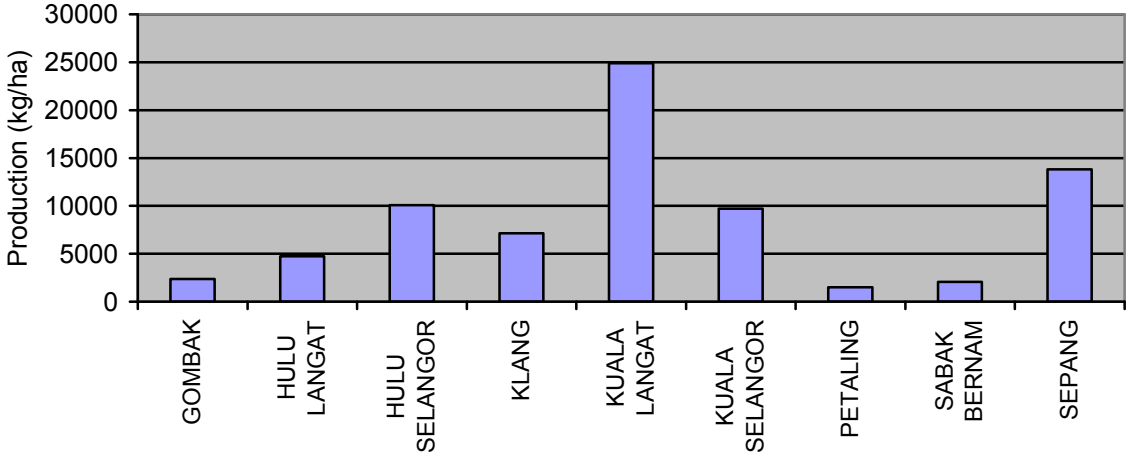

Figure 2. Average production (kg/ha)

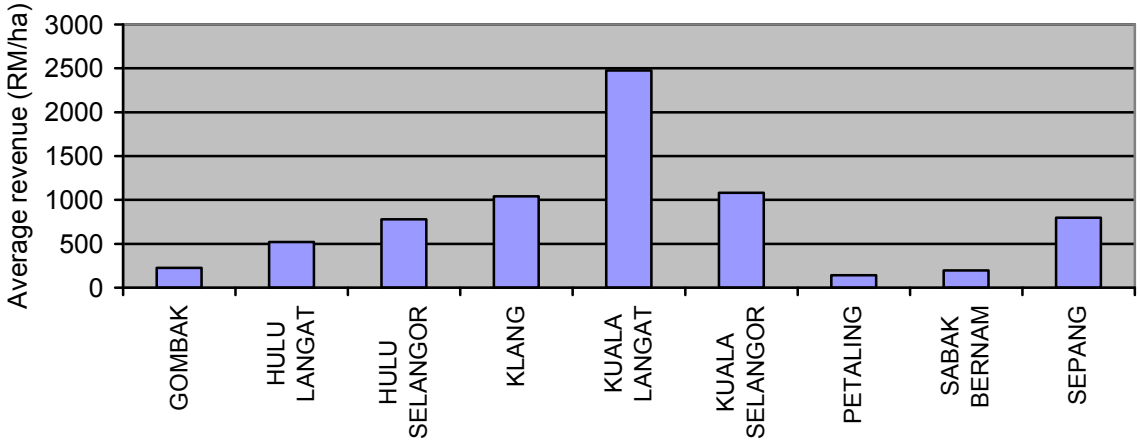

Figure 3. Monthly average revenue

Conclusions

Several factors are presumed to affect the distribution of fruit crop production projects. These factors include the type and fertility of soils, the availability of natural resources such as water and market vicinity for higher value of produce. Following the implementation of high impact projects and new incentives for farmers, fruit production is projected to further grow in coming years.

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Production and Postharvest Handling of Organic Vegetables

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Introduction

Vegetables farming is the cultivation of herbaceous plants for foods, fibers and other products use to sustain life. In the farming system, basic requirements are soil, water, nutrients (macronutrients and micronutrients), minerals and organic matters. Soil plays a role to support plant roots to anchor to as well to hold water, nutrients and minerals. Organic matters, nutrients and water are very crucial for plant optimum growth and health. The vegetable cultivation started with traditional practices of nutrients recycling. Crop rotation and composting are the main practices in managing the soil fertility and pest management. As the time goes by, there is remarkable shift of the farming practices in response to the development of science and technology. New technology improves the farming techniques such as the production of synthetic fertilizers, inorganic pesticides and the usage of growth regulators and hormones which replace the old agronomic practices. The blooming of these technology have resulted in mass produce of the agriculture products as to fulfill the market demand due to the increase of the world human population.

However, excessive use of these chemical fertilizers has caused some negative impact on the environment and the farmers. There is a growing concern on the soil quality related to soil deterioration and degeneration. Inorganic fertilizers, when applied for long periods on the same soil, cause soil acidification, soil compaction, mineral volatilization and phosphorus fixation (Brady and Weil, 2008). Prolong use of the fertilizers also reduces the population of soil organisms like earthworms and beneficial microbes (Atiyeh et al., 2000) as well as contaminate groundwater with heavy metals and nitrate that are hazardous to humans and animals. Such an example is the baby blue syndrome which occurs to infant that drink nitrate contaminated water (Brady and Weil, 2008). Widespread use of inorganic pesticides could bring harm to farmers that deal with the pesticides directly, while the inorganic pesticide residues could affect consumers' health. Therefore, now the focus has been shifted to organic vegetables production.

Organic vegetables production

People tend to think that organic farming is farming without chemical fertilizers and pesticides, but it is a production of holistic approach. It is not only exclude the use of chemical compound fertilizers, pesticides and growth regulators, but it also depends heavily on composting, crop rotation and aspects of biological pest control, to maintain soil fertility and control pest. One of its important principles is soil fertility. Soil constitutes the center of natural ecosystem as it is a habitat for plants, animals and microorganisms. Therefore, managing soil fertility is important in organic production. The soil must be fertile and biologically active, whereby, it has the combination of complete plant nutrients for maximum growth, continuous supply of organic matter and acceptable pH. Organic matter provides habitat and food for huge numbers of soil organisms which resulted in excellent soil structure, capable of taking up and storing large quantities of water and nutrients. Compost and green manure are added to supplement nutrients supplied by soil minerals and organic matter.

Seeds, vegetative propagation materials and transplants must be produced using organic methods. Genetically modified organisms and products are strictly prohibited to use in the organic vegetables production. Also, seeds or propagation materials are not allowed to be treated with any prohibited substances such as wetting agents. As the organic vegetables production stress on the environmental friendly and sustainability practices, managing pests in a natural way, through prevention instead of curatively, is the call. The idea is to use natural enemies, such as predators and parasitoids, to control insect pest. Spiders, assassin bugs, praying mantis and lady-bird beetles are examples of predators species that are usually found in the garden. These predators normally attack their prey, such as caterpillars or aphids, by direct feeding or sucking out the body juices. Parasitoids, like wasp and tachinid flies, commonly attack the prey by laying their eggs on or in the host body, and the immature parasitoids will feed on the host.

Many adult parasitoids and predators feed on pollen and nectars of flowering plants, hence, plant diversity is required to continuously provide the food sources to the natural enemies. At the same time, companion plants and trap crops can be planted to prevent the pest from attacking the main crops. In addition, crop rotation or mixing crops also help to control diseases and weeds. Crops with resistant varieties also can be selected to combat with diseases. Instead of using chemical pesticides, organic pesticides, such as pyrethrums, *Bacillus thuringiensis*, and neem leaves extract spray, are used. The farmers also have to pay attention to water management. Water source should be free from any chemical contaminants or pathogen. Also, water is used properly so that there is no water wastage due to excessive use.

Advantages of organic vegetables production

Organic vegetables have advantages over the conventional vegetables production. Through organic production practices such as crop rotation, cover crop and composting, the fertility of soil is conserved and maintained. Soil rich in organic matter and humus improves soil structure and aggregates which resulted in the better root penetration and water holding capacity. As a result, plants efficiently take up the available nutrients in soil. Hence, leaching out of nutrients to underground water is reduced compared to conventional practices. Application of integrated pest management such as biological control, trap crop, selection of resistant varieties and bio-pesticides reduce pesticides residues in food and environment. This leads to less pollution to the environment and protect the health of humans and animals. Incorporation of bio-fertilizers reduce the utilization of non-renewable external inputs and energy as less petrol fuel is used in manufacturing synthetic fertilizers and pesticides. In addition, these practices generate original ecosystem through promoting biodiversity. Also, many studies have been reported that organic produce is more nutritious, as they have significantly higher vitamin C, iron, calcium, magnesium and lower nitrate and heavy metals than inorganic produce (Worthington, 2001; Rembialkowska, 2004).

Harvesting

Harvesting is usually done during the coolest time of the day to maintain low product respiration. Care is taken to avoid unnecessary wounding, bruising, crushing, or damage from humans, equipment, or harvest containers. In the field, harvested products are placed under shade to keep them cool. Covering harvest bins or totes with a reflective pad greatly reduces heat gain from the sun and reduces water loss and premature senescence. If possible, move product into a cold storage facility or carry out postharvest cooling treatment as soon as possible. It is also important to harvest plant at their proper maturity stage. If plant is harvested at the immature or over matured age, the shelf life is shorter and yield decreases compare to mature ones. For an example, over matured cabbage easily spilt and are prone to rot incidence. While immature ones have puffy head due to incomplete development, the inner leaves make

them more susceptible to damage (Bautista and Acedo, 1987). Physiological age of the vegetable is also another crucial consideration as it affects the postharvest quality.

Postharvest handling

After harvesting, the vegetables are commonly put into collection containers like bamboo baskets and plastic crates. Harvested vegetables should not be exposed to heat of the sun as high temperature speed up quality deterioration due to water loss and respiration. Hence, they have to be transferred to packing house as fast as possible. On the contrary, postharvest quality of pak choy increases if it is laid under the sun for 30 minutes, immediately after harvest (Jiang and Pearce, 2005). Temporary wilting of pak choy significantly reduced its mechanical damage. After that, it rehydrated and washed by dipping into water. Also, drying out the cabbage head can reduce the incidence of soft rot, and they require no washing.

Defect on leaves or any part of the vegetables, are trimmed off including some outer wrapper leaves of cabbages. However, not all the outer leaves are removed at once. Few outer leaves are left to protect head of the vegetables from physical injury without any decrease in product quality and increase farmers' profits. Sometime, there is also a second trim to take off mechanically damaged leaves during transport. Then, harvested produce is sorted and graded. Poor produce is separated from good produce, followed by classification of the good produce based on quality parameters, such as size and appearance. Grading is done according to set rule or standard by agencies or industry. Another step is washing to remove dirt and debris, or any surface contaminants. Sanitation is required in controlling disease spreading through the natural surface contours, openings and wounds of the harvested vegetables. The treatments with chlorine, hydrogen peroxides, ozone, alum, lime and botanical and leaf extracts are not allowed.

In maintaining postharvest quality, packaging should properly be design to avoid premature deterioration, reduce physical and supply good ventilation for cooling and heat to escape due to respiration. Also, to minimize water loss from harvested vegetables, produce packages should be rigid containers such as bamboo basket, crate and carton. In addition, the packages have to be vented for air ventilation. Before the produce are marketed, cooling and storing is crucial to extend the produce shelf life. Cooling slows down the physiological change rate and inhibits the microorganism growth, while storage is required to control temperature, relative humidity and air circulation. For example, optimum storage temperature for tropical vegetables is less than 10 °C and RH for leafy vegetables is 90-98% (Antonio and Acedo, 2010).

Conclusions

Consumer awareness, go green and sustainability, cause a high demand for organic products. To maintain postharvest quality and extent shelf life, none or less chemical compounds should be allowed, postharvest handling come to play important role-market value and profits.

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SRI Rice Crop Establishment

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Introduction

Agro-ecological based farming methods are now touted to be the methods of choice to address food security for the poor, decrease environmental degradation and increase resilience to climatic shock. Being a low-carbon, resource-preserving type of agriculture, agro-ecology has proven to be able to double food production within 10 years (UN-HRC, 2010). An alternative rice management system known as System of Rice Intensification or SRI which requires less water, seeds and chemical fertilizers is now recognized internationally as the preferred agro-ecological green technology that employs improved water management and organic inputs and therefore substantially reduces adverse ecological impacts while, at the same time, raising crop yield. The UN-DESA (2011) report has listed SRI as one of the sound examples of creative innovations that has had large-scale impacts on agriculture and natural resource management. This paper discusses the current knowledge of rice crop establishment that may be applied to SRI. The paper also reports some findings on a comparative study between direct seeding and manual transplanting under SRI as well as some initial findings of the use of mycorrhiza in the presence of biochar in augmenting rice crop establishment.

Materials and Methods

The paper highlights the plant physiological advantages of SRI rice crop establishment and the benefits of rice seed treatment. A comparative study between direct seeding and manual transplanting under SRI was conducted based on a completely randomized block experiment of 5 X 5m plots in 4 replication. A second experiment comparing rice seedlings grown in a soil medium inoculated with mycorrhiza and mycorrhiza plus rice husk biochar was conducted based on a split plot experimental design. The mycorrhiza inoculant was a commercial product by FELCRA Research and Development. Both experiments were based on the performance of the rice variety MR219.

Results and Discussion

There are many benefits of good crop establishment and associated practices. SRI is a method of rice planting that focuses on realizing the full genetic potential of the plant through practices that encourage the health of the whole plant and soil health. Many physiological advantages such as more productive tillers per hill, heavier grains, more spikelets per panicle and more efficient photosynthesis and water use are noted for the SRI method (Tables 1 and 2). Mycorrhizal rice root colonization positively influences the uptake of phosphorus and potassium (Hajiboland et al., 2009). Incorporation of well composted rice straw can reduce weeds, supply silica and nutrients for the plant and microorganisms, making the rice plants strong and upright. Additionally, the plants become more photosynthetically efficient with increasing yield, reducing susceptibility to biotic stress such as chewing insects and abiotic stresses such as drought, metal toxicity and salt, whilst increasing phosphate availability (Ma, 2004; Massey and Hartley, 2006). Proper land leveling is a must for SRI cultivation since in most parts of the season, the field is kept just wet without flooding thereby decreasing the risk of dessication and inefficient use of water. Seed treatment for the SRI method, which is vital in reducing seed borne diseases and instrumental in increasing yield, does not include methods that are detrimental to beneficial microrganisms (Table 3).

In the SRI method, 8-12 days old seedlings are cultivated on dry raised nursery beds or trays, giving enough space between seeds to reduce the possibility of fungal attack. Table 4 lists the various crop establishment practices associated with SRI method. Although SRI manual transplanting is less laborious than conventional manual transplanting, the current scarcity of labour calls for a consideration for other less laborious methods of rice crop establishment. Under conventional rice cultivation, direct seeding is more attractive than transplanting because it is cheaper and can result in an earlier harvest (Balasubramanian and Hill, 2002). The results of the comparative study of manual transplanting and direct seeding under SRI showed that manual transplanting was significantly higher than direct seeding in all the yield components ($p<0.05$) (Table 5). Under manual transplanting, the yield obtained was 12.36 tonne/ha, whereas under direct seeding, the yield was 6.91 tonne/ha. Table 6 shows the results of the productivity analysis of the two methods which reveals that the benefit cost ratio for transplanting was higher (3.39) than direct seeding (2.94). Based on the yield components and productivity, it can be concluded that manual transplanting is better than direct seeding. The experimental results of the effect of mycorrhiza on rice seedling establishment indicated that plant height at eight days after sowing was significantly higher for mycorrhiza treated rice seedlings (mean = 10.3 cm) than the control (mean = 5.8cm) at $p<0.05$ (Table 7). In this experiment, biochar does not seem to have a positive effect on the growth of the mycorrhiza treated rice seedlings. While it cannot be assumed that biochar amendments will always result in a net benefit to plant productivity (Warnock et al., 2007), the effect of biochar on rice seedling establishment needs further investigation.

Table 1. Physiological advantages of SRI

Plant Growth Stage	Physiological Advantage
Vegetative stage	More tillers more open plant architecture with more erect and larger leaves Higher xylem exudation rates deeper and better-distributed root systems Higher water use efficiency; higher photosynthetic rate; lower transpiration
Ripening stage	higher leaf chlorophyll content; delayed senescence; greater fluorescence efficiency
Harvest Stage	longer panicles, more grains per panicle and higher % of grain-filling; heavier grains

Source: Thakur et al., 2009

Table 2. Maximum grain weight from organic SRI rice plots

Site	Variety	1000-grain weight[+]	Source
Beranang	MR219	39.6	Experimental, 2009
Beranang	UKMRC2[++]	30.2	Experimental, 2009
Tanjung Karang	MR219	29.6	Experimental, 2009
Tanjung Karang	UKMRC2[++]	28.5	Experimental, 2009
Tunjong	Hijrah	31.0	Farmer's trial, 2011
Tunjong	Sintanur	29.2	Farmer's trial, 2011
Kg. Lintang	MRQ74	22.6	Farmer's trial, 2011

[+]1000-grain weight at 14% moisture. [++]UKMRC2 seeds were provided by Wickneswari Ratnam

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Table 3. Rice seed treatment

Seed Treatment	Action	Advantage
Sunbathing	Exposing the seeds to sunlight for one or two days	Higher transparency of seed skin; higher transpiration; better oxygen supply: reduction in anti-germination substances: better germination rate and speed; ultraviolet light kills germs: eliminates harmful CO_2 gas produced during confinement
Specific gravity selection	Putting seeds in saline water and getting rid of light, floating seeds	Sorting for good quality seeds. Eliminating immature grains and disease infected grains
Seed disinfection	Soaking seeds for 5 min in 45 – 47 ^oC, then 50 – 52 ^oC for 10 min and rinsing with cool water immediately	Kills seed borne pathogens (and nematode disease)
Soaking/seed priming	Soaking seeds in water for 24 h	Activates enzymes, hastens changes of the albumen storage component into a soluble component, and reduces germination inhibiting substances
Seed priming	priming in KCl for 48h. osmohardening with KCl or $CaCl_2$ for 24 h, or vitamin priming (ascorbate 10 ppm) for 48 h and seed hardening for 24 h.	Decreases lipid peroxidation and increases superoxide dismutase (SOD), and catalase (CAT) activities, starch breakdown (Ella et al., 2011); ensures rapid and uniform seed germination; improves crop stand establishment, growth, yield and quality; increases in activities of α-amylase
Sprouting at proper temperature	Turning over the sprouts at 90% sprouting. Bring the temperature down to 28-32^oC. Turn the seeds everyday and sprinkle water 2-3 times a day to lower the temperature. Adapting to normal temperature: when the length of the roots become 1 - 2 x the length of the seeds, and when the sprouts grow half the size of the seeds, spread the seeds and expose them to ambient temperature.	Bursting out at high temperature for fast seed whitening; bursting usually starts within 12 h.
Others	Soaking in EM solution	Controls various seed, soil and seedling diseases.

Sources: Konayaga (2000), Farooq et al. (2006), IRRI Rice knowledgebank website and AFSC (2009).

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Table 4. Crop establishment in SRI

Crop Establishment	Explanation	Place
Manual transplanting	Shallow transplanting of young seedlings, singly and in wide spacing. Most common SRI practice	Most countries where SRI is practised
Machine transplanting	Using machine transplanters modified to plant 1 seedling at shallow depth	Cuba, India
Semi-mechanical transplanting	Several workers aboard a moving tractor conducting the task	Pakistan
Kadiramangalam	Re-transplanting 30 day old seedlings, singly, which were transplanted earlier at 15 days	Cauvery Delta, India
Direct seeding	Direct seeding of pre-germinated seeds, thinning by weeding at 20 days to acquire a square spacing pattern of single plants	Sri Lanka
Broadcasting seedlings	Broadcasting 10 day old seedlings thinning by weeding 10 days later to acquire a square spacing pattern of single plants	Tamilnadu, India
No-till raised beds	Transplanting on no-till raised beds	Sichuan, China

Source: Uphoff (2007).

Table 5. Yield component comparison between transplanting and direct seeding

Crop establishment method	Plant height (cm)	Panicles per hill	Spikelets per panicle	% filled grains	100 grain weight (g)	Yield
Transplanting	116.2(10.1)	25.1(4.1)	175.3(35.2)	87.8(5.80)	2.88(0.11)	12.36(3.21)
Direct seeding	100.1(9.2)	17.8(6.3)	149.5(35.2)	88.2(4.81)	2.62(0.21)	6.91(2.96)
L.S.D $_{0.05}$	4.3*	2.4*	15.7*	2.37 n.s	0.6*	1.4*

Standard deviations are given in parentheses (n = 40, variety MR219) * =significant, n.s = not significant

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Table 6. Productivity comparison between transplanting and direct seeding

Particulars	Transplanting	Direct Seeding
Seed rate kg/ha	5	25
Days to transplant	8-12	0
Cost of raising nursery (RM)	50	0
Labour required for transplanting @RM50/manday	15-20	1
No of effective tillers / sq. meter	279	198
No. of filled grains / panicle	154	133
Average yield (kg/ha)	12.36	6.91
Total cost of cultivation RM / ha	3400	2150
Gross returns (RM) @ RM1000/tonne	12360	6910
Net returns per ha (RM)	8960	4760
Benefit cost ratio	3.39	2.94

Table 7. Influence of mycorrhiza on seedling growth

Treatment	Plant Height (cm)
Control	5.8(1.7)
Mycorrhiza	10.5(2.1)
Mycorrhiza plus biochar	5.4(1.6)
$L.S.D_{0.05}$	0.95

Standard deviations are given in parentheses (n = 29, variety MR219)

Conclusions

In conclusion, proper crop establishment is absolutely essential for the success of SRI cultivation. Based on the yield components and productivity, it can be concluded that manual transplanting is better than direct seeding. These findings also hold promise for the use of mycorrhiza in rice crop establishment to enhance the yield potential of the rice plant.

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Quality Assesments of F_1 Hybrid Rockmelon (*Cucumis melo* L.) Cultivars Grown on Soiless Culture

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Introduction

Rockmelon had become the most demand fruit not only because of its sweet and juicy tastes but also because of its ability to provide the consumers with lots of nutritional value. Hybrid plant can be defined as a selective breeding product after undergo cultivation process and it consists with lots of interested gene which allow them to further propagate actively. F_1 Hybrids plants that include with selective gene may produce large, sweet, high resistance product. Usually all those hybrid seeds that present in market were supplied from other countries. Some of the imported were genetically unable to survive in certain area with different climate, growth media and more other factors. Roach and Wulff (1987) stated that it is important to determine the seed dormancy, dispersal and germination rates.

Mohd Razi (1994) proved that growing plant by using soilless culture with controlled environment to be beneficial compared with open field cultivation. In this experiment, soilless culture system was applied with efficient usage of water and nutrients for the plant growth. β-carotene contributes to orange color for the fruits and act as antioxidant where it used to protect our body from free radicals. This antioxidant taken in diet may lower risk of having heart disease and cancer (Gabrielle et al., 2000 and Bjelakovic et al., 2007). It also is a source for vitamin A for the body that needed for good vision, eye health (Herrick et al., 2000) and strong immune system (Sluijs, 2009).

Comparative studies between F_1 Hybrid rockmelon cultivars had never been conducted before and the qualities of the fruit were examined to ensure the beneficial effect that the hybrid fruit could bring on. This research include the study on seed germination rate, survival rate, total soluble solid, vitamin C and β-carotene content for four different rockmelon cultivars planted on soilless culture irrigated through fertigation technique.

Materials and Methods

All imported seeds (Glamour, Japan; Honeymoon, Sunshine sweet and Champion, Europe) used were supplied from MARDI, Serdang. Hundred seeds from each cultivars of F_1 hybrids rockmelon were sown on peat moss and a week after, germinated seedling were transferred to polythene bags filled with coco peat used as growing medium. The experiment was carried out under rain shelter at MARDI, Serdang with mean daily air temperature ranged from 37 to 49 °C from March to May. After four weeks, the numbers of plant that survived were measured.

After two months, the fruits of each cultivar was harvested and weighed by using measuring balance. Fruits were cut and the fruits flesh color was observed subjectively. Brix test were done to test the sugar content for each type of F_1 hybrids rockmelon cultivars by using Agato PR-1 hand refractometer. A drop of homogenized fruit pulp was used and placed on the refractometer. Direct reading was taken and repeated for three times. The mean value data was presented in Table 2.

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Dichlorophenolindophenol (DCPIP) test also were done in order to test the Vitamin C content on the fruit. For the test, 0.1% ascorbic acid is used to decolorize blue DCPIP solution (Ranganna, 1977). Steps were continued and the percentages of Vitamin C were calculated as below:

$$\text{Vitamin C (\%)} = \frac{\text{Number of drops of ascorbic acid to decolorize blue DCPIP}}{\text{Number of drops of sample's juice to decolorize blue DCPIP color}} \times 0.1\%$$

Beta carotene analysis was done on the fruit juice and the method used was proposed by Cyanotech Corporation (2002). Each treatment has conducted with three replicates, and the results were presented as mean ± SD (standard deviation). The statistical analysis of experimental data was done on ANOVA one-way by using Statistical Analysis Software (SAS). Each of the experimental values was compared to its corresponding control. Statistical significance was accepted when the probability (P) is less or equal than 0.05 ($P \leq 0.05$).

Results and Discussion

Germination and survival rate

During germination process, seeds undergo imbibitions where the seed coat was soften by the water surround it and then successfully germinated. Table 1 below indicated mean of seed germination rate and seedling survival rate. Glamour lead with highest germination rate (99±2.00%) followed by Honeymoon, Champion and Sunshine Sweet. There was a significant difference ($P \leq 0.05$) on the seed germination rate among the F_1 hybrid rockmelon cultivars.

Successfully germinated seedlings were transferred into coco peat growth media. The plant was adapted to the new environment and the numbers of plant that survived was calculated (Table 1). After second week, all the transferred plants from each cultivar were successfully survived (100%).

Table 1: Mean value of seed germination rate and seedling survival rate and for different types of F1 hybrid rockmelon ($P \leq 0.05$).

Hybrids	Germination rate (%)	Survival rate (%)
Glamour	99±2.00	100
Honeymoon	94±5.16	100
Sunshine sweet	85±5.03	100
Champion	90±5.16	100

The survival of rockmelon cultivar seedlings on coco peat might be influenced by micronutrient and macronutrient that being supplied using fertigation technique. Sheldrake (1989) and Steidman (1988) stated that the coco peat could hold very high water availability and this may produced a good aeration to root zone. It is also are environmentally sustainable and there are other research were succeeded on applying coco peat as the plant growth media (Reynolds, 1990; Meerow, 1994). From this study it was proved that coco peat was able to be used in growing F_1 hybrids rockmelon cultivars and substitute soil which is the general plant growth medium used in plant cultivation.

Fruit fresh weight

After 2 months, the fruits were successfully harvested and collected. Fresh weights of the fruits were taken and data values were shown by using mean value ± sd (Table 2). Sunshine sweet gave the highest fresh weight (2.61±0.098 kg) and statistical analysis indicated that there was significant difference ($P \leq 0.05$) among four hybrids fresh weight.

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Table 2: Fruit's color, percentage of ascorbic acid and total soluble solid for different types of F_1 hybrid rockmelon ($P \leq 0.05$).

Hybrids	Fresh weight	Flesh color	Ascorbic acid (%)	TSS (% Brix)
Glamour	1.86±0.055	Slightly orange	28±1.00	14±0.82
Honeymoon	2.24±0.038	Whitish orange	14.58±1.53	12±0.82
Sunshine sweet	2.61±0.098	Pale green	N/A	8.5±1.80
Champion	2.23±0.187	More orange	30.43±2.31	16±1.41

Total Soluble Solids (TSS), Ascorbic acid and β-carotene

Fruits color, TSS, ascorbic acid and β-carotene content of the fruits were measured and data were presented in Table 2. Champion gave the highest value for TSS (16±1.41%) followed by Glamour, Honeymoon and Sunshine sweet. High TSS value indicated that Champion could provide a large number of sugar content. Champion also showed the highest vitamin C percentage (30.43±2.31%) compared to other hybrids. On the other hand, for β-carotene content Honeymoon had the highest value ($9.5x10^{-4}$±0.70%), followed by Champion and Glamour (Table 3). From statistical analysis, result had shown significant differences ($P \leq 0.05$) for every cultivar on TSS, ascorbic acid and β-carotene content.

Table 3: Beta carotene content for different types of F_1 hybrids rockmelon ($P \leq 0.05$)

Hybrids	β-carotene content (%)
Glamour	$5.2x10^{-5}$±0.92
Honeymoon	$9.5x10^{-4}$±0.70
Sunshine sweet	N/A
Champion	$3.4x10^{-4}$±0.46

Among all four hybrids studied, Champion produced the best quality fruit based on its vitamin C value and TSS. The F_1 hybrid rockmelon fruits quality can be arranged in descending order as follow: Champion > Glamour > Honeymoon > Sunshine Sweet. Champion was the most recommended to be planted and further commercialized as it could gave high fruit quality for consumers satisfaction.

β-carotene is a pigment that found in plants especially carrots, colorful fruit and vegetables (Jaswir et al., 2011). There was a positive relationship between fruit orange color intensity with β-carotene content (Krinsky, 1998; Russell, 1998). Studied done by Englberger et al. (2003) and Amorim et al. (2009) found that Micronesian and Brazilian bananas with yellow to orange color gave the highest β-carotene content. However, in this study, Honeymoon with low orange color intensity had given the highest value of β-carotene content compared to Champion with high orange color intensity. Howard et al. (2003) and Islam et al. (2003) stated in their study that the different quantity on formation of secondary metabolites (β-carotene) from different cultivars could be influenced by genetic factors and plant growing condition.

Conclusions

As conclusion, Glamour is the best F_1 hybrid rockmelon seed that could perform fine seedling growth. On the other hand, Champion fruits gave the best quality among those hybrids with high vitamin C and total sugar content preferences. However, Honeymoon may contribute the largest β-carotene source within the fruit and could further used as antioxidant. More research on the plant anatomy, chlorophyll content and photosynthesis are recommended for each type of F_1 hybrid rockmelon cultivar.

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Mango Flowering Improvement with Chemical Treatment

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Introduction

In Malaysia, mango plants grow best in areas of low rainfall and low relative humidity for flowering, and with a warm to hot climate during fruiting. Usually mango trees produce flowers a month after drought season and achieve peak in the second month after the drought season. The dry weather during the flowering period is considered ideal for mango production (Yee, 1979). The mango trees in Malaysia generally exhibit two flowering peaks in a year i.e. in Jan-February and July- September. In Peninsular Malaysia, mango flowering one or two times in a year depend on its clone and cultivation area. In some area, the mango trees only produce more shoots than flowers and fruits. Furthermore, the pattern of weather changes from year to year. Thus, flowering habit of mango trees are inconsistent.

Good flowering is necessary to obtain consistent mango production in Malaysia. Therefore, it is important to explore other possible chemical inducing substances, which is not only necessary for flowering induction but also for yield increase. A number of diverse chemicals that have growth regulating properties in plants have been tested for promoting/inhibiting flower production in mango in different countries. Paclobutrazol, a strong gibberellin biosynthesis inhibitor, had found effective in promoting flowering in many fruit crop species including mango. Chemicals such as ethrel and potassium nitrate (KNO_3) may also be used to stimulate bud break and modify the flowering behavior of mango. However, (Chacko, 1991) stated that the responses of plants to different flower inducing treatments differ according to cultivar, climatic conditions and geographical location.

The objective of this study was to determine the effectiveness of chemical treatment on enhancement of flowering in young Mango *(Mangifera indica*) clone "Chok Anan" (MA 224) and ultimately, the fruit production.

Materials and Methods

A field experiment was conducted at Ladang Pertanian 2, UPM Serdang, Selangor. The young mango trees clone "Chok Anan" (MA 224), aged about 12 months old were used as planting materials. Four treatments were imposed onto the tree i.e. T-1: controlled trees (Normal practices); T-2: KNO_3 sprayed at concentrations of 1%, 2% and 5%; T-3: Paclobutrazol applied as soil drench followed by 2% KNO_3 spray and 2% Ethephon spray; and T-4: Ethephon sprayed at concentrations of 1%, 2%, and 5%. The young mango trees selected for this experiment had never produced flowers before. Only mature young plants with dark green and fully expanded leaves were subjected to the treatments. Foliar spray was applied at 2 weeks intervals until flower initiation. Sapol was used in the spray solution as wetting agent. The experiment was arranged in Randomized Complete Block Design (RCBD) and plants were blocked according to the tree size. Treatments were applied in early May, 2011.

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The parameters recorded were panicle length (cm), flowering intensity, and fruit number. Flowering intensity in terms of the percentage of shoots flowered on canopy was assessed on selected branches. Panicle length was measured after full bloom by taken randomly a few panicles. Number of fruit produced per tree will be recorded. Then, the collected data were analyzed by using SPSS Statistical Package Version 16.

Results and Discussion

Foliar spray of 1% KNO_3 (T-2) induced floral bud break in mature mango shoots five weeks after application while 2% and 5% KNO_3 only produced new leaves flushes two months after application. The mango shoots only response to induce flowering at lower rates of KNO_3 due to its capability to uptake the molecules that may involve in the plant metabolism. Only little flowering was observed on treatment with 1% KNO_3 sprayed (Figure 1).

The dormant mango buds treated with paclobutrazol drench (T-3) initiated earlier flowering in response to 2% Ethephon spray (Figure 2) within 3 weeks after treatment compared to other treatments. Few days after Paclobutrazol application, leaves of treated trees had turned to dark green in color compared to control tree. Paclobutrazol treated-trees (T-3), produced high percentage of flowering intensity, compacted flowers (Figure 3) and shortened panicle length compared to other treatment (T-2 and T-4). Shorter flower panicles (Figure 4) give an indication of the fact that Paclobutrazol has been taken up by the trees. (Winston, 1992) showed that panicle size reduction (Table 1) is due to effect of Paclobutrazol, but not caused by an increase in number of panicles. Eventhough this tree fully flowered, all developing fruits dropped prematurely (Figure 5). This is because the tree cannot support the growth of fruits possibly may be due to low levels of carbohydrates reserves in storage to support fruit development.

Flowering was observed on mango shoots treated with 1% Ethephon (T-4); however there was no stimulation in flowering at concentrations of 2% and 5% Ethephon, due to failure in chemical uptake in mango shoots. Most of flowers dropped due to anthracnose and susceptible to insect and pest attacks, thus low fruit set was formed as result showed in Table 2. No flowering occurred in control tree (T-1). Other chemicals such as Ethephon and KNO_3, although were found effective in increasing flowering, the number of panicles and fruits per tree were very much lower than trees treated with Paclobutrazol as result showed in Table 2.

In this study, Paclobutrazol was able to induce almost all mango shoots to flower completely and profusely: in contrast, those sprayed with KNO_3 (T-2) and Ethephon (T-3) alone resulted only 14.29% and 20% flowering intensity with sparse flowers. This indicated that paclobutrazol has the ability to enhance early flowering response of mango to ethephon spray. Researchers have shown that flower initiation in mango is inhibited or delay by application of high concentrations of gibberellic acids (Tomer, 1984; Oosthuyse, 1996). Gibberellic acid prevents the accumulation of starch because it promotes starch breakdown and mobilization (Jacobsen and Chandler, 1987). Application of Paclobutrazol, a potent GA_3 biosynthesis inhibitor, should induce higher starch accumulation, and decrease GA levels are definitely needed to influence flowering on mango.

Flowering has found related with metabolic drifts in the stem and the leaves of plants under inductive conditions. During inductive condition, the sugar level increases and the activity of hydrolyzing and oxidative enzymes also increase in plants. DNA and RNA content are high probably due to an increase in number of growth centre involving continued mitotic activity. It would appear that with the induction of floral buds and consequent increase in the number of growth centres, the metabolic machinery is geared up to increase the mobilization of the food materials by increasing the activity of the degradative

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enzymes. Sugars produced act as respiratory substrate for the increased release of energy that is needed for synthetic activity leading to the synthesis of proteins and nucleic acids to build up new cells and tissues in the shoot.

Table 1. Effect of flower induction on panicle length.

Treatment	Average panicle length (cm)
*KNO3	
1%	18
2%	form new flushes
5%	form new flushes
*Ethephon	
1%	20.17
2%	at dormant stage
5%	at dormant stage
**PBZ	
*2% KNO3	17.5
*2% Ethephon	24.57
Control	no flower produced
* Foliar spray at every 2 weeks intervals.	
** Soil drench around the base of tree (apply once)	

Table 2. Effect of flower induction application on flowering intensity and fruit number.

Treatment	Average percentage of inflorescence/ tree	Average no of fruit/ tree
*KNO3		
1%	14.29%	10
2%	no flower produced	no fruit produced
5%	no flower produced	no fruit produced
*Ethephon		
1%	20%	13
2%	no flower produced	no fruit produced
5%	no flower produced	no fruit produced
**PBZ		
*2% KNO3	52.63%	24
*2% Ethephon	79.59%	51
Control	no flower produced	no fruit produced
* Foliar spray at every 2 weeks intervals.		
** Soil drench around the base of tree (apply once)		

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Figure 1. Little flowering initiation in tree treated with 1% KNO_3 sprayed (Treatment 2).

Figure 2. Tree treated with paclobutrazol and 2% Ethephon sprayed.

Figure 3: Compacted flowers.

Figure 4: Short panicle length in trees treated with paclobutrazol.

Figure 5: Formation of fruit set (Treatment 3).

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Conclusions

In conclusion, it can be deduce that shoot maturity may influence tree internal factors such as assimilation production in order to transform shoot from its vegetative phase (dormant terminal bud) to reproductive phase (flowering). Paclobutrazol followed by 2% ethephon spray was found to be effective on enhancing mango flowering in young clone "Chok Anan" (MA 224) but the concentrations rate of paclobutrazol applied need to be altered.

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Harvesting Evaluation of *Gigantochloa albociliata* (Buluh Madu) on Shoot Productivity

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Introduction

The two most popular bamboo species planted for bamboo shoots production are *Dendrocalamus anper* (Buluh betung) and *Gigantochloa albociliata* (Buluh madu). In Peninsular Malaysia, *D. asper* is an indigenous large size species and widely distributed. The largest area planted with *D. asper* for shoot production is situated in Kulai, Johor with acreage of 70 acres. The harvested shoots are sold locally and exported to Singapore. Whereas *G. albociliata* is considered as a small size bamboo that was introduced from Thailand and recently it is widely planted by smallholders in Perlis and Kedah. Locally, the only information on *D. asper* was based on research conducted that focuses on the establishment techniques and site suitability (Abd. Razak, 1999).

In bamboo shoot harvesting activity, still there is no procedure or standard to harvest bamboo shoots in terms of its height or duration after sprouting above the ground level (Winarno, 1992). Most of the harvesting activities of the resources are unsystematic and haphazard in nature (Azmy *et al*. 1997). With proper harvesting techniques, it will help to increase the production of bamboo stock (Numata 1979). Production of good quality of shoots depended on culm density and also the age of shoots to be harvested and retained in the harvesting cycle (Yudodibroto, 1985). With systematic harvesting techniques, shoot height manipulation and culms density manipulation, the shoots production can be enhanced.

The bamboo shoots of *G. albociliata* locally known as buluh madu is widely consumed in the states of Perlis and Kedah. Most of the shoots production is harvested from planted stands. Due to its importance, trial plots have been established to assess the harvesting technique for shoot production.

Materials and Methods

The experiment was carried out at Lanchang Agriculture Department food production area in Pahang. The average annual rainfall is about 2789 mm with the mean temperature of 27 ºC and relative humidity of 80%. The method of planting used was open planting with the density of 450 bamboo clumps per ha.

Four harvestable heights of bamboo shoots (70, 90, 120 and 150 cm) from the ground level were harvested and recorded to determine the shoot production. Ten shoots were harvested for each harvestable height treatment. The parameters observed are duration to reach the harvestable height, the basal diameter, raw weight and edible weight of shoot according to harvestable height treatments. This study will give the estimation of shoot productivity and recommended shoot height for harvesting purposes.

Results and Discussion

The mean duration of harvesting, diameter, raw and edible weight and percentage of shoot recovery weight according to harvestable height of *G. albociliata* is given in Table 1. The results show a negative relation between harvesting height and percentage of shoot recovery weight. Hence, as the harvesting height increased the recovery rate is reduced and resulted in lower productivity. The harvesting height of 70, 90, 120 and 150 cm produced 49.8%, 39.1%, 31.6% and 25.7% of shoot recovery weight,

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respectively. However, the highest mean of edible weight recorded for the shoot production is at the harvestable height of 120 cm and 9 days after sprouting from the ground, followed by 90, 150 and 70 cm shoot height producing 222.9, 218.7 and 202.2 g, respectively. This result showed that shoot productivity can be determined by the height of bamboo shoot to be harvested. Result also showed that there is not much different in diameter of shoot recorded during harvesting and the mean diameter is 45 mm.

Table 1. The mean duration of harvesting, diameter, raw and edible weight and percentage of shoot recovery weight according to harvestable height of *G. albociliata.*

Shoot height (cm)	Duration of harvesting (days)	Diameter (mm)	Raw weight (g)	Edible weight (g)	% of shoot recovery weight
70	5	46.8	406.1	202.2	49.8
90	6	45.5	569.4	222.9	39.1
120	9	43.5	788.8	249.6	31.6
150	10	43.1	850.6	218.7	25.7

The statistical analysis as shown in Table 2 below revealed that harvestable shoot height had highly significant influence on the weight productivity of bamboo shoot. However, there is no significant effect of harvestable height of bamboo shoots on the shoot basal diameter.

Table 2. Analysis of variances on the mean diameter, raw weight and edible weight of shoots harvested according to harvestable height of *G. albociliata.*

Source of variation	F - values			
	df	Diameter	Raw weight	Edible weight
Height	3	0.519ns	0.001**	0.002**

ns – not significant at P< 0.05; ** *- highly significant at P < 0.05*

Results in Table 3 showed that the raw weight of bamboo shoots increased according to harvestable height. The bamboo shoots can be harvested up to 150 cm in height, however the best harvestable height to produce optimum edible weight is 120 cm (produced 235.5 g of shoot). This shows that the harvesting height is closely related to shoot production and will affect the recovery of bamboo shoots. It is also interesting to note that the diameter of shoots have no effect on shoot production.

Table 3. Effects shoot harvesting height on diameter, raw and edible weight of *G. albociliata.*

Harvesting height of shoot (cm)	Mean values		
	Diameter	Raw weight	Edible weight
70	46.8a	405.59a	196.29a
90	45.5a	565.07b	221.55ab
120	43.1a	765.77c	235.46b
150	43.0a	843.45c	219.75ab

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

Conclusions

The studies showed that the recommended harvesting height of *G. albociliata* (Buluh madu) shoots is 120 cm and it took 9 days after sprouting from the ground. It can produce the mean of 235.5 g of edible weight per shoot.

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Optimum Nutrient Solution for Growth of Maize Seedlings

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Introduction

In Malaysia, maize is an important commodity to the livestock industry. However, Malaysia could only produce 27,000 tonnes of maize in 2008 and depends heavily on maize importation to sustain the local demand (Anonymous, 2009). Therefore, innovation on the current maize cultivation practice is needed. Studies have shown that the use of plant growth promoting rhizobacteria (PGPR) as biofertilizers can increase maize growth and yield (Piromyou et al., 2011). Growing maize in a soilless culture condition is an efficient and rapid method to conduct nutrient related research. Hoagland's nutrient solution is a commonly used plant medium which contains all the essential plant nutrients required for plant growth. However, its effective concentration needs to be determined to prevent the occurrence of deficiency or toxicity effects on maize seedlings (Hoagland and Arnon, 1950). This experiment was conducted to determine the optimum concentration of Hoagland's nutrient solution in a soilless culture environment, as well as the effects of inadequate and excessive nutrient supply, on the growth of maize seedlings. This experiment provides a preliminary basis for further investigations on the effects of PGPR on maize growth and yield via growth chamber, pot and field trials.

Materials and Methods

The study was conducted in a growth chamber (28/26 ^{o}C on 12/12 hours day/night cycle) over duration of two weeks at the Soil Microbiology Laboratory, UPM, Serdang. Maize variety used was Masmadu variety, obtained from Malaysian Agricultural Research and Development Institute (MARDI). Five seeds were sowed in a loosely sealed Kilner jar with three layers of filter papers at the base. Hoagland's nutrient solution concentration was provided according to the five treatments imposed at the beginning of the experiment (Hoagland and Anon, 1950). The experiment was conducted in a completely randomised design (CRD) with three replicates and a total of 15 experimental units. There were five treatments; 0% (distilled water), 25%, 50%, 75%, and 100% HS. Parameters measured were the chlorophyll index by a SPAD meter (Minolta SPAD-502), plant height, root length, and fresh and dry weights of tops and roots. Analysis of variance (ANOVA) was done to determine any significant differences between treatments. Significant difference between the treatment means was established by using least significant difference (LSD).

Results and Discussion

Figure 1 shows the mean chlorophyll index (SPAD value) of maize seedlings grown at various concentrations of HS. 100% HS had the highest SPAD value at 23.59, followed by 50%, 75%, and 25% HS at 22.11, 21.66 and 20.46, respectively. 0% HS had the lowest SPAD value at 13.90. These differences in SPAD values could be due to the differences in the amount of available nitrogen in the various HS. Plant chlorophyll content correlates to the amount of plant available nitrogen absorbed by maize roots and any deficiencies or excess of plant nitrogen are reflected in the leaf chlorophyll (Shapiro, 1999).

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The height of maize seedlings grown in various HS were significantly different (p=0.05) as shown in Figure 2. The 50% HS concentration had the tallest maize seedlings (16.3 cm) but was not significantly different from 75% HS (12.7 cm). The shortest seedlings were from 0% HS at 6.6 cm but had the longest root length at 15.4 cm. Root lengths due to 25%, 50%, 75%, and 100% HS, were 12.0 cm, 13.3 cm, 11.8 cm, and 10.4 cm, respectively. The elongated and extended roots in 0% HS could be due to the need of the plants to source for more plant nutrients. Inadequate and excessive nutrient supply can cause stunted seedlings as observed in 0% and 100% HS, respectively (Hoagland and Arnon, 1950).

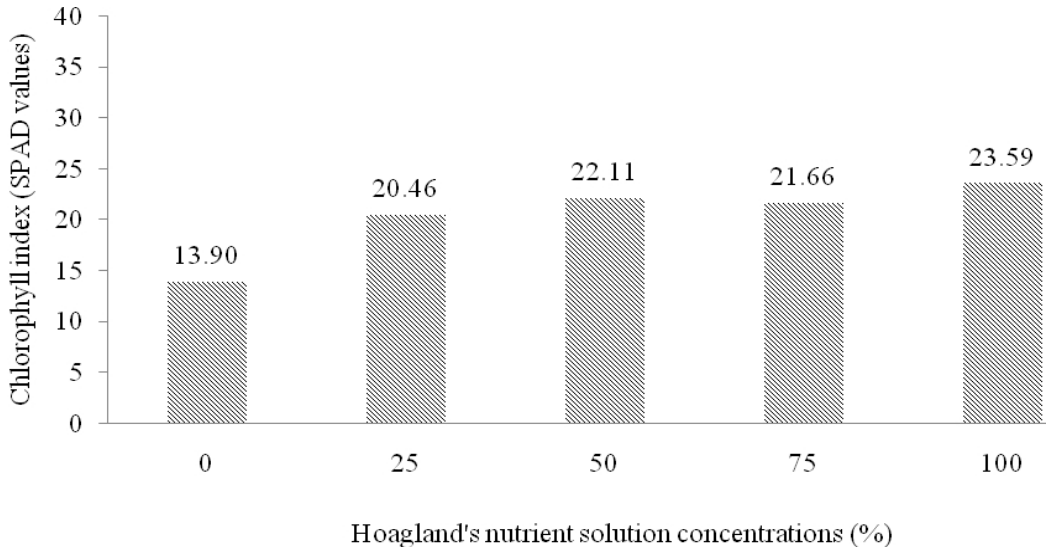

Figure 1. Chlorophyll index (SPAD value) of maize seedlings grown at various concentrations of Hoagland's nutrient solution. (Mean values are not significantly different at p=0.05).

The fresh and dry weights of tops and roots showed similar trends, except at 0% HS (Figures 3 and 4). A concentration of 50% HS produced the highest fresh and dry weights of tops and roots, with 0.508, 0.453, 0.034, and 0.023 g, respectively. In Figure 3, low fresh weight of tops were observed at 0% (0.253 g) and 25% HS (0.308 g). The 0% (0.293 g), 25% (0.240 g), 75% (0.290 g), and 100% HS (0.253 g) treatments also produced low fresh root weights. In Figure 4, dry weight of tops from 0%, 25%, and 100% HS, were at 0.021, 0.022, and 0.021g, respectively. Dry weight of roots from 25% HS was the lowest at 0.011 g. Results showed maize seedlings with 50% HS received sufficient level of essential nutrients to produce optimum growth of plant tops and roots as also observed by Piromyou et al. (2011). Therefore, 50% HS concentration can be used as a medium in future determinations of PGPR effects on seedlings growth and yield of maize, under controlled conditions.

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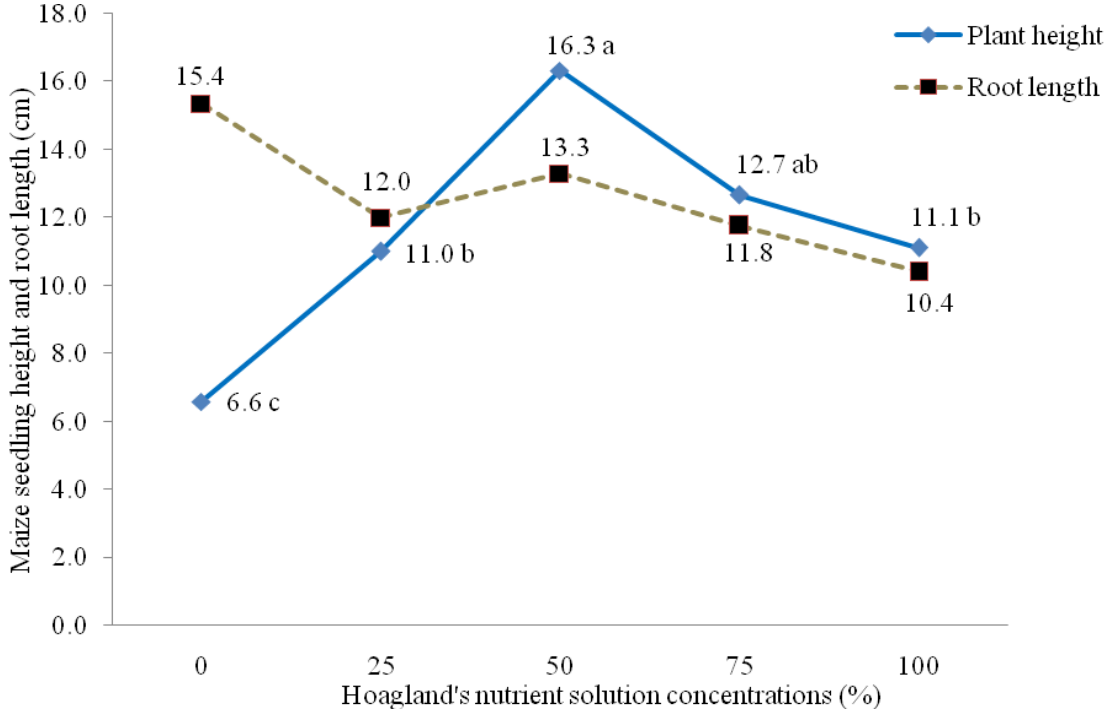

Figure 2. Height and root length of maize seedlings grown at various concentrations of Hoagland's nutrient solution. (Mean values with different letters on the lines are significantly different at p=0.05).

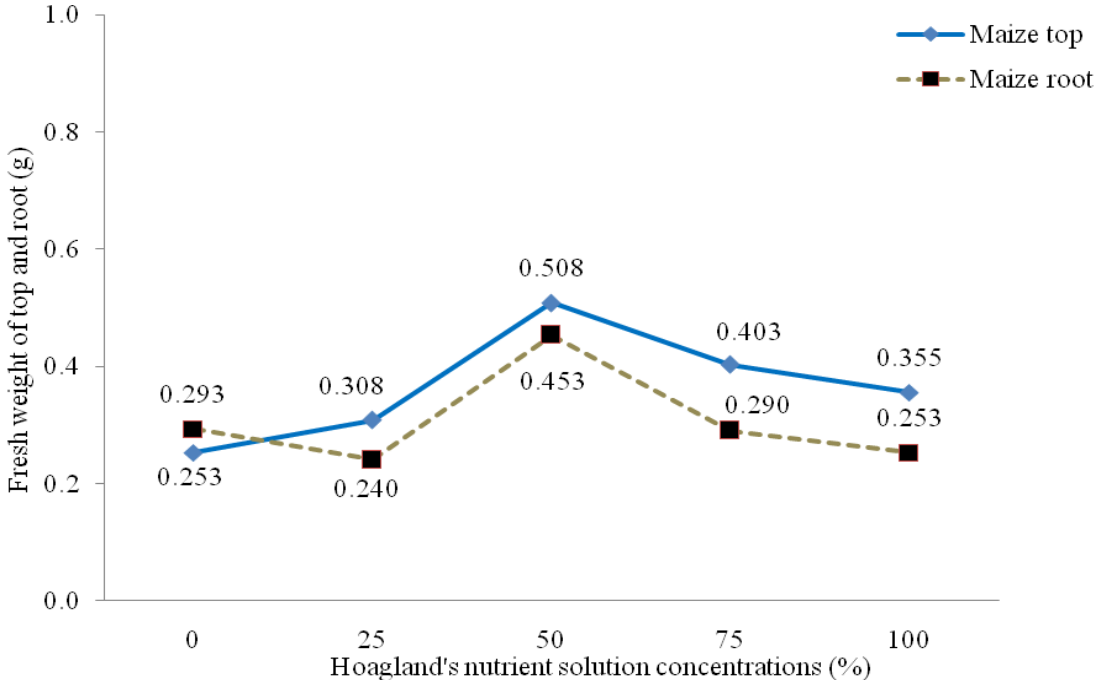

Figure 3. Fresh weight of maize tops and roots grown at various concentrations of Hoagland's nutrient solution. (Mean values are not significantly different at p=0.05).

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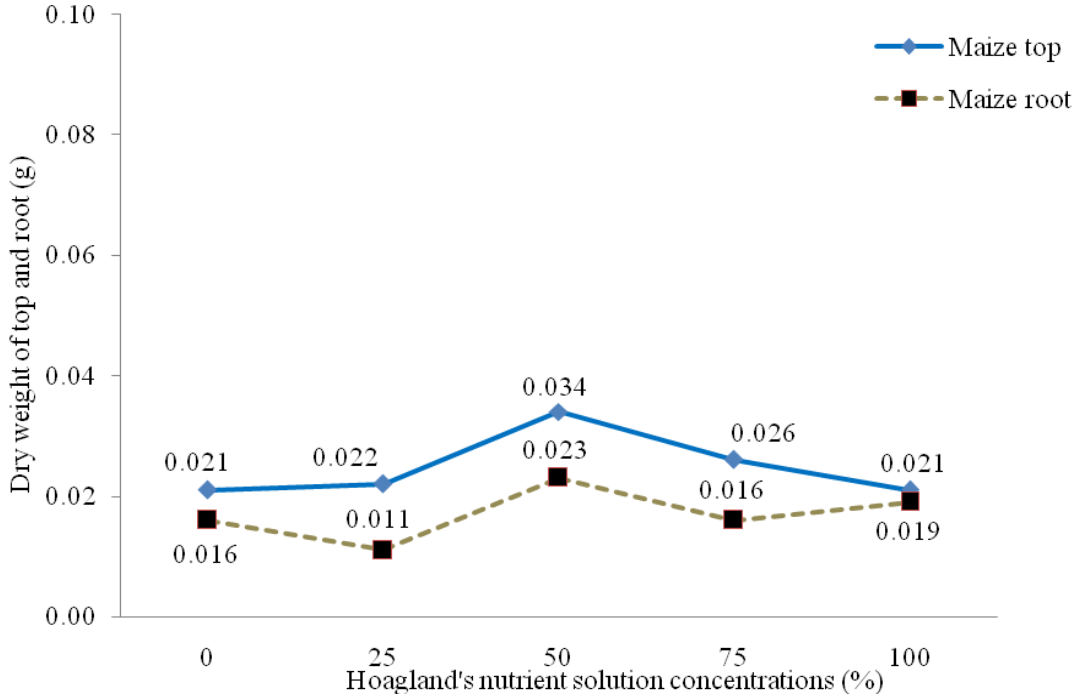

Figure 4. Dry weight of maize tops and roots grown at various concentrations of Hoagland's nutrient solution. (Mean values are not significantly different at p=0.05).

Conclusions

The experiment showed that the most suitable concentration of Hoagland's nutrient solution for growth of maize seedlings in a soilless culture condition is at 50%, which produced the maximum plant height, fresh weight of top and root, dry weight of top and root, SPAD value and root length. In addition, growth of maize seedlings is reduced by inadequate or excessive nutrient supply. Further investigations on the effects of PGPR and other plant nutrient sources on seedlings growth and yield of maize under controlled conditions are suggested.

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Effect of Pyroligneous Acid on Growth, Yield and Quality Improvement of Rockmelon in Soilless Culture

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Introduction

Melon (*Cucumis melo* var. Cantalupensis) is an important commercial crop in many countries. Rockmelon fruits are highly consumed in the summer and are popular because of its sweet pulp and the pleasant aroma (Villanueva et al., 2004). In Malaysia, the cantaloupe type, especially the cultivar 'Glamour' (with the striking golden yellow color) is the favourite. Pyroligneous acid or wood vinegar is the crude condensate produced from the distillation of volatiles substances generated in the process of making charcoal. This by-product is rarely used and often disposed off as waste. Chemically, it occurs as a complex mixture of water, guaiacols, catecols, syringols, vanillins, furan carboxaldehydes, isoeugenol, pyrones, acetic acid, formic acid and other carboxylic acids. Major groups of compounds present in pyroligneous acid includes; hydroxyaldehydes, hydroxyketones, sugars, carboxylic acids, and phenolics (Guillén and Manzanos, 2002). Pyroligneous acid has been used in traditional agriculture to increase seedling vigour and crop stands (Modi, 2002). Pyroligneous acid has have also been shown to induce new branches, elongate roots, and increase plant height and rice yield (Tsuzuki et al., 1989). The aim of this study was to compare the effects of three fertilizer formulations (M, CS and BEN) in combinations with different concentrations of pyroligneous acid on plant growth, fruit yield and quality of rockmelon (*Cucumis melo* L. cv. 'Glamour').

Materials and methods

Plant materials

The experiment was conducted under rain shelter facilities of Kumpulan Pertanian Kelantan Berhad, Bachok, Kelantan, Malaysia (Latitude: 6.067, Longitude: 102.400). Rockmelon seeds (*Cucumis melo* L*, cv*. 'Glamour') were germinated in seed trays containing peat-moss as sowing medium. Trays were watered twice daily (1 liter per tray) to ensure healthy seedling germination and growth. After one week of establishment seedlings were transplanted into 30 x 25 cm polybags with one seedling per bag. The pyroligneous acid supplied by MM Charcoal Company in Taiping, Perak, Malaysia, is a by-product resulting from the burning of mangrove wood to produce charcoal. Samples were taken in triplicate to determine heavy metals by Inductively Coupled Plasma Mass-Spectrometry (ICP-MS; Model DRC-e, PerkinElmer Inc.) and analysis of semi-volatile organics by Gas chromatography-Mass spectrometry (GC-MS) fingerprinting (Agilent Tech 5973, Inert MSD and HP 7694 Headspace Sampler), (Table 1).

Treatments and experimental design

The three types of fertilizer formulations evaluated were local formulation (M), Cooper Standard (CS) and Benoit (BEN) (Table 2). The growth medium was a 3:1 v/v mixture of coconut dust and empty oil palm fruit bunch fibres (Table 3). Dilutions were made by adding 10, 20 or 30 ml of concentrated pyroligneous acid to 90, 80 or 70 ml of distilled water respectively, to obtain 10, 20 and 30% concentrations of pyroligneous acid. Ten ml of 0, 10, 20 or 30% of pyroligneous acid was added once a week to the planting media from transplanting to the third week of crop growth. In all fertilizer storage

tanks, the electrical conductivity (EC) of the solutions was maintained within the range of 1.0 to 3.0 dS/m. The nutrient solutions were pumped through a drip fertigation system, twice a day with a total of two litres per plant. The polybags were weeded once per month to ensure normal plant growth without weed competition. Insecticides (malathion, chloropyrifos and deltamethrin) and fungicides (chlorothalonil, thiram and mancozeb) were applied in alternate sequence as and when necessary to control insect pests and diseases. Treatment combinations of four levels of pyroligneous acid (including control) and the three fertilizer formulations were arranged in a Randomized Complete Block Design with eight replicates. Preliminary quantitative and qualitative analysis of semi-volatile organics in pyroligneous acid was performed. Crop growth and fruit characteristics recorded include plant height (at 0, 10, 17 days after transplanting), number of leaves, fruit weight, diameter of fruits, and fruit sugar content (% Brix).

Table 1. Semi-volatile organics in pyroligneous acid (GC-MS fingerprinting using Agilent Tech 6890N GC with Agilent Tech 5973 inert MSD and HP 7694 headspace sampler)

No.	Identified	Hit list (%)
1.	Pyrogallol 1,3-dimethyl ether (ester group)	93
2.	2-methoxy-benzeneethanol (alcohol group)	90
3.	1,2,3-trimethoxy-5-methyl benzene (ester group)	87
4.	Methyl 3-methoxy-4-hydroxybenzoate	72
5.	3-(o-Azidophenyl) propanol (alcohol group)	72
6.	N-(dimethylthiophosphinyl)-3-amino pyridine	72
7.	2-pyridinepropanoic acid (acid group)	64
8.	2,5-dimethylphenol (alcohol group)	64

Values are means of three replications

Table 2. Chemical composition of fertilizer formulations

Elements	Local Formulation (%)	Cooper standard (%)	Benoit (%)
Stock A			
Calcium nitrate	24.36	22.03	12.72
Ferum chelate (EDTA)	0.42	1.76	2.60
Potassium nitrate			0.12
Stock B			
Potassium nitrate	22.26	12.98	3.32
Magnesium sulfate	12.54	11.41	3.20
Manganese sulfate	0.04	0.08	0.08
Boric acid (Boron)	0.14	0.14	0.14
Cooper sulfate	0.08	0.08	0.08
Ammonium molybdate	0.01	0.01	0.01
Zinc sulfate	0.08	0.08	0.08
Monopotassium	5.84	5.84	2.21
Potassium sulfate			0.29

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Table 3: Available macro and micro nutrients in the growth medium (ppm)

Media	N	P	K	Mg	Ca	Fe	Zn
Coconut dust with empty fruit bunch (3:1, v/v)	7700††	900	8700	9000	11500	16.73	40.56

Nitrogen=N, Phosphorus=P, Potassium=K, Magnesium=Mg, Calcium=Ca, Iron=Fe, Zinc=Zn.
Values are means of 3 replications

Statistical analysis

The data were statistically analyzed using the ANOVA procedure in the SAS Statistical Software Version 9.0.Tukey's studentized range test was used to compare differences between treatments.

Results and Discussion

Constituents of pyroligneous acid fraction

Inductively Coupled Plasma Mass-Spectrometry (ICP-MS) analysis of the pyroligneous acid samples revealed the presence of 15 elements. The elements detected were calcium, cadmium, chromium, copper, iron, potassium, magnesium, lead, aluminum, sodium, zinc, arsenic, boron, molybdenum and phosphorus. Highest concentrations were recorded for calcium, iron and sodium with levels of 8.82, 5.80 and 3.93 ppm, respectively (Figure 1). Cadmium, copper, lead, arsenic, zinc, boron, molybdenum and phosphorus were present at very low levels, while chromium, potassium, magnesium and aluminum were present at intermediate levels.

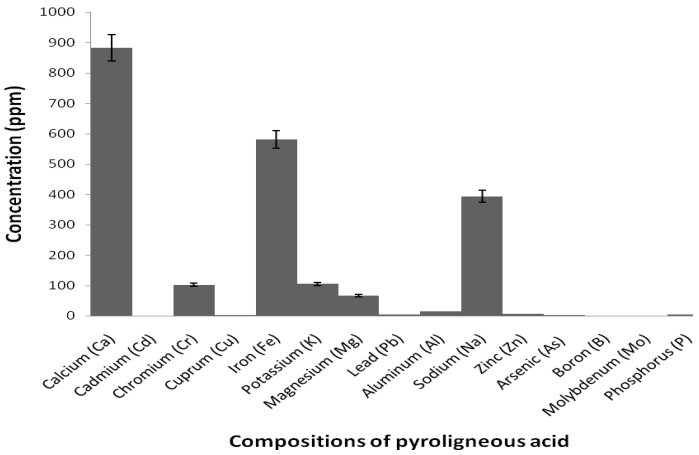

Figure 1. Quantitative analysis of pyroligneous acid using inductively coupled plasma-mass spectrometer (ICP-MS, Model DRC-e).

Effect of treatments on plant height

At 17 day after transplanting (DAT), the tallest plants were recorded in treatments with formulation M (188.83 cm), followed by BEN (177.29 cm), while the shortest plants were observed with the CS formulation (175.23 cm). The taller plants at 10 DAT were recorded with the 0, 10 and 20% concentration of pyroligneous acid (86.78, 95.90 and 86.06 cm, respectively). However, there were no significant differences between fertilizer formulations. The use of the local fertilizer formulation (M) resulted in

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taller plants compared to the other fertilizers throughout the growth stages, indicating that this fertilizer formulation and pyroligneous acid had positive effects on growth and development of rockmelon plants (Figure 2). The increased plant height in M fertilizer formulation compared to other formulations was possibly due to the better growth conditions provided by the media (Shinde et al., 1999). The increased plant height can be attributed to the higher nutrient composition in M formulation, especially calcium nitrate, potassium nitrate, magnesium sulfate and mono potassium. Calcium is an important element for cell division and root development and functioning (Evans and Sorger, 1966; Zekri, 1995a: Zekri, 1995b). The high level of potassium in the M fertilizer formulation would have enhanced cell division and plant growth. There were significant increases in plant height with the addition of 10% pyroligneous acid at 10 DAT. These results revealed that pyroligneous acid promoted growth. Similar results were reported in three vegetable crops (tomato, okra and bean) with significant improvement in seedling vigour and growth in comparison to controls when treated with pyroligneous acid (van Staden et al., 2006). In addition, pyroligneous acid is rich in nutritional components (Narwal, 2000; Tsuzuki et al., 1989) that attracts microbes including bacteria and fungus to roots of plants where symbiosis occurs. The positive effects of bacterial and mycorhizal symbiosis can be attributed to an improved nutritional state (due to N supplied by bacteria and P by mycorhizae), which in turn leads to increased photosynthetic rates and improved plant growth (Kaschuk et al., 2009).

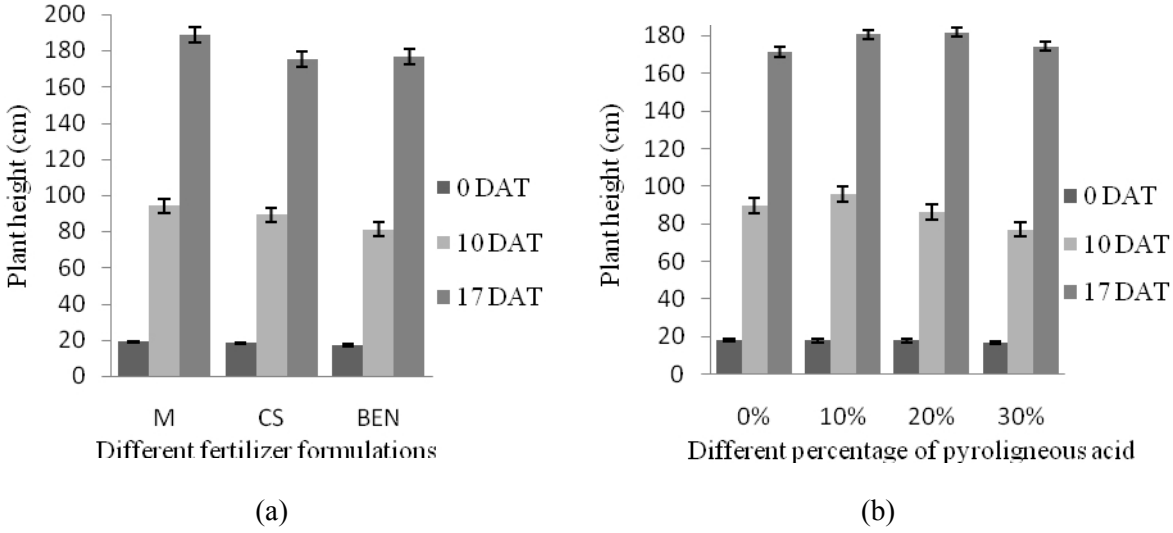

Figure 2. Effect of (a) different fertilizer formulations, and (b) concentrations of pyroligneous acid, on height of rockmelon plants during the first 17 DAT. (M: Local formulation, CS: Cooper standard, BEN: Benoit fertilizer formulation, Means over eight replications).

Effect of treatments on number of leaves

Irrespective of treatments, the number of leaves increased with increasing plant age (Table 3). However, M fertilizer formulation produced significantly higher number of leaves per plant compared with other fertilizer formulations, with mean values of 24 and 42 at 10 and 17 DAT, respectively. No significant variation in the number of leaves was observed with the pyroligneous acid treatments at 10 and 17 DAT. At 17 DAT, the lowest number of leaves was observed with the CS fertilizer formulation. The number of leaves per plant increased rapidly from 10 to 17 DAT. There was no significant variation observed in the number of leaves in different concentrations of pyroligneous acid. However, there were significant differences in pyroligneous treatments in combination with fertilizer formulations. It has also been previously reported that with nitrogen (N), phosphorus (P), potassium (K) and pyroligenous acid, more

leaf biomass was obtained (Singh et al., 2009). In the present study plants treated with M formulation produced significantly higher number of leaves at 10 and 17 DAT. The higher leaf number recorded in the M formulation was probably because of the high calcium content in the medium which boosted leaf production. Ruiz et al., (1999) had reported similar results in tobacco (Nicotiana tabacum cv Sevilla) where the application of calcium chloride gave rise to greater production of leaf and root dry matter compared to the control.

Table 3. Effect of fertilizer formulations and pyroligneous acid on number of leaves produced per plant.

Fertilizer formulations	0 DAT	10 DAT	17 DAT
M	13a	24a	42a
CS	12a	23ab	37b
BEN	12a	23ab	40ab
Pyroligneous acid (%)			
0	12a	23a	40a
10	12a	22a	37a
20	12a	23a	41a
30	12a	22a	39a
CV	14.98	17.39	10.76

M=Local formulation, CS=Cooper standard, BEN=Benoit fertilizer formulation and CV=Coefficient of variation. Values are means of eight replications; Means within columns followed by the same letter(s) are not significantly different at P<0.05 (Tukeys test).

Effect of treatments on fruit production

Fruit weights were recorded at 110 DAT (Table 4). Results revealed that fresh fruit weight was significantly higher with M fertilizer formulation, indicating that this formulation had positive effects in increasing fruit weight. The highest fruit weight of 1.86 kg was observed with 10% of pyroligneous acid in combination with the M formulation followed by CS and BEN fertilizer formulations and 20, 30 and 10% pyroligneous acid treatments, respectively. The lowest fruit weight of 1.43 kg was obtained with BEN fertilizer formulation without pyroligneous acid. Fruit diameter was significantly different in different fertilizer formulations. Fruit diameter varied significantly with different percentages of pyroligneous acid (Table 1). The larger fruit diameter of 14.43 and 14.25 cm were recorded in M and CS fertilizer formulation with 10 and 20% of pyroligneous acid, respectively. The smallest fruit diameter of 13.24 cm was recorded in BEN fertilizer formulation with 0% pyroligneous acid. Local (M) and CS fertilizer formulations resulted in sugar contents of 12.68, 12.63% (Brix). The results on sugar content of rockmelons revealed significant differences between treatments. Pyroligneous acid treatments at 10, 20 and 0% resulted in fruit sugar contents of 13.00, 12.89 and 12.64% Brix, respectively. Higher fruit weights produced with the 10% pyroligneous acid is attributed to the better root system produced with this treatment. A similar increase in fruit yield obtained in fertigation with 100% water-soluble fertilizer (WSF) was attributed to lesser leaching of nitrate-N and K to deeper layers of soil (Hebbar et al., 2004). Fruit weight and size were better in the M fertilizer formulation probably due to presence of more nutrients than in other fertilizer formulations. These include calcium nitrate, potassium nitrate, magnesium sulphate, and monopotassium phosphate. Highest fruit weight, fruit size and sweettness in M fertilizer treatments are attributed to the beneficial effects of pyroligneous acid, which enhanced fruit production and quality (Figure 1 and Table 3). Large amounts of assimilates produced (Gifford and Evans, 1981) and the associated high sink strength were important factors in determining high yields in crop production as shown in the M fertilizer treatments with 10% of pyroligneous acid. Fruit weight increased by 37.3 and 27.5% with 10 and 20% pyroligneous acid compared with control, respectively. Pyroligneous acid is known to be rich in nutritional components that increase fruit yield and size (Narwal,

2000; Tsuzuki et al., 1989). The local formulation resulted in only a 4% increase in fruit weight compared to the CS fertilizer formulation. However, the BEN formulation resulted in 21% lower fruit weight than the M fertilizer formulation. Interestingly, pyroligneous acid enhanced the fruit weight of rockmelon plant due to the better rooting system, flowering and growth of the plant. Furthermore, sink stimulation of photosynthesis could possibly lead to an increased period of leaf activity or delayed senescence (Paul and Peliny, 2003), which in turn could increase the potential period for plant growth and fruit weight or yield. Harris et al. (1985) suggested that C sink strength of symbioses stimulated the rate of photosynthesis. The pyroligneous acid contains several esters, alcohols, acids and several heavy metals which may have contributed to better growth and production (Figure 1 and Table 4). Previous studies have also shown that pyroligneous acid interacts with gibberellins, cytokinins, abscisic acid and ethylene in photoblastic and thermodormant seeds (van Staden et al., 2000). It has also been suggested that the active principles in pyroligneous acid behave in a manner similar to that of other plant growth regulators (Senaratna et al., 1999; Gardner et al., 2001). However, it is not yet clearly understood how pyroligneous acid promotes growth or how it interacts with other plant hormones. In general, crop productivity may be increased or decreased depending on inhibitory or stimulatory effects of different factors on each other under non-limiting growth resources, such as light, water, nutrients and space (Narwal, 2000). The sugar content in all fertilizer formulation treatments in combination with pyroligneous acid were between 12-13%. In terms of cost, CS fertilizer formulation is more costly followed by M and BEN. The highest total weight of fertilizer used was the local formulation with almost 33 kg. The lowest total weight of fertilizer used was with the BEN fertilizer formulation (14 kg). With the higher total weight of fertilizer extra transportation cost will be involved. In terms of nitrate content, BEN fertilizer formulation was more environmental friendly, followed by CS and M with 13, 17 and 24 kg, respectively.

Table 4. Effect of fertilizer formulations and pyroligneous acid on fruit yield and quality

Treatments	Fertilizer formulations			Pyroligneous acid (%)				CV
	M	CS	BEN	0	10	20	30	
Fruit weight (kg)	1.73a	1.66b	1.43c	1.34d	1.86a	1.71b	1.52c	4.46
Fruit diameter (cm)	14.43a	14.25a	13.77b	13.24c	14.62a	14.64a	14.08b	2.76
Sweetness (% BRIX)	12.68a	12.63ab	12.25b	12.64a	13.00a	12.89a	11.53b	2.97

Means within columns followed by the same letter(s) are not significantly different at P<0.05 (n= 8)
M=Local formulation, CS=Cooper standard, BEN=Benoit fertilizer formulation and CV= Coefficient of variation.

Conclusions

In conclusion, the addition of pyroligneous acid as organic substance was effective in increasing fruit yield. Addition of 10% percent pyroligneous acid was suitable for increasing fruit weight of rockmelons in combination with M fertilizer formulation. The M formulations in combination with 10% pyroligneous acid had the ability to improve the productivity of rockmelon. Better fruit size and sweetness was also obtained in combinations of M and CS formulations with 10 and 20% of pyroligneous acid.

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CHAPTER 2

POSTHARVEST TECHNOLOGY AND QUALITY CONTROL

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Postharvest Quality of Harumanis Mango after Ethephon Dipping

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Introduction

Harumanis mango (*Mangifera indica* L.) is a well known fruit found in Indonesia and Malaysia (Kusumo et al., 1984). It grows healthily in Bukit Bintang and Paya Kelubi, Perlis (Abdul Razak Shafiai, Pers. comm.). A Harumanis mango fruit weigh about 500 g and the skin remained green although the pulp has softened and taste sweet (Yuniarti, 1980). This causes a low demand for this fruit because consumers prefer a yellow skin as compared to green skin mango fruit when ripened.

According to Kays (1991), the appearance of fresh fruits is a primary criterion in making purchasing decisions. Colour, size, shape, form, condition and absence of defects are the main characteristics of product appearance. Appearance is utilized throughout the production – storage – marketing – utilization chain as the primary means of judging the quality of individual unit of product (Kays, 1999). Fruit appearance is of critical importance to consumers because it sets up expectations of what the product will taste like (Jaeger and MacFie, 2001). In mango fruits, consumers prefer yellow skin because it reflects the fruits have ripened and ready for consumption. However, a 'green-riped' fruits affect consumer perception and preference and price in marketplace (Janave and Sharma, 2006).

Ethephon (2-chloroethyl phosphonic acid) is a synthetic plant growth regulator which acts by releasing ethylene when it penetrates plant tissues. Ethephon has been widely used in improving fruit colour. By treating pepino fruits with ethephon, the colour of fruits change from green to yellow or orange with purple stripes (Lopez et al., 2000). It has also been reported that treatment with ethephon enhanced red skin of Cripp's Pink apple (Whale et al., 2007). Ethephon has also been reported to stimulate and advance ripening and reduce the storage potential of the apple fruits (Wang and Dilley, 2001 and Stover et al., 2003). However, studies on effects of ethephon application on Harumanis mango is still lacking. Thus, this study was carried out to evaluate the effects of ethephon on postharvest quality of Harumanis mango fruit.

Materials and Methods

The material used in this study was Harumanis mango fruits (*Mangifera indica* L.) from Department of Agriculture, Bukit Bintang, Perlis, Malaysia. The fruits were harvested from 8 to 10 years old plants with 9 m x 9 m planting distance. Green-matured Harumanis mango fruits at weeks 13 after flower anthesis were harvested manually in the morning. Only uniform size and free from defects fruits were used. Three replications with 40 mango fruits were selected for each replication. The fruits were washed to remove debris and latex stains. Then, the fruits were dipped in hot water at 50 ºC for 10 min to control the postharvest pathogens. After dipping with hot water, the fruits were dried using a blower before being packed. The fruits were arranged in a fibre board carton of 60 x 30 x 30 cm^3 and transported within 24 h directly to Postharvest Laboratory, Universiti Putra Malaysia, Selangor.

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The fruits were randomly divided into five groups. Each group consisted of eight fruits and treated by immersion in solutions of ethephon at five different concentrations; 0 (control), 2000, 4000, 6000 and 8000 mgl^{-1}. A commercial wetting agent (Tween 20, 0.1% (v/v)) was added to all ethephon solutions. The fruits were dipped for 10 min in the solutions. Then, the fruits were allowed to ripen for 5 days at 25 ^{o}C and 67% RH. Analysis was carried out on day 0, 1, 3 and 5 using two fruits/treatment to determine skin and pulp colour, pulp firmness, soluble solids concentration (SSC), pH, titratable acidity (TA) and vitamin C. The experimental design was a complete randomized with three replications. Data were analysed using the analysis of variance (SAS Institute, Cary, NC) and means were separated by Duncan's multiple range test.

Results and Discussion

The ethephon treatment had no significant effects on the skin and pulp colour of Harumanis mango as evidenced by the F-test of the lightness (L*), chroma (C*) and hue (h^{o}) values (Table 1). As ripening day progressed, there was no significant difference on L* values of the skin colour. The C* values decreased significantly as fruit ripen to day 5. The h^{o} values of Harumanis mango skin colour decreased significantly as fruit ripen.

For the pulp colour of Harumanis mango, there were significant differences in L*, C* and h^{o} values as ripening progressed (Table 1). The decrease in values of L* and h^{o}, accompanied by an increase in the value of C* indicated that the pulp colour became orange as ripening day progressed. There were no significant interactions in skin and pulp colour between the ethephon concentrations and ripening day.

Table 1. Skin and pulp colour (L*, C* and h^{o}) of Harumanis mango after treated with five different concentrations of ethephon and ripened for 5 days at 25 ºC, 65% RH.

	Skin colour			Pulp colour		
Factor	L*	C*	h^{o}	L*	C*	h^{o}
Concentration of ethephon ($mg\ l^{-1}$), E						
0	56.65	32.22	117.59	75.90	49.48	91. 88
2000	56.69	33.37	114.49	74.69	49.93	91.45
4000	56.22	33.60	114.92	74.79	49.68	91.19
6000	56.74	33.04	114.77	73.05	50.19	89.53
800	56.48	33.53	115.42	75.06	49.00	91.59
F-significant	NS	NS	NS	NS	NS	NS
Ripening Day, D						
0	56.58	34.38 a	119.66 a	79.16 a	46.34 a	94.52 a
1	57.26	33.21 a	118.26 a	78.37 a	45.90 b	94.52 a
3	56.70	33.72 a	114.09 b	71.11 b	52.73 a	89.09 b
5	55.59	31.30 b	109.72 c	70.15 b	53.66 a	86.38 c
F-significant	NS	*	**	**	**	**
Interaction						
E x D	NS	NS	NS	NS	NS	NS

n = 120
Mean separation within columns and factors by LSD at $P \leq 0.05$.
*NS, *, ** Non significant or significant or highly significant at $P \leq 0.05$, respectively.*

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The ethephon treatment had no significant effect on the pulp firmness of Harumanis mango (Table 2). As ripening day progressed, there were highly significant effects in the pulp firmness. The pulp firmness was significantly higher at day 0 and decreased as ripening progressed. On the other hand, there were no significant interaction between the ethephon concentration and ripening day. Similar result was reported in Jonagold apple where ethephon application did not affect pulp firmness (Awad and Jager, 2002).

The ethephon treatment had no significant effect on the SSC of Harumanis mango (Table 2). As ripening days progressed, SSC increased significantly. However, there were no significant interaction between the ethephon concentration and ripening day in SSC. A similar result was obtained in Macoun apple which was treated with 300 mgl^{-1} of ethephon treatment (Autio and Krupa, 2006). However, in the study of Keitt (Sergent et al., 1993) on Kensington Pride (Singh and Janes, 2001) and Kent mangoes (Centurion et al., 1998), the SSC was significantly higher in ethephon treated fruit as compared to control.

The ethephon treatment had no significant effect on the pH of Harumanis mango (Table 2). As ripening day progressed, there were significant effects in the pH of Harumanis mango fruit. However, there were no significant interaction between the ethephon concentration and ripening day on pH.

The TA of Harumanis mango was not significantly affected by ethephon treatment (Table 2). As ripening day progressed, there were significant effects in the TA of Harumanis mango. There were no significant interaction between the ethephon concentration and ripening day on TA. However, the TA of berries was significantly lower in ethephon-treated fruit as compared to control (Ban et al., 2007). Besides, the TA was significantly reduced in ethephon-treated Kensington Pride (Singh and Janes, 2001) and Kent (Centurion et al., 1998) mangoes.

Table 2. Pulp firmness, soluble solids concentration (SSC), pH, titratable acidity (TA) and vitamin C of Harumanis mango after treated with five different concentrations of ethephon and ripened for 5 days at 25 ºC, 65% RH.

	Firmness (N)	SSC (%SSC)	pH	TA	Vitamin C (mg 100 g^{-1})
Concentration of ethephon (mg l^{-1}), E					
0	44.65	9.23	4.32	1.21	7.34
2000	49.48	9.69	4.49	1.01	8.67
4000	43.87	11.34	4.23	1.33	6.56
6000	41.04	10.04	4.41	1.35	5.77
8000	48.67	10.99	4.37	1.39	6.70
F-significant	NS	NS	NS	NS	NS
Ripening Day, D					
0	101.47 a	5.11 d	4.00 b	1.63 a	11.86 a
1	55.78 b	7.20 c	3.90 b	1.09 bc	5.92 b
3	14.29 c	12.13 b	4.69 a	0.89 c	5.32 b
5	10.65 c	16.60 a	4.86 a	1.43 ab	4.93 b
F-significant	**	**	**	**	**
Interaction					
E x D	NS	NS	NS	NS	NS

n = 120
Mean separation within columns and factors by LSD at $P \leq 0.05$.
*NS,** Non significant or highly significant at $P \leq 0.05$, respectively.*

The vitamin C content of Harumanis mango was not significantly affected by ethephon treatment and also no significant interaction between the ethephon concentration and ripening day. However, as ripening day progressed, there were highly significant effects on the vitamin C of Harumanis mango. In Kent mango, ethephon treatment reduced vitamin C content of fruit (Centurion et al., 1998). The vitamin C content increased with the increase in ethephon concentrations and higher values were obtained in the fruits treated with 600 mgl^{-1} of ethephon in papaya (Bal et al., 1992).

Conclusions

The ethephon treatment used as dipping solution for Harumanis mango fruit did not affect physical and chemical quality characteristics of postharvest. Possibly, ethephon is not suitable to be used to degreen Harumanis mango. Therefore, the objective of this study was not achieved using ethephon treatment to degreen Harumanis mango.

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Fruit Development Affecting Phytonutrient Contents of Red-Fleshed Pitaya

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Introduction

There is an increasing demand and appreciation from consumer on fresh horticultural produce quality. They are not only expecting fruit having good taste, texture and appearance but should contain antioxidants. Antioxidants are class of compounds that exert their effects in humans by preventing oxidative processes which contribute to the onset of several degenerative diseases (Kanner et al., 2001). Antioxidants are also relevant in postharvest research because they play an important role in the natural defence of fruit (De Gara et al., 2003; Hodges et al., 2004). Red-fleshed dragon fruit is potentially a good source of antioxidants as it produced red-violet colour pigments that known as betacyanin. Therefore, the objective of this study was to determine the content of total phenolic content and antioxidant activities of red-fleshed dragon fruit during development.

Materials and Methods

The study was conducted using 4-year-old dragon fruit plants by tagging 11 pollinated flowers to allow calculation of the number of stages of fruit development. Day 0 was the day where hand-cross pollination was carried out. Starting from 5 days after pollination (DAP), three fruits were harvested every 5 days until 35 DAP. The harvesting was done in the morning and the fruit was transported to the Postharvest Laboratory and analysis was carried out immediately after fruit arrival.

Total phenolic compounds in the red-fleshed dragon fruit extracts was determined using Folin-Ciocalteu reagent according to the method of Wolfe et al. (2003) with gallic acid as a standard phenolic compound. The concentration of total phenolic compounds in the extracts was determined as microgram of gallic acid equivalent (GAE)/100 g of sample by using an equation that was obtained from standard gallic acid graph. The DPPH (1,1-diphenyl-2-picrl-hydrazil) assay was carried out according to the method of Brand-Williams et al. (1995) with some modifications. The DPPH radical concentration was calculated using the following equation:
DPPH Scavenging effect (%) = $100 - [(A_0 - A_1)/A_0) \times 100]$ where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample. The ferric-reducing antioxidant power (FRAP) assay was done according to Benzie and Strain (1996) with some modifications.

Statistical analysis

The data from four replicates were processed by one-way ANOVA.

Results and Discussion

Total phenolic content (TPC) of red-fleshed dragon pulp fruit showed a rapid decrease (by 65.5%) when fruit developed from 5 to 25 DAP (Figure 1). The highest (228.9 mg GAE/ 100 g FM) pulp TPC was recorded at 5 DAP. There was 59% reduction in pulp TPC from 5 to 35 DAP. Studies reported that a decrease in the TPC reduces the astringency of pomegranate (Kulkarni and Aradya, 2005) and guava (Bashir and Abu-Goukh, 2003). According to Kays and Wang (2000), the decrease in astringency is due

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to polymerization of the existing flavonoids and the concentration of water-soluble tannin diminishes, eventually giving fruit with an excellent flavour during maturation and ripening.

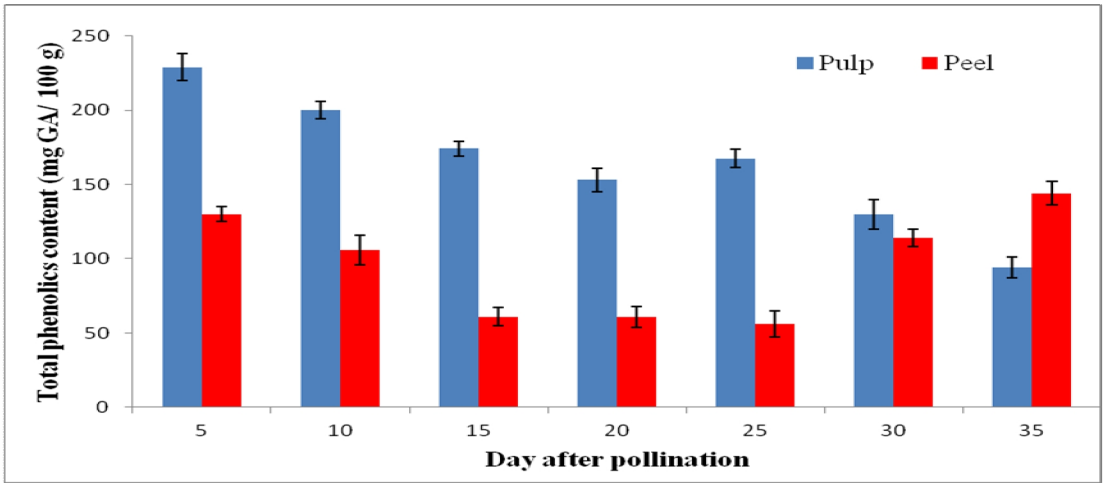

Figure 1. Change in peel and pulp total phenolic content of red-fleshed dragon fruit as fruit developed from 5 to 35 day after pollination. Data shown are the means of four replicates. Vertical bar represents the standard error.

Plant phenolics are biosynthesized using different routes but the shikimic acid pathway being the most involved route. This pathway, as reported by Ritenour and Khemira (1997) is thought to be an acclimatization mechanism of plants to external stress (temperature, injury, infections, etc.). Differences in TPC occur due to variation in the methods of analysis, condition of sample, temperature of extraction, solvent concentration and species of fruit which may play roles in increasing the yield of TPC (Lata et al., 2005). This might be an explanation of TPC reported in this study which was two times higher than those reported by USDA on red-fleshed dragon fruit (107.6 mg GAE/ 100 g FM) at ripening stage (USDA, 1992). The distribution and concentration of TPC also influenced by other factors such as genetics, cultivation practices, environmental, growing condition and maturation (Pandjaitan et al., 2005).

The antioxidant activities of red-fleshed dragon fruit measured using DPPH are shown in Figure 2. The peel antioxidant activity shows an increases from 5 to 10 DAP while the pulp antioxidant activity shows a decreases from 5 to 15 DAP, which immediately replenished to its peak activity with 45% increment at 20 DAP. The highest antioxidant activity of 78 and 98% was recorded at 20 DAP for both peel and pulp, respectively.

The antioxidant activities of red-fleshed dragon fruit using FRAP assay are shown in Figure 3. The pulp antioxidant activity shows an increase from 5 to 35 DAP. The lowest absorbance was recorded at 5 DAP, whilst the highest at 35 DAP. This indicates a stronger ferric reducing ion activities occurred at 35 DAP compared to other stages of fruit development.

DPPH is scavenged by polyphenols and betacyanin through the donation of hydrogen, forming the reduced DPPH-H (Li and Liu, 2009). The colour changes from purple to yellow after reduction can be quantified by its decrease of absorbance at wavelength 517 nm (Chang et al., 2007).

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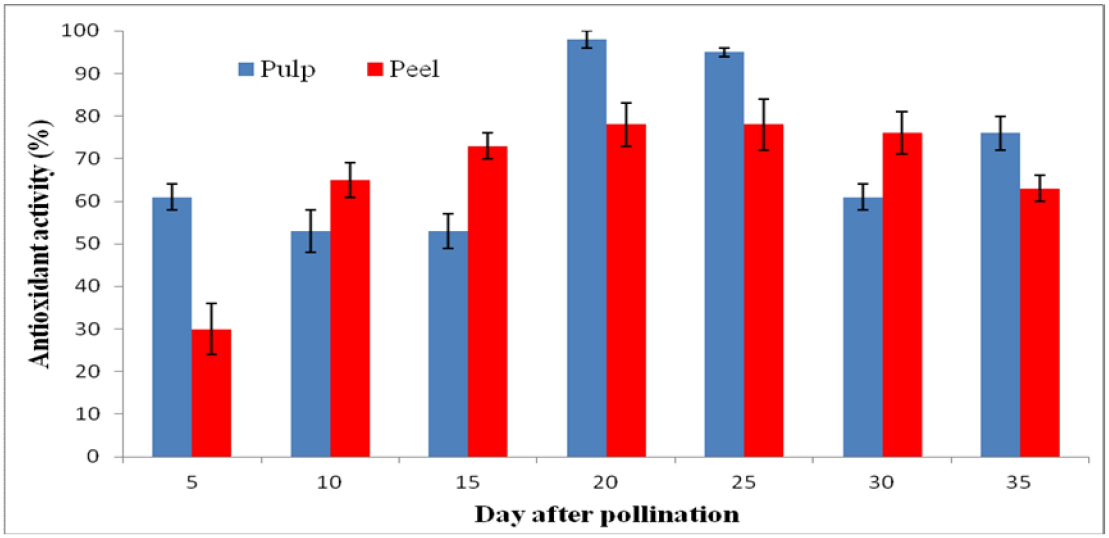

Figure 2. Change in peel and pulp antioxidant activity of red-fleshed dragon fruit as fruit developed from 5 to 35 day after pollination. Data shown are the means of four replicates. Vertical bar represents the standard error.

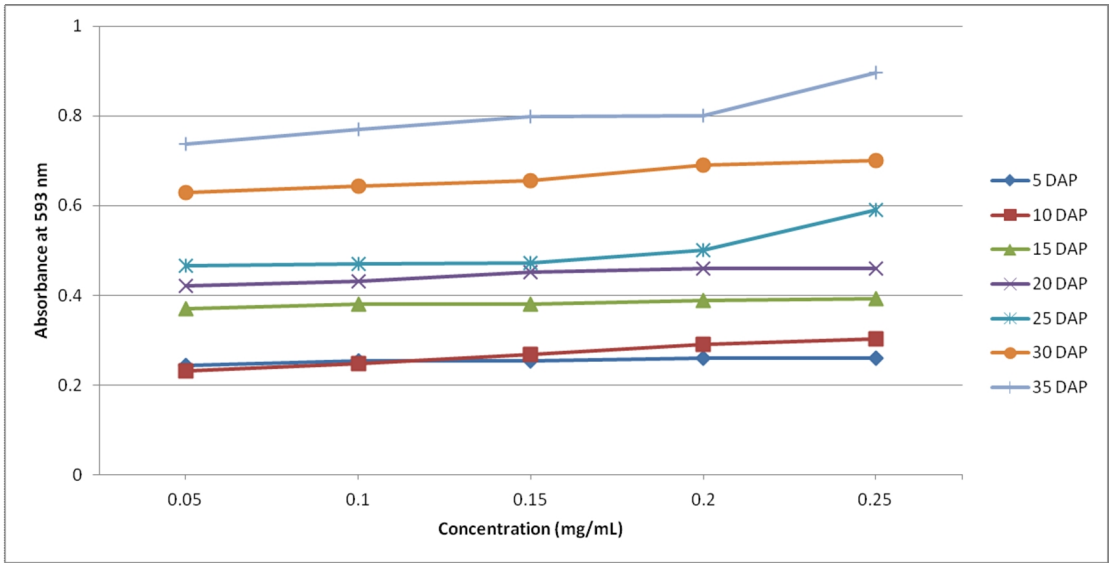

Figure 3. Change of ferric-reducing antioxidant power (FRAP) in pulp extracts of red-fleshed dragon fruit as day after pollination (DAP) progressed. Data shown are the means of four replicates.

Conclusions

From this study, it is concluded that red-fleshed dragon fruit is not a good source of TPC. Peel and pulp of red-fleshed dragon fruit have high content of antioxidant activity, yet relatively low levels of TPC. With the low TPC in this fruit, thus browning is not an issue for red-fleshed dragon fruit. Discarded red-fleshed dragon fruit peel could be utilized as value-added ingredient to assist in prevention chronic disease as it is a good source of antioxidant.

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Postharvest Quality Characteristics of Lemongrass (*Cymbopogon citratus*) at Three Maturity Stages

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Introduction

Maturity is generally considered as the state at which the particular plant part has characteristics that are preferred for processing and/or consumption by consumers (Beveridge, 2003; Kader, 2003). An edible flower, buds, stems and leaves vary greatly in metabolic activity during maturation (Wills et al., 2007). Stem and leaves often senescence rapidly and thus lose their attractiveness and nutritive value when harvested at the wrong maturity stage. Harvesting at the proper stage of maturity is essential for optimum biomass yield, quality and often for maintenance of quality after harvest. Postharvest handling is a very important activity to maintain the quality of produce. Any improper handling after harvest will cause losses of the products which had utilized labour, material and capital to produce. Knowledge on optimum harvesting time of lemongrass is important to ensure high and good quality produce. Joy et al. (2006) reported that lemongrass can be harvested at 4 to 8 months after planting. However, harvesting at 4 months may not be suitable if the lemongrass is for extraction of essential oil or fresh consumption, since at such an early age, the plant had not accumulated sufficient amount of active ingredients. Agronomic practices such as harvesting at optimum maturity stage of lemongrass for high postharvest quality characteristics (antioxidant content, soluble solids concentration, pH, titratable acidity and vitamin C) have not been studied in Malaysia. Therefore, the objective of this study was to determine the effects of maturity stages at harvest on total phenolic content (TPC), soluble solids concentration (SSC), titratable acidity (TA) and pH of lemongrass.

Materials and Methods

The lemongrass was planted at the University Agricultural Park, Universiti Putra Malaysia (UPM), Serdang, Selangor. An experimental plot size of 20 m x 32 m was used for planting the lemongrass. The planting materials used were lemongrass stalks of ±25 cm in length. The lemongrass stalks were planted at a spacing of 1 m x 1 m. The plot was divided into 12 subplots with each subplot measuring 4 m x 4 m and planted with 16 clusters of lemongrass. For each planting point, 4 stalks were planted directly into the soil by placing the basal part of the stalk within a depth of 2 cm. Lemongrass were harvested at three maturity stages, such as 5.5, 6.5 and 7.5 months after field planting. After harvesting, the outer leaf sheath including the roots was trimmed off. The lemongrass stalks were packed into black plastic bags and transported to the Postharvest Laboratory, Faculty of Agriculture, UPM, and stored at 10±2 °C until ready for postharvest quality characteristics determination. TPC was determined using Follin-Ciocalteau assay of Alothman et al. (2009). The results were expressed as gallic acid equivalents (mg GAE/100 g dry weight). SSC (%) was determined with a digital refractometer (Baush Lomb Abbe 3 L, Rochester, NY). Juice pH was determined by using a pH meter (Crison Micro pH 2000, Crison Instruments, Spain). Titratable acidity was analyzed using titration with 0.1% NaOH. The results were expressed as percentage of malic acid per 100 g fresh weight.

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Results and Discussion

Effects on total phenolic content

Figure 1 shows a significant positive quadratic relationship between TPC and maturity stage at harvest (months after planting) of lemongrass indicating that TPC increased as maturity stages at harvest increased. Variability in TPC could be due to varieties, climatic and storage conditions and cultural practices (Podsedek, 2007). Sreelatha and Patma (2009) reported that TPC was 21.40% higher in mature leaves of *Moringa oleifera* compared to young and tender leaves. A similar result was cited on tea shoot leaves (Erturk et al., 2010). However, TPC of red raspberry leaves were 21% higher in young leaves compared to mature leaves (Wang and Lin, 2000).

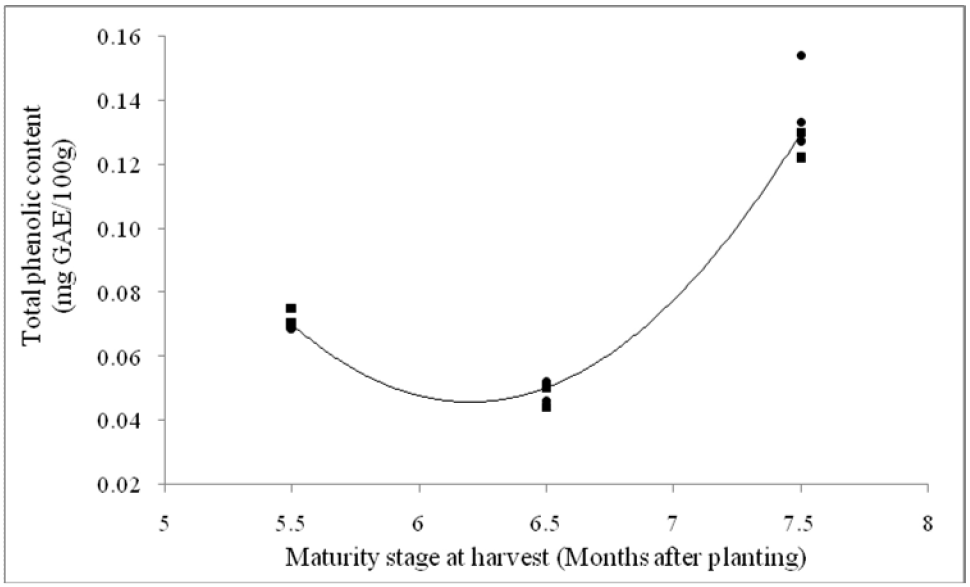

Figure 1. Relationship between total phenolic content (mg GAE/100 g dry weight) and maturity stage at harvest (months after planting of lemongrass), $y=0.06x^2-0.68x+2.16$, $R^2=0.96$. Each point represent 4 lemongrass clumps per replicate with a total of 4 replicates.

The increase in TPC as maturity stages at harvest increased could be due to the length of exposure to sunlight and daytime (Harbowy and Balentine, 1997). The biosynthesis of phenolic compound on tea shoots can be effectively induced by stronger sunlight and length of daytime. Tea shoots and leaves show a significantly different TPC when exposed to longer sunlight and daytime compared to shaded tea shoots and leaves (Mahanta and Baruah, 1992).When plants are exposed to biotic and abiotic stress, high levels of reactive oxygen species accumulate, causing oxidative damage at the cellular and molecular levels that lead to a decrease in plant productivity (Vranová et al., 2002). Reactive oxygen species can rapidly oxidize membrane lipids, proteins, and other cellular organelles, leading to their dysfunction. To protect themselves from reactive oxygen species, plant possess a number of free-radical scavenging enzymes such as ascorbate peroxidase (APX), catalase, and superoxide dismutase, and low-molecular weight antioxidants, like ascorbate and tocopherols.

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Effects on soluble solids concentration, titratable acidity and pH

Figure 2(a) shows a positive quadratic relationship between SSC and maturity stage at harvest of lemongrass. Figure 2(b) shows a negative quadratic relationship between malic acid and maturity at harvest of lemongrass. While Figure 2(c) shows a negative quadratic relationship between pH and maturity at harvest of lemongrass. Flavour is a combination of acidity (TA and pH), SSC (individual sugars and sugar alcohols) and aroma volatiles. Flavor also can be significantly affected by maturity at harvest. Since sugar is usually a major component of soluble solids and the increase in SSC when lemongrass was harvested at 6.5 months was probably due to the rapid respiration that occurred in the plants, causing more polysaccharides to be broken down into sugars.

However, the respiration was slowed down at 7.5 months compared to at 6.5 months after planting as indicated by the lower SSC. Koukounaras et al. (2007) reported that there was a significant difference of 7.36 % SSC between young and fully expanded leaves of rocket salad (*Eruca sativa* Mill.). However, there was no significant difference between leaves of rocket salad harvested at fully expanded and mature leaves.

When there was an increase in the SSC of *Hippophae rhamnoides* L., the titrable acidity was decreased (Raffo et al., 2004). A similar trend is shown in the current study, when the SSC increased, malic acid and pH tended to decrease. The decrease in acidity when plants reached the mature stage could be due to a rapid utilization of organic acids for respiration. Acids were known to be used quickly during respiration as compared to other compounds. The decrease in acidity could also be due to the consumption of organic acids as substrates in the metabolic processes (Ackermen, 1992).

The pH value gave a measurement of the acidity or alkalinity of the lemongrass. However, pH value was not directly related to titratable acidity, but is dependable on the concentration of free hydrogen ions and buffering capacity of the extracted juices (Wills et al., 2007). pH is a measure of the free hydrogen ions concentration of the crop. During maturity or senescence stage, the organic acids were respired or converted to sugars. The organic acids, including malic acid, contain H^+ ions. The concentrations of H^+ ions affect the pH value, where higher concentration of H^+ ions will cause a drop in the pH value. According to Coseteng and Lee (1987), the decrease in pH throughout maturation was due to the metabolic process in the plant products that resulted in the decrease of organic acids.

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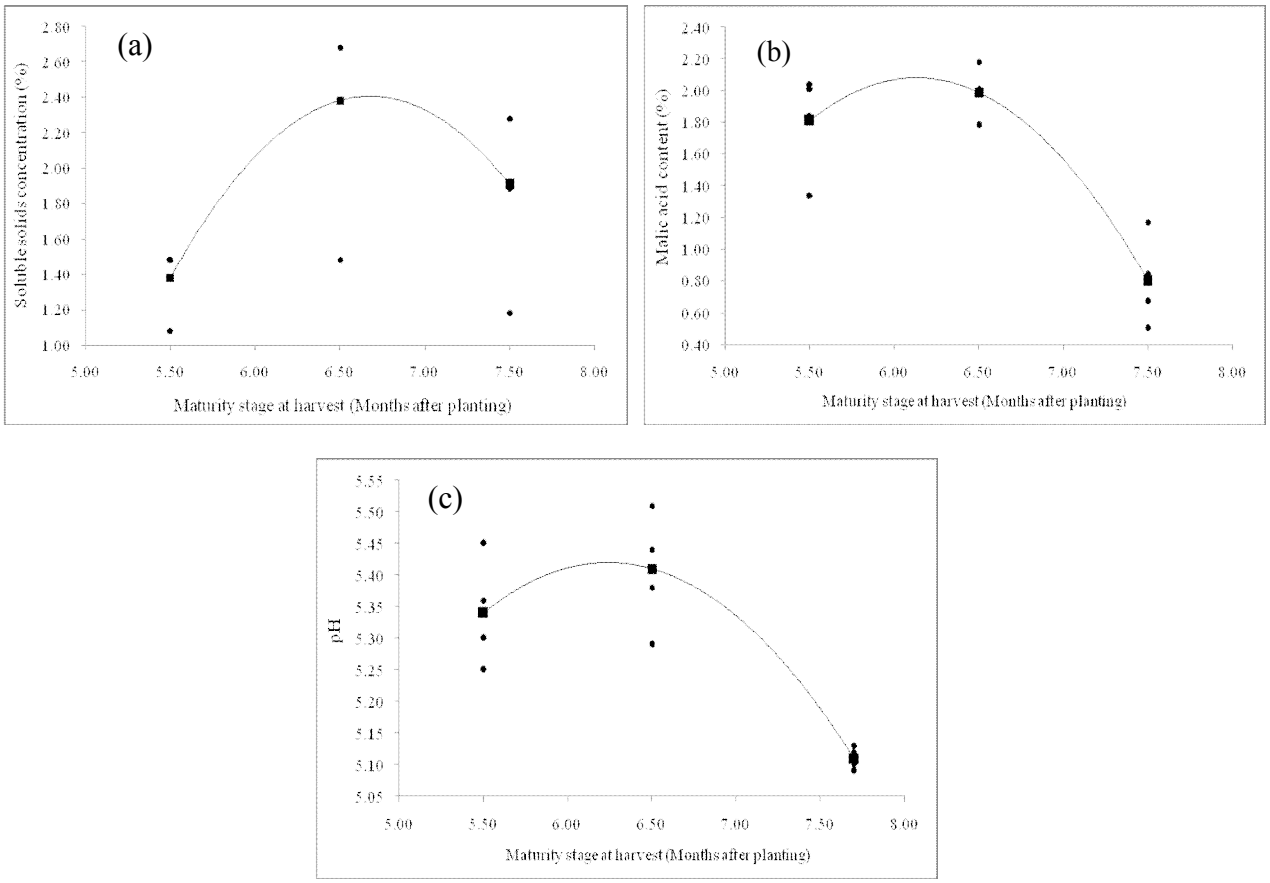

Figure 2. Relationships between (a) soluble solids concentration (y= -0.55x^2+7.50x-23.23, R^2=0.60) (b) titratable acidity (y= -0.81x^2+10.06x-29.39, R^2=0.80) and (c) pH (y= -0.14x^2+1.80x-0.13, R^2=0.89) and maturity stage at harvest (months after planting). Each point represent four lemongrass clumps per replicate with a total of four replicates.

Conclusions

TPC, SSC, TA and pH showed significant differences when lemongrass was harvested at different maturity stages. The optimum percentage of SSC, malic acid and pH was obtained when lemongrass was harvested at 6.5 months after planting and decrease when lemongrass was harvested at 7.5 months after planting. However, TPC was highest when lemongrass was harvested at 7.5 months after planting and lowest when lemongrass was harvested at 6.5 months after planting. Thus, lemongrass should be harvested between 6.5 to 7.0 months after planting to obtain optimum postharvest quality characteristics.

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Ripening Characteristics of Vapour Heat Treated 'Paiola' Papaya (*Carica papaya*) as Affected by Ethylene Gas

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Introduction

The papaya industry in Malaysia is rejuvenated with the introduction of the new F1 hybrid variety called 'Frangi' or commercially known as 'Paiola'. The fruit is the size of a palm, with golden-yellow peel and sweet tasting flesh or pulp. It can remain longer than other papayas due to the thick skin and firmness of the fruit. The production of papaya has become more prominent in Malaysia, due to the high demand in the domestic market and for export mainly to China and Japan (Chan, 2009). However, fruit fly infestation has become a serious problem especially when the papaya fruit peel has turned 25% yellow (Seo et al., 1982). Damages caused by fruit flies include minor surface blemishes, destruction of the edible flesh and spoilage from decay (Couey, 1989). Thus, China and Japan have made the vapour heat treatment (VHT) as an export demand for disinfestation of fruit flies in Malaysia.VHT is a technique of heating fruit with water vapour at temperatures of 40-50 ˚C to kill insect eggs and larvae as a quarantine treatment before fresh market shipment (Luries, 1998). Too high temperatures or prolonged exposure time to high temperature results in adverse effects on fruit quality such as water loss, skin discoloration, increase susceptibility to contaminating microorganisms and decrease in shelf life (Barkai-Golan and Phillips, 1991). In fact, internal fruit quality is strictly related to the changes occurring during the ripening and maturation stage of the plant. Fruit internal quality is represented by fruit texture sugars and organic acids content, flavour, sweetness, pH and eating quality (Costa et al., 2001).

Thus, the right combination of time-temperature treatment is essential to reduce or only cause minimal damage to the commodity in which at the same time can reduce the insect pest as well as maintain the quality of the fruits. Responses of papaya fruit to heat treatment varies with maturity and cultivar (Paull, 1995). Since Paiola papaya is a new hybrid cultivar, there is still no research on the effect of VHT on the fruit. Thus, the objective of this study was to determine the effect of VHT on papaya fruit (*Carica papaya* var. Paiola) quality characteristics and life storage. This study is necessary to determine whether the quality of Paiola papaya can be maintained after VHT. The Paiola papaya could be a potential variety for export and thus contribute to the export earnings.

Materials and Methods

Papaya fruits (*Carica papaya* var. Paiola) were obtained from a farm in Lancang, Pahang. The fruits were treated with 0.1% Octave fungicide for 3 to 5 min. Then, fruits were individually wrapped with a plain white paper and packed into corrugated paper boxes before being VH treated.

Vapour Heat Treatment (VHT)

In the VHT system (EHK-500MC, Sanshu Sang You Co.L.TD in Japan), 20 fruits were arranged onto a tray, and then six trays were loaded into the chamber. A thermostat, to measure fruit core temperature was inserted into selected fruits in each chamber. The VHT system was turned on. The temperature of the chamber and fruit core was raised to 48 and 46.5 ˚C, respectively. The respective temperatures were maintained for 20 min followed by air cooling (35 min), water cooling (10 min) and fruit drying (5 min).

All the fruits were removed from the VHT system and repacked into corrugated paper boxes, with nine fruits per box.

Ethylene treatment

After VHT, the fruits were treated with 100 µL/L ethylene gas at 20 ˚C for 24 hours in a ripening room. Non ethylene treated (control) fruits were also kept at 20 ˚C for 24 hours but in a different ripening room. After 24 hours, all the papaya fruits were removed from the ripening room and stored at ambient temperature (25 ± 2 ˚C) for 2, 4, 6 and 8 days. Measurements for quality characteristics of two fruits that reached maturity stage 4, 5, and 6 from three boxes (one box/replication) were taken for the quality characteristics determination (firmness, soluble solids concentration, titratable acidity, ascorbic acid and pH).

Experimental design and statistical analysis

This study was carried out in a factorial experiment (2 ethylene treatments x 4 storage days), with three replications. The data were analyzed using analysis of variance (ANOVA) and means were separated by using Duncan's Multiple Range Tests (DMRT) at ($P \leq 0.05$).

Results and Discussion

The results showed that after VHT, there were no significant differences in ripening characteristics between fruits that had been treated with or without (control) ethylene (Table 1).This indicated that after VHT, treatment with ethylene gas had no effect on the ripening process and the fruits were able to ripen normally during storage at ambient temperature. Exposure to temperatures >40 ˚C for a short period, could result in a rapid loss of 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) in papaya, but after moving out from heat for 3 days, ACC oxidase activity recovered (Paull and Chen, 1990). This could explain why ethylene treatment had no effect on the ripening characteristics of the papaya.

Table 1. Effects of ethylene gas treatment on firmness (N), soluble solids concentration (SSC), titratable acidity (TA), ascorbic acid and pH on vapour heat treated Paiola papaya.

	Firmness (N)	SSC (%)	TA (% malic acid)	Ascorbic acid (mg/100 g)	pH
With ethylene	42.66[a]	9.93[a]	0.05[a]	63.95[a]	5.87[a]
Without ethylene (control)	35.96[a]	9.86[a]	0.05[a]	62.94[a]	5.83[a]

[a] *Mean separation within a column by DMRT at ($P \leq 0.05$).*

However, there were significant effects of storage day on pH, SSC and ascorbic acid content. There was a significant quadratic increase of pH in fruits treated with ethylene, but a significant quadratic decrease of pH in fruits without ethylene treatment with increasing storage day (Figure 1a). Figure 1b showed that there was a significant quadratic decrease on SSC from day 2 up to day 8 of storage for both treated and control fruits. This finding is contrary with the review shown by Ding and Tee (2010) where exogenous ethylene treatment increased SSC during storage on dabai fruits. Figure 1c showed that both treated and control fruits gave a similar pattern of ascorbic acid content where ascorbic acid was increased during the 8 days of storage. It has been reported that, in many horticultural crops, DHA (L-dehydroascorbic acid) that represent less than 10% of total vitamin C tend to increase during storage (Lee and Kader, 2000). However, the ascorbic content for fruits treated with ethylene was much lower, compared to control fruits on day 4 and 6. Meanwhile, the highest ascorbic acid content was observed in ethylene treated fruits, on storage day 8.

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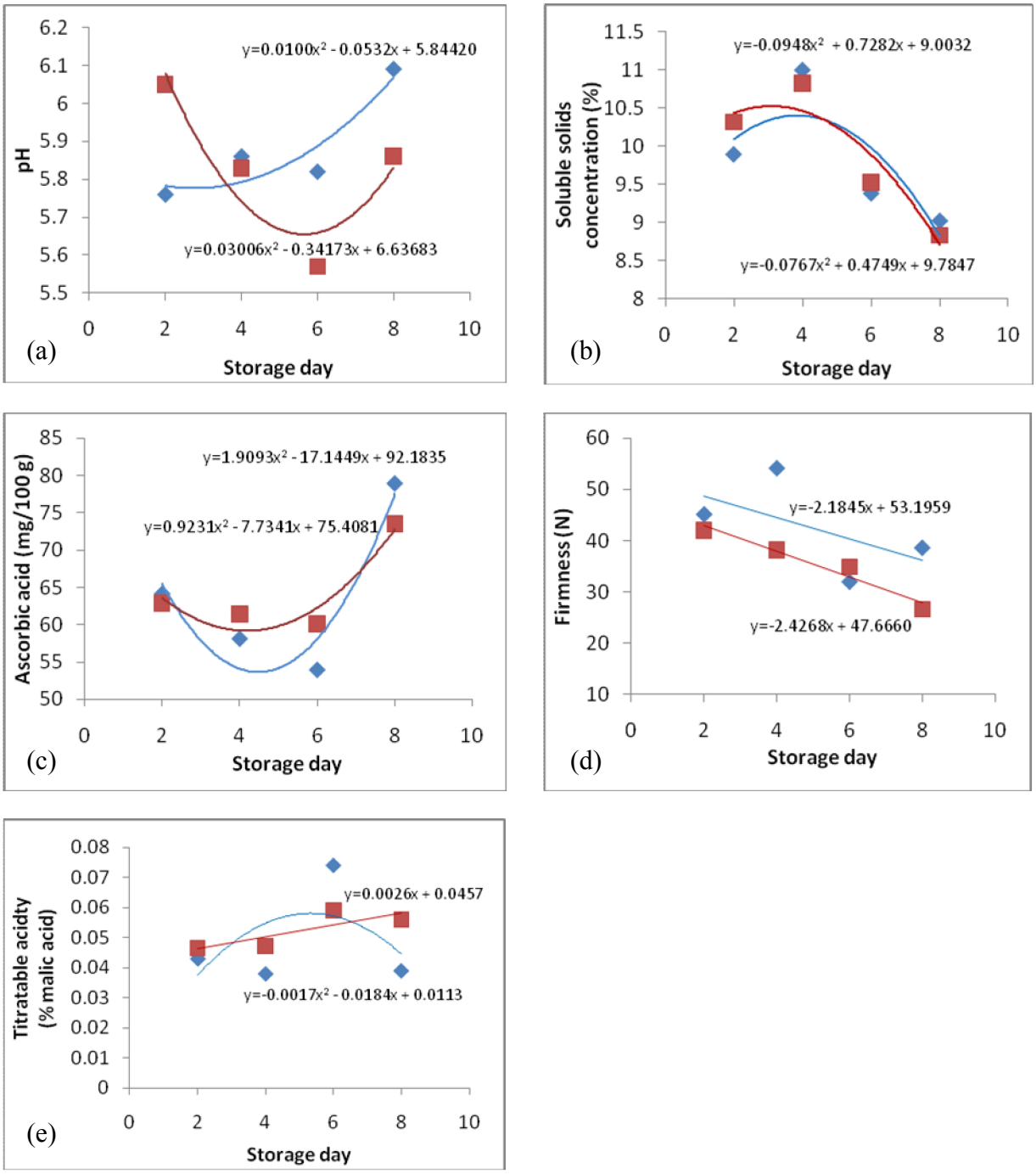

Figure 1. The relationship between storage days and a) pH b) soluble solids concentration c) ascorbic acid d) firmness e) titratable acidity , where (◆)with ethylene and (■) without ethylene (control).

During the 8 days of storage, fruit firmness of both ethylene and control fruits were significantly decreased (Figure 1d), indicating loss of flesh firmness. In this study, fruits treated with ethylene gas were more firm compared to control fruits. The decrease in firmness was due to the increasing maturity stage of

the papaya as the fruit tended to ripen during the storage. According to Almora et al. (2004), during the ripening process, hydrolytic enzymes are initiated, resulting in breakdown of cell walls and that lead to fruit softness. This result was supported by a previous study done on mango hybrids in which at ambient temperature, firmness of the fruits was decreased as storage duration increased (Jha et al., 2010). TA of the fruits was expressed in terms of malic acid (%). There was a linear increase in TA for ethylene treated fruits, while control fruits showed a quadratic decreased of TA (Figure 1e). A previous study cited that TA in 'Pluk Mai Lie' papaya ranged from 0.11 to 0.13%, and there was no significant difference in TA during the ripening process (Fuggate et al., 2010).

Conclusions

In this study, it can be concluded that Paiola papaya fruits with or without ethylene gas treatment following VHT were able to ripen normally during storage at ambient temperature (25 ˚C).

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Effects of Anti-Browning Agents on Ambarella (*Spondias dulcis*) Quality during Storage

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Introduction

Ambarella (*Spondias dulcis* or *Spondias cytherea* Sonn.) also known as kedondong, hog plum or golden apple, is a tropical fruit belongs to the Anacardiaceae family. It is native to Melanesia, and it has been introduced to tropical areas such as Malaysia, India and Ceylon through Polynesia (Siti Aishah et al., 2005). The fruit is round or oval in shape and the pulp tastes a sweet and sour mango-like flavour. It is considered to be a good source of minerals and vitamin C, and it has been suggested that the fruit may have some values in aiding diabetes, heart ailments and urinary problems (Siti Aishah et al., 2005; Koubala et al*.,* 2008). Normally, ambarella is freshly eaten or used for making pickles and mature green ambarella with crunchy pulp is mostly like by Malaysian. Nowadays, consumer demands for good healthy convenience foods such as minimally-processed fruit had increased. However, it had been reported that the action of polyphenol oxidase (PPO) on phenolic compounds released during the process of cutting which is known as enzymatic browning leads to discolouration of the minimally-processed produce (Gorny et al., 2002). Enzymatic browning can be controlled by various methods. Surface treatments by dipping minimally processed products in the appropriate anti-browning agents can effectively help to delay discoloration (Suttirak and Manurakchinakorn, 2010). The naturally safe and non-toxic anti-browning agents such as ascorbic acid (AA), citric acid (CA) and oxalic acid (OA) are widely used as anti-browning agents. The uses of anti-browning agents will not harm the flavour and aroma of the product as it is very effective in the enzymatic browning reduction as well as safe, cheap and well accepted by the consumers (Cortez-Vega et al., 2008). It had been proven that the uses of ascorbic acid and citric acid were effective to control fresh-cut browning in several fruits such as apple (Cortez-Vega et al., 2008), pear (Gorny et al., 2002) and mango (Puthmee et al*.,* 2010). However, there is still lack of information on the effects of anti-browning agents on the minimally processed ambarella. This study was carried out to determine the effect of combination of ascorbic acid and citric acid at different ratio in controlling browning of minimally processed ambarella.

Materials and Methods

Plant material and treatment solution

Ambarella was harvested from an orchard in Johore (Southeast region of Peninsular Malaysia). Mature green fruits with uniform size, free from blemishes and discolouration and no edge browning were selected. The fruits were minimally processed (washed with distilled water, disinfect with 200 ppm of sodium hypochlorite, dried and peeled). The peeled ambarella was then dipped in anti-browning agents, 1% of ascorbic acid (AA) and 1% citric acid (CA). These chemicals treatment were at different ratios of AA:CA, 0:0 (control), 100:0, 50:50 and 0:100 (v/v) with three replications for each treatment. They were then air dried and packed in PE-wrapped-foam tray and stored at 5 °C for 0, 2, 4, 6, 8 and 10 days. Each tray consisted of three fruits. They were then evaluated for weight loss (%), skin colour (L*, C* and h°), flesh firmness (N), soluble solids concentration (%), pH value, titratable acidity (% citric acid), vitamin C content (mg/100 g) and browning index as the storage day progressed.

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Experimental design and statistical analysis

The experiment was conducted in Completely Randomize Design (CRD). The data were analysed using Analysis of Variance (ANOVA) and the means were separated using Duncan's Multiple Range Tests (DMRT) at $p \leq 0.05$. Regression analysis was carried out to look at the relationship between soluble solids concentration of the treatments and storage days.

Results and Discussions

The effects of anti-browning agents were significant for hue angle and browning index (Figures 1A and 1B). Figure 1A showed that control and 100:0 were significantly lower in h° value than other treatments. This indicated that the colour of fruits flesh from the former two treatments were more yellow compared to latter two. Hue indicates colour changes from green (180°) to yellow (90°) and the higher values of h° in the presence of citric acid indicated that ripening was delayed. This is similar to a previous study by Jiang et al. (2004) where fresh-cut apple treated with 0.1M citric acid remained partially bright colour as compared to control by the end of storage. Browning index (BI) of control was significantly lower than the treatments with anti-browning agents (Figure 1B). However there is no significant different in BI between treatments with anti-browning agents. Higher value of BI showed the higher degree of browning. In this study, fruits treated in anti-browning agents showed higher browning as compared to control. This is contrary with the review shown by Suttirak and Manurakchinakorn (2010) where 1% of ascorbic acid treatment increased the PPO inhibition from 36.1% to 87.1%. So, the uses of anti-browning agents were not affective in order to prevent cut-surface browning of minimally processed ambarella.

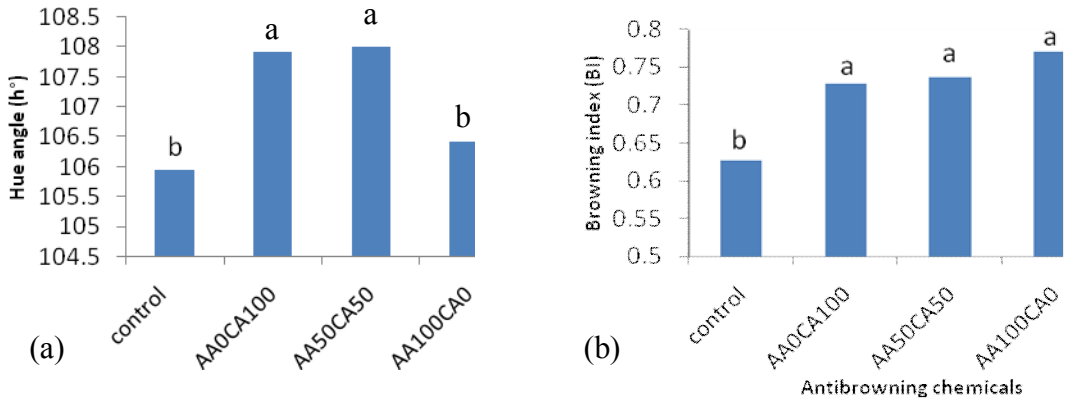

Figure 1: (A) Hue angle and (B) browning index of minimally processed ambarella as affected by 1% of anti-browning chemicals at different ratios of ascorbic acid (AA) and citric acid (CA). Means with different letter were significantly different between treatments. Mean separation by DMRT at $p \leq 0.05$.

The changes of skin colour were expressed by the values of L*, C* and h°. Table 1 showed the L^* value of minimally processed ambarella on day 8 was significantly higher as compared with other storage days. However, the other storage days were not significantly different between each other. The higher value of L* indicates lighter fruits flesh. However, in this study, L* value cannot indicate the browning degree because the colour was for the remaining peel together with the flesh. The green colour indicated lower L* value initially and increased as storage day progressed. This is probably related to the ripening of the minimally processed ambarella during storage. C* value is indicating the colour intensity which was significantly higher during the early storage days, 0 and 2. This result contrary with the results shown by Ding et al. (2007) where the C* value of minimally processed carambola increased significantly during

storage. However, the h° values were significantly decreased with increasing storage days. The higher values of h° were observed during day 0 and 2, which were significantly higher as compared to other storage days. Higher h° value indicates greener colour whereas lower value is more yellow. This is probably due to the ripening process of the fruits during storage. This result is supported by Ding and Tee (2010), where the flesh of the papaya decreased from 97° to 65° as the ethylene exposure duration increased.

A positive increased in weight loss with significant linear relationship (R^2= 0.964) showed that there was loss of weight during storage (Figure 2A). This indicated that the longer the storage duration, more water will be loss, thus reduced the fruits quality. The weight loss is possibly due to translocation of soluble products to the axis for respiration (Choon et al., 2010). There was significant quadratic decrease of vitamin C content with increasing storage day (Figure 2B). The decrease in vitamin C content is similar to the findings on carambola where vitamin C content decreased from day 0 to day 5 (Ding et al., 2007). According to Ding et al., (2007), it could be due to the autoxidation of vitamin C by ascorbate oxidase. Figure 2C showed that there was a significant linear increased of SSC as the storage day progressed. It may be due to the increasing maturity stage of the fruits during ripening process. Ambarella is a climacteric fruits that can produce endogenous ethylene during ripening even after detachment from the mother plant. During ripening, hydrolysis of starch into sugar contributes to high soluble solids concentration (SSC) and also could be due to the breakdown of acid compounds into sugar or being utilized during respiration (Ding et al., 2007).

Table 1. L* (Lightness), C* (Chroma) and h° (hue) value of minimally processed ambarella during 10 days of storage.

Storage days	Colour		
	L*	C*	h°
0	62.40^{b}	34.52^{a}	109.54^{a}
2	61.38^{b}	33.95^{a}	110.28^{a}
4	61.24^{b}	25.83^{d}	107.25^{b}
6	62.87^{b}	28.17^{c}	107.02^{b}
8	68.47^{a}	31.52^{b}	105.48^{c}
10	63.65^{b}	26.98^{cd}	102.87^{c}
F-test significant	*	*	*

L = lightness; C* = chroma; h° = hue angle*
Mean separation within columns and factors by DMRT at p≤0.05.
**, significant at p≤0.05, respectively*

Based on Figure 3, due to the interaction between anti-browning agents × storage days, there were significant positive quadratic relationships between SSC percentage and storage days of minimally processed ambarella for all treatments. However for treatment 0:100 there was significant linear increased of SSC percentage during storage duration. The significant relationship indicated that SSC for the control and 100:0 gradually increased up to 10 days of storage. However, after 4 days of storage, SSC of 100:0 was higher compared to control. Meanwhile, SSC for treatment 50:50 showed a slight decreased during 4 days of storage followed by gradually increase from day 6 up to day 10. From the result obtained, SSC of all treatments increased during storage. The increase of SSC indicated that the sweetness of minimally processed ambarella increased during storage. This results was contrary to the results obtained by Ding et al. (2007) where there were no significant different of SSC for interaction between ascorbic acid × storage duration.

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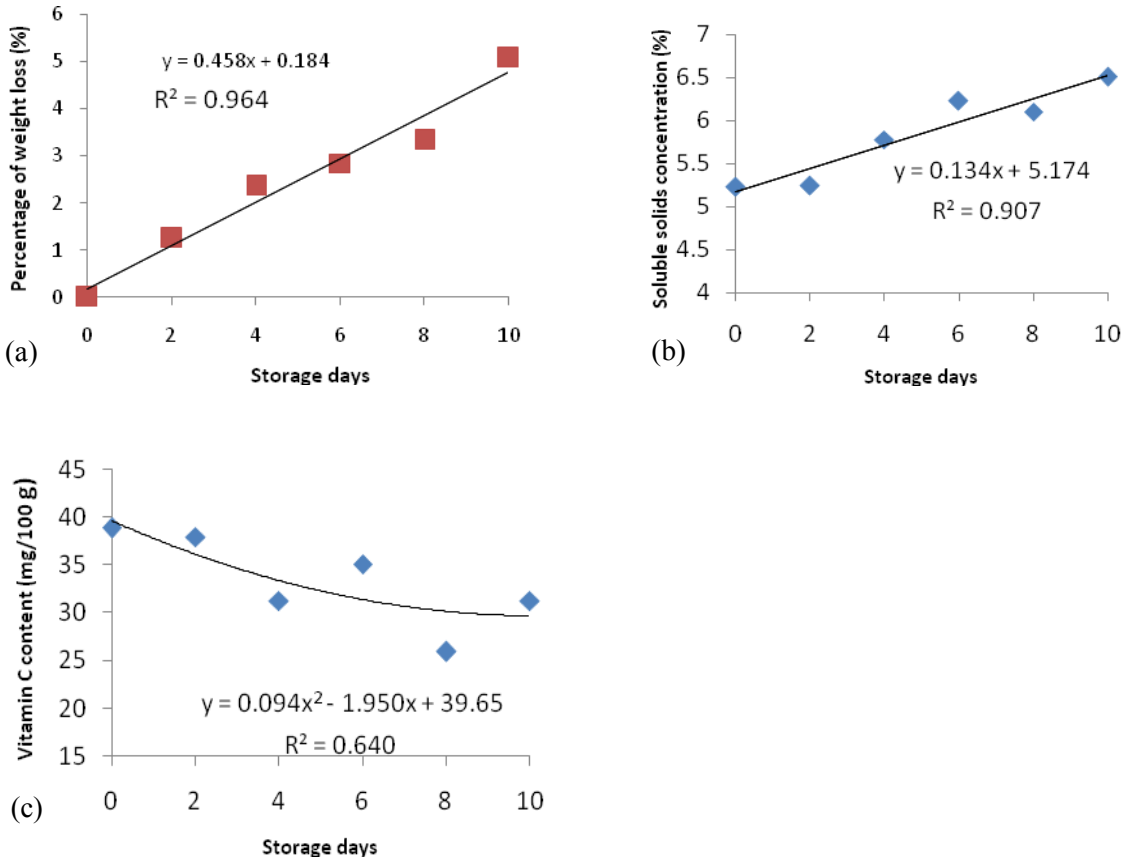

Figure 2: (A) Weight loss, (B) hue value and (C) vitamin C content concentration of minimally processed ambarella during 10 days of storage in 5 °C. Mean separation by DMRT at p≤0.05.

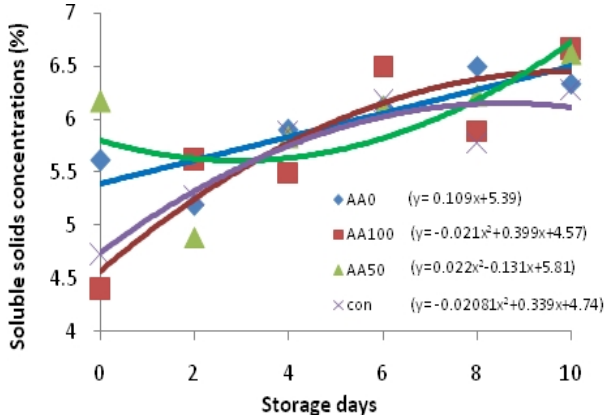

Figure 3: Effects of storage day × anti-browning agents on soluble solids concentration (%) of minimally-processed ambarella. AA:CA, (◆) 0:100, (■) 100:0, (▲) 50:50, (×) 0:0. Mean separation by DMRT at p≤0.05.

Conclusions

From this study, control fruits were found to have the lowest BI as compared to other ratios, thus, the combination of anti-browning agents were not effective in controlling browning of minimally processed ambarella. However, in term of h° value, 50:50 and 0:100 treatments were able to delay the ripening process. The uses of anti-browning agents had not much effect on quality of minimally processed ambarella during storage. It was much affected by the duration of the storage.

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Response of Papaya Seedlings (*Carica papaya* L. cv. Eksotika II) to Preharvest Foliar Application of Calcium

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Introduction

Papaya belonging to family *Caricaceae* is a large perennial plant with rapid growth (Paull and Odilo, 2011). It is an important fruit in Malaysia, ranking third after durian and banana. The Eksotika II cultivar is a high yielding good quality F_1 hybrid released by Malaysian Agricultural Research and Development Institute (MARDI). Although the cultivar has gained popularity in the domestic and export market (Shukur and Shokri, 1997), the harvested fruit is susceptible to anthracnose infection. The most common intervention measures of the disease are by the use of hot water treatment, fungicides and biological material like chitosan. Calcium, a major plant nutrient, affects cell wall and plasma membrane formation and plays a key role in growth and biomass production in plants. It can be used to increase root development, decrease fruit decay and increase firmness and storage duration. It can also be added to the soil or sprayed directly to the foliar to increase fruit quality (White and Broadly, 2003). So far, little or no attention has been paid to investigate the effects of foliar application of calcium on papaya under controlled environment especially with respect to variety Eksotika II. Therefore, this study was conducted to look at the effect of different sources and concentrations of calcium on growth and calcium uptake of papaya.

Materials and Methods

Seeds of Eksotika II were obtained from MARDI. The seeds were germinated in 25-cells polypropylene filled with coco peat and paddy husk. Six weeks old papaya seedlings, each having more than four fully expanded leaves and uniform size, were transplanted into 5L black polyethylene bags containing coco peat and paddy husk (50:50 v/v). The seedlings were arranged in rows at 120 cm distance between bags and 200 cm between rows. The experiment lasted for a period of 90 days after transplanting in a shelter at Agrotech Unit, University Agriculture Park (TPU), Universiti Putra Malaysia, Serdang, Selangor.

Standard nutrient solution was used to fertilize the plants. The electrical conductivity (EC) and pH of the solution throughout the experiment were 2.5 and 6 dS m^{-1}, respectively. A total volume of 600 ml day^{-1} of the nutrient solution was applied using an automatic drip irrigation system. Maximum and minimum temperature in the shelter was 35 and 23 ºC, respectively while relative humidity was 80-90%. Photosynthetic active radiation (PAR) was 700-800 $\mu molm^{-2}sec^{-1}$. Treatments were started at one month after transplanting. Treatment included three sources of calcium, calcium chloride, calcium nitrate and calcium propionate at four concentrations (0, 60, 120 and 180 mgL^{-1}). They were applied every two weeks in the form of foliar spraying using a manual sprayer. Sprays were applied until drippings occurred from the foliage and Tween 20 was used as a surfactant to maximise calcium absorption.

At ninety days after transplanting, all treatments were completed. Measurements of plant parameters were carried out two weeks after all treatments were finalized, i.e. 104 days after transplanting. Plant height was measured from the media surface to the apical part of plant. Plant diameter was measured at three fixed locations of the plant (10 cm, 60 cm and 120 cm from the base of the plant) and the average was calculated. The fresh and dry weights of the shoots were also determined. The roots were carefully

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washed to remove the media prior to fresh and dry weight determination. Dry weight of shoots and roots was obtained by oven drying at 70 ºC for 120 h.

Nutrient measurements

Wet digestion methods were used to determine nutrient concentrations in the tissues. Four fully matured leaves per treatment were harvested from the middle of the crown of each plant and then oven dried in 70 °C and ground with 2 mm mesh grinder. Then, 2 mL H_2SO_4 were added to the ground tissue. After that, 2 mL H_2O_2 was added to the mixture. Samples were put into the digestion flasks at 280 ºC for 40 minutes. Following completion of digestion, the solution was made up to 100 ml with deionised water and then analysed using the Atomic Absorption Spectrometer (AAS) (Perkin Elmer Model 3110) to determine the concentrations of potassium, calcium and magnesium in the samples. Nitrogen and phosphorus concentrations were analysed using Auto Analyser (AA) (Perkin Elmer Model 403).

Statistical analysis

Three sources of calcium at four concentrations were arranged in factorial experiment in Randomized Complete Block Design (RCBD) with four replicates. Data were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA). The means were compared by the Duncan's Multiple Range Test (DMRT) at significance level of 0.05. Interaction effects were also determined.

Results and Discussion

For nitrogen, there was interaction between sources and concentrations where the effects of increased concentrations were different with sources (Figure 1). Phosphorus content was higher when the source of Ca was calcium nitrate and increased with concentrations (Table 1). Calcium stimulates P absorption by plant root by increasing the electronegative charges on the roots (Robson et al., 1970). Potassium was also higher when the source was calcium nitrate (Table 1). However, the content was higher when the concentration was 60 mgL^{-1} but not significantly different from the control and 120 mgL^{-1}. Sources did not affect calcium absorption significantly (Table 1). However, the content increased significantly with concentrations. As expected, these were also the findings of other researchers where increased calcium in the media or foliar application caused calcium uptake to be higher (Tomar and Awasthi, 1995; Awada et al., 1975). Magnesium content was not affected by sources but significantly higher when the concentrations were 60 and 120 mgL^{-1} (Table 1).

In papaya tree, calcium lowered Mg uptake (Awada et al., 1975)**.** Stem height and diameter, root fresh and dry weights were not significantly different when treated with different sources of calcium (Table 2). However, all these parameters increased with concentrations. Shoot fresh and dry weights were higher when the sources were calcium nitrate and propionate (Table 2). They also increased with concentrations.

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Table 1. Main and interaction effects of three sources of calcium and four concentrations on mineral nutrients absorption in leaves of Eksotika II sprayed one month after transplanting for four times at two weeks interval. Ca spraying was carried out for four times at every two weeks for two months.

Sources (S)	Macronutrients (%)				
	N	P	K	Ca	Mg
$CaCl_2$	1.76b[z]	0.50 b	6.08 b	1.17 a	0.16 a
$Ca(NO_3)_2$	1.88a	0.61 a	7.19 a	1.23 a	0.17 a
Ca $(C_2H_5COO)_2$	1.78b	0.59 ab	5.85 b	1.24 a	0.16 a
Concentrations (C) (mgL^{-1})					
0	1.92a	0.26 c	6.49 ab	1.11 c	0.14 b
60	1.82ab	0.59 b	7.2 a	1.16 cb	0.19 a
120	1.77b	0.66 ab	6.27 ab	1.24 b	0.17 a
180	1.72b	0.75 a	5.55 b	1.34 a	0.15 b
Interactions (S×C)	**	ns	ns	ns	ns

[z]*Means followed by the same letter in the same column are not significantly different at ($p \leq 0.05$) at DMRT*
[ns,**] *Non-significant and highly significant at $p \leq 0.05$*

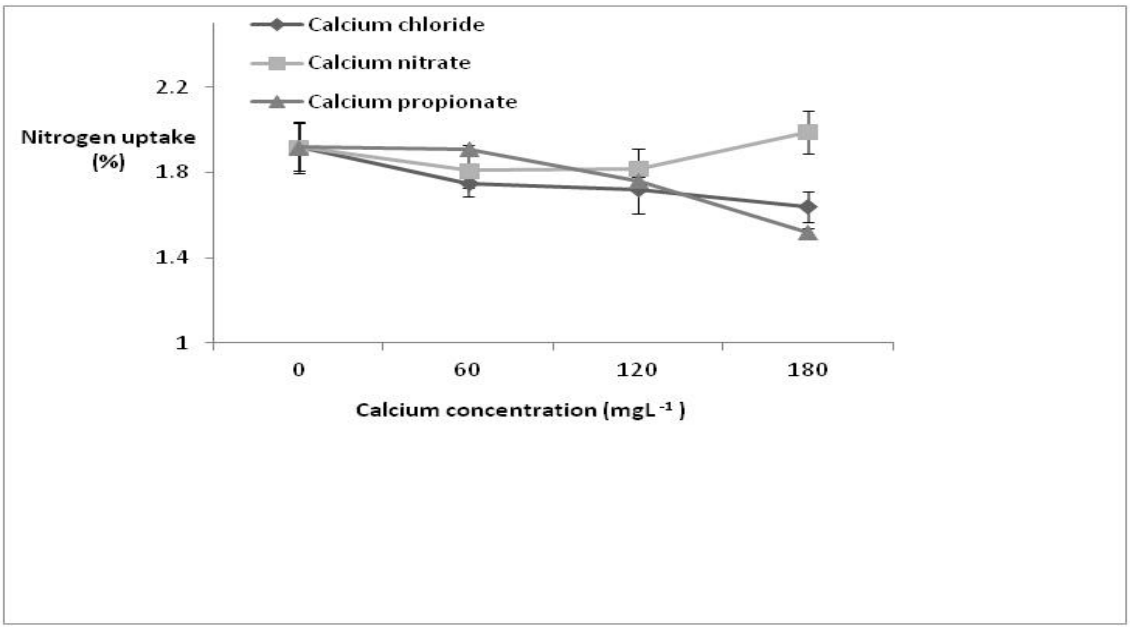

Figure 1. Effect of sources and concentrations of Ca on nitrogen concentration in leaves of papaya seedlings. Vertical bars are standard error of the means (SEM).

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Table2. Main and interaction effects of three sources of calcium and four concentrations of calcium on growth of Eksotika II

Sources	Growth parameters					
	Stem height (cm)	Stem diameter (mm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
$CaCl_2$	145.8a*	28.09a	893.75b	236.25a	96.25b	19.12a
Ca $(NO_3)_2$	148.93a	27.53a	1040.63a	274.69a	110.62a	20.93a
Ca propionate	144.56a	27.71a	1003.13a	258.13a	106.87a	19.37a
Concentrations (mgL^{-1}) (C)						
0	131.25b	25.91b	737.5c	200c	87.5b	15b
60	148.41a	27.77ab	1008.33b	230c	105.83a	15b
120	151.4a.	27.57ab	1037.50b	275b	108.33a	21.25b
180	154.66a	29.87a	1133.33a	320a	116.66a	28a
Interaction (S×C)	ns	ns	ns	ns	ns	ns

[z]Means followed by the same letter in the same column are not significantly different at ($p \leq 0.05$) at DMRT
*[ns,**] Non-significant and highly significant at $p \leq 0.05$.*

Conclusion

The results can be concluded that different sources and concentrations affect mineral uptake differently. This was also true with some of the growth parameters. Thus, based on what are required, the choice of the source and concentration will be subsequently identified.

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CHAPTER 3

ECOPHYSIOLOGY AND STRESS BIOLOGY

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Assessment of *Ficus microcarpa* on Particle Removal Capability in Urban Environment

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Introduction

Kuala Lumpur is a highly industrialized city where the main sectors of industries and administrations are centralized in this city. Kuala Lumpur has experienced rapid urbanization since decades ago where its inhabitants live in the modern lifestyles with numerous advanced amenities. Despite of all this, the increase of vehicle usage and the bloom of power plants and industries have degraded the atmospheric condition of the city by emitting various types of hazardous pollutants into the air (Ilyas, 2007). Sulphur dioxide, carbon monoxide, nitrogen oxides and particulate matters are some of the significant pollutants contaminating the atmosphere. This situation will give harmful effects towards human health and other biotic ecosystems.

In order to improve the air quality of the polluted atmosphere of urban environment, various species of plants have been planted along the roads, walkways, highways, parks, settlements, inside and around the buildings. These plants are known as urban forest where they provide numerous benefits to the society in terms of economic, health, aesthetic and environment (Knuth, 2005; Nowak et al., 2008; Bista, 2009). Plants have the capability in removing the particulate matters from the anthropogenic sources, especially the leaves which have significant morphological traits that are able to accumulate airborne pollutants (Shan et al., 2007). Leaves with abaxial hairy surface and broad surface area would be advantageous as leaves will filter great amount of airborne particulates. Naturally, there is a boundary layer surround the leaf's surface which creates a removal mechanism that aids in capturing the pollutants. This mechanism occurs during dry deposition where the particles are transported through the quasi-laminar, stagnant air layer near the leaf surface (Picardo and Ghosh, 2010).

Ficus microcarpa from Moraceae family is a woody shrub when young and grows to an evergreen tree when reaches maturity. It is one of trees which is commonly planted in streets worldwide. The leaves are usually 3–5cm wide, 5-8cm long with elliptic to broadly elliptic or obovate (Starr et al., 2003). The main morphological characteristics that well describe this species are pavement cells, stomata and trichomes or known as leaf hairs (Khan et al., 2011). This species is a hardy plant which is suitable for landscape purpose (Wong, 2007). This study was aimed to access morphological characteristics of *F. microcarpa* in deposition of urban particulates.

Materials and Methods

Sampling location and description

Samples were collected at Jalan Hang Tuah, Kuala Lumpur (latitude 3°8'17"; longitude 101°42'18"), subject to plant availability and road access. There are several plants species planted along road dividers as well as the roadsides. The roads have three lanes on both sides and there are a number of buildings, mosque, car parks, constructions and Light Railway Transit station around there.

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Sampling and physico-chemical analysis of airborne particulates

Three shrubs of *F. microcarpa* planted along the road divider of the study site were chosen for the study. Two young and mature leaves from each tree were randomly selected and tagged. Only leaves which were highly exposed to the roads and 1.5-2.0 m from ground were selected. The surface of the leaves was cleaned wisely by using Nylon Art Brush 34mm for 20 seconds and then left for 3 days of exposure.

After 3 days of exposure, the selected leaves were cut on the petioles, inserted into plastic bags separately and brought to the lab for screening analysis using SEM/EDX on the same day. The leaves were cut into small size of 0.5 cm x 0.5 cm and stabbed onto double-sided carbon tape on a specimen stub. Then, the samples were coated under 25 ng gold conductor. Samples were viewed using scanning electron microscope at an accelerating voltage of 5-15kV under a back-scattered electron detector (Santos et al., 2007). Two to four photomicrographs were taken in different fields for each sample with magnification varying from 1.00k to 5.00k depending on the individual size and distribution size of the particles (Umbria et al., 2004).

In order to obtain the chemical composition of the samples, Energy Dispersive X-Ray (EDX) was used and technically combined with the SEM. The detected chemical elements from the samples were automatically presented in a form of graph and table together with the microphotographs of the morphologic analysis.

Results and Discussion

Morphologic analysis

The size, texture and shape of the particles depended on the particle origin and they way they responded to the climate as well as reaction upon vehicles' combustion besides other factors in a microscopic world (Umbria et al., 2004). The results shown by SEM were the organization of the particles' structures, not the elementary contained by the particles. Figures 1 and 2 show the texture of the leaf samples' surface for both mature and young leaves respectively. The surface texture was rough indicating the possible presence of trichomes, stomata and wax layer. These features enhanced particles deposition onto leaves (Figures 3-6). Most of the particles were in a form of cluster in which they agglomerated in the air before being accumulated on the leaf resulting in bigger size of particles. Generally, airborne particles originate from both natural and anthropogenic sources. Figures 3 and 4 show particles originated from combustion of vehicle petrol due to their irregular and amorphous shapes (Umbria et al., 2004) while Figure 5 is a particle originated from natural sources like soil or sea sand due to its highly complex structure with regular distribution. Figure 6 is a pollen grain which has symmetric structure (Umbria et al., 2004). Even though studies have found several structures to define particles and the sources, further studies have to be done as the particles may undergo different physical-chemical reactions such as recondensation and division.

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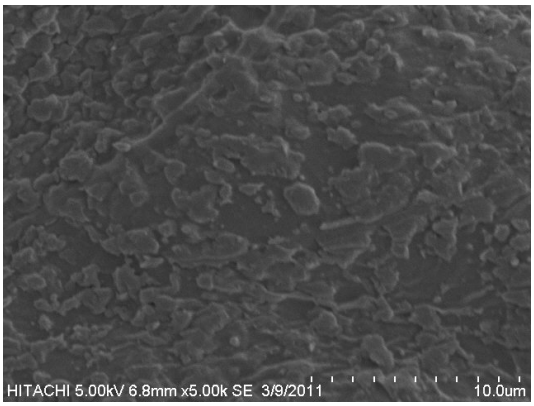

Figure 1. Texture of the abaxial surface of *F. microcarpa*'s leaf (low magnification)

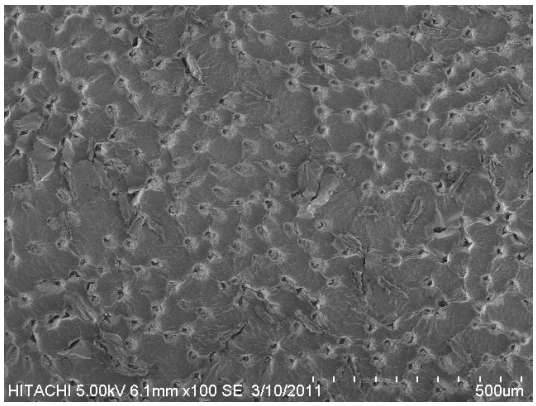

Figure 2. Texture of the abaxial surface of *F. microcarpa*'s leaf (high magnification)

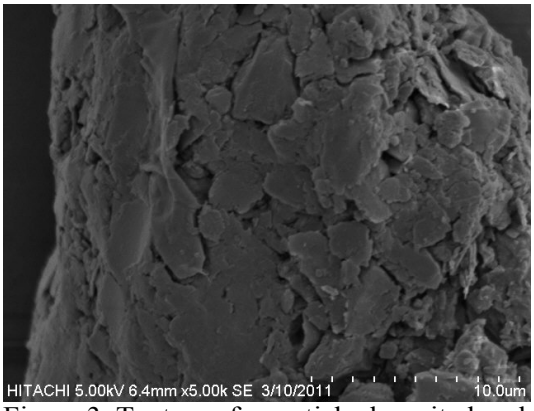

Figure 3. Texture of a particle deposited on leaf

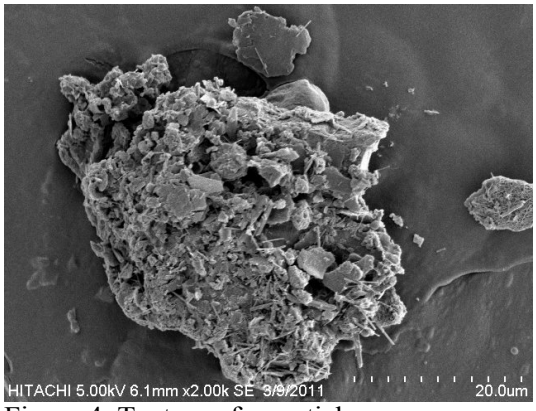

Figure 4. Texture of a particle

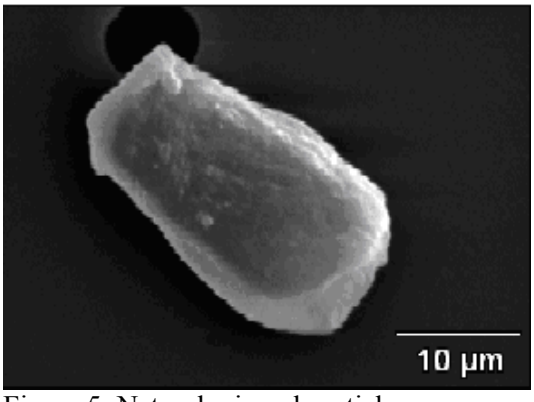

Figure 5. Natural mineral particles

Figure 6. Pollen grain

Chemical analysis

Complete description on a particle must include the analyses of morphology and chemical as they are complementary to each other (Fruhstorfer and Niessner, 1994). Chemical analysis by means of EDX enhances some information and proof on the particles' origin and elementary composition. Natural particles usually contain higher chemical elements compared to the anthropogenic particles (Umbria et al., 2004). Figure 7 shows that the particle might be originated from biomass where there are sodium (Na) and chlorine (Cl) detected. A particle formed by combustion process, aluminium (Al), carbon (C), silicon

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(Si) and sulphur (S) are the major elements and sometimes these elements will react together to form combined particles due to different methods of burning and its composition.

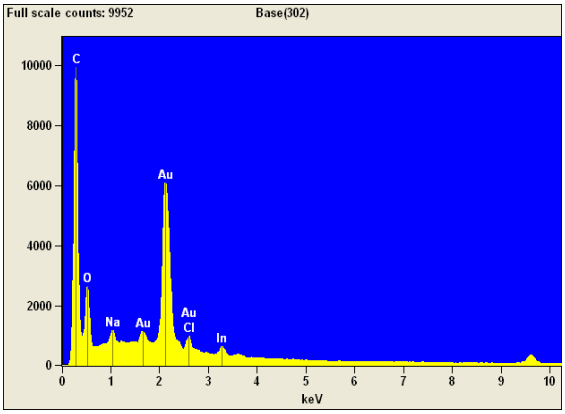

Figure 7. EDX of a natural particle

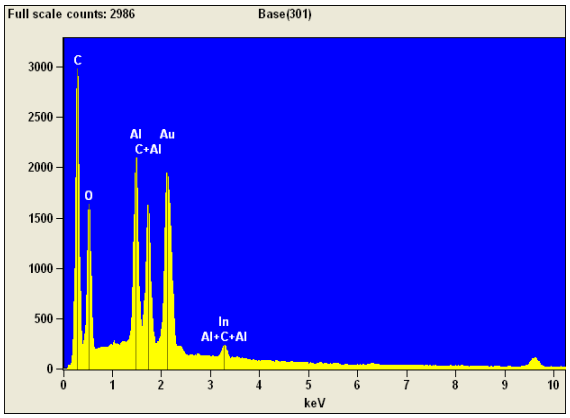

Figure 8. EDX of a combustion particle

Conclusions

Ficus microcarpa is a suitable species to be planted in an urban environment as it can tolerate harsh situation. The morphological characteristics of its leaf enhance the accumulation of airborne particles and hence increase the removal of hazardous atmospheric pollutants. Trichomes and wax layer of the leaves are able to entrap large amount of particles that are passing through the leaves due to the turbulence of winds, raindrops and emission from the vehicles' exhaust. A turbulent layer transport during dry deposition enhances the transportation of particles to the leaf surface with the aid of aerodynamic resistance, quasi-laminar resistance and surface resistance (Picardo and Ghosh, 2010).

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Growth of *Zingiber officinale* Rosc. var. Bentong under Different Irrigation Frequencies under Controlled Environment Structure

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Introduction

Ginger is the most important and widely used spices in the world. Botanically known as *Zingiber officinale* Rosc., this plant belongs to the family Zingiberaceae and order Sciminatae. The main ginger growing countries are India, China, Jamaica, Taiwan, Nigeria, Bangladesh etc. but India remained as the highest producer of ginger and contributed about 45% of the total global ginger production (Ai et al., 2004). The demand for fresh ginger in Malaysia is more than the supply, due to low production. The local demand is expected to increase from 15,575 tonnes in 2005 to 20,000 tonnes by the 2010 (Jaafar, 2007). In 1999, Malaysia imported 5,671 tonnes ginger from China, Indonesia, Thailand, Myanmar and United States of America. By the year 2000, the import has increased to more than 8,000 tonnes and continued to increase to more than 9,000 tonnes in 2001. Although the demand for ginger increased locally, the area of ginger plantation keeps decreasing. In 1998, the area was about 316 ha, then reduced to 283 ha in 1999, and continued to decrease to 244 ha in 2000 (Firdaus, 2008).

The open cultivation of ginger is beset with a number of constrains that affect the potential production. Ginger has usually been cultivated in hilly and good drainage areas such as Bentong - Janda Baik, Cameroon Highland and Tambunan. However, the efficiency of rhizome production in this hilly area reduced due to difficult harvesting process along the hilly area. This problem can be solved by planting ginger in lowland area under controlled environment system (CES). This is possible because microclimate condition, especially water amount and irrigation frequency, can be manipulated to become condusive for enhanced plant growth due to increased water and nutrient deficiency. Besides, off season planting is possible to enable production throughout the year. However, some important agronomic practices, such as irrigation frequency, need to be resolved to find the best irrigation frequency for ginger growth under CES because water deficit poses as one major factors affecting ginger production in Malaysia (Jaafar, Pers. comm.). Therefore, the objective of this study was to evaluate the growth performance of ginger as affected by different irrigation frequencies and to determine whether there was interaction between irrigation frequency and weeks after planting.

Materials and Methods

The experiment was conducted in Agrotechnology Park, Bukit Ekspo University in an experimental glasshouse. Young ginger (*Zingiber officianale* var Bentong) rhizomes were germinated for two weeks in 10.5 cm^2 growing pots and then transferred to 15 cm x 18 cm polyetylene bags with a soiless mixture of cocopeat and burn paddy husk in 1:1 ratio. Uniform ginger rhizomes were chosen for the experiment and fed with nutrient solution in a fertigation system based on formulation of Hoagland and Arnon (1950). Randomized Complete Block Design (RCBD) factorial design with 4 replicates was used where two factors were studied, i.e. irrigation frequency (2x, 4x, 6x) and time after treatment (4, 8, 12, 16 weeks after treatment). Total leaf area was determined by using leaf area meter (Model LI –3100A Lincoln Inc, Nebraska, USA). Rhizome fresh weight was taken using a sensitive electronic weighing scale (Model CDS 125, Mitutoyo Inc, Japan).Total dry matter accumulation per plant was taken by calculating the dry weight of roots, rhizomes and leaves per plants. The plant parts were placed in paper bags, and oven-dried

at 80 °C until constant weight (i.e. three days) was reached. Plant total dry weight was taken using the same method. Growth analysis was calculated based on an individual plant basis through measurement of total plant leaf area and total plant dry weight. The parameters of growth analysis included relative growth rate (RGR), shoot: root and CGR according to the formulas reported by Gardner et al. (1986). Fiber content of ginger was determined by using method from Asmah (Pers. comm.) by heating dried fiber in neutral detergent (NDF). The data were subjected to ANOVA at 5% probability level by using statistical analysis system (SAS) software and treatment means were compared using Standard error of differences between means (SEM).

Results and Discussion

Interaction between irrigation frequency and time significantly ($p \leq 0.01$) influenced total leaf area, total plant dry weight, rhizome fresh weight and relative growth rate of ginger. However, fiber content and cumulative growth rate was influenced by irrigation frequency. In the present study, the total leaf area, total plant dry weight, shoot:root, rhizome fresh weight, RGR and NAR were enhanced on ginger that received ≥ 4X (Figures 1a - 1g). The growth enhancement might be contributed by the increase of soil relative moisture content. According to Kun (1999), as soil relative water content in the range between 40 – 80 %, the growth of the ginger was elevated and this has been justified by enhancement of plant height, tiller number and leaf area. Although soil relative water content was not measured during the experimental period, it can be assumed that the soil relative moisture content was within the range. The constant elevated moisture levels in the root zone of ≥ 4x enhanced the hydraulic conductivity and water availability of the plant (Ravindran and Nirmal, 2004). Furthermore, Xu et al. (1993) reported that growth enhancement of plant under high irrigation frequency was due to a higher availability of nutrients. The ginger fiber was observed to be enhanced with 2X irrigation frequency than the other treatments. The fiber content in ginger was found to be negatively correlated with total plant biomass (Xu et al., 1993).

In the present study, ginger treated with ≥ 4x irrigation frequency had higher growth than 2X irrigation frequency. This was justified with high RGR and CGR of the plant (Schroider and Lieth, 2002). At end of week 16, rhizome fresh weight was observed to be highest with ≥ 4x irrigation frequency. There was also 55% increase in total dry weight and increment of RGR and CGR by 9 and 63% respectively as compared to 2x irrigation frequency. Ginger showed to have partitioned more of the biomass to shoot compared to the root. However, as the experimental period was encroaching, the difference between shoot and root biomass was shown to be balanced. More biomass was partitioned to shoot to produce more leaf area to intercept more light as the ginger was at the active stage as observed from week 4-16 after treatment (Zhengxian et al., 2000).

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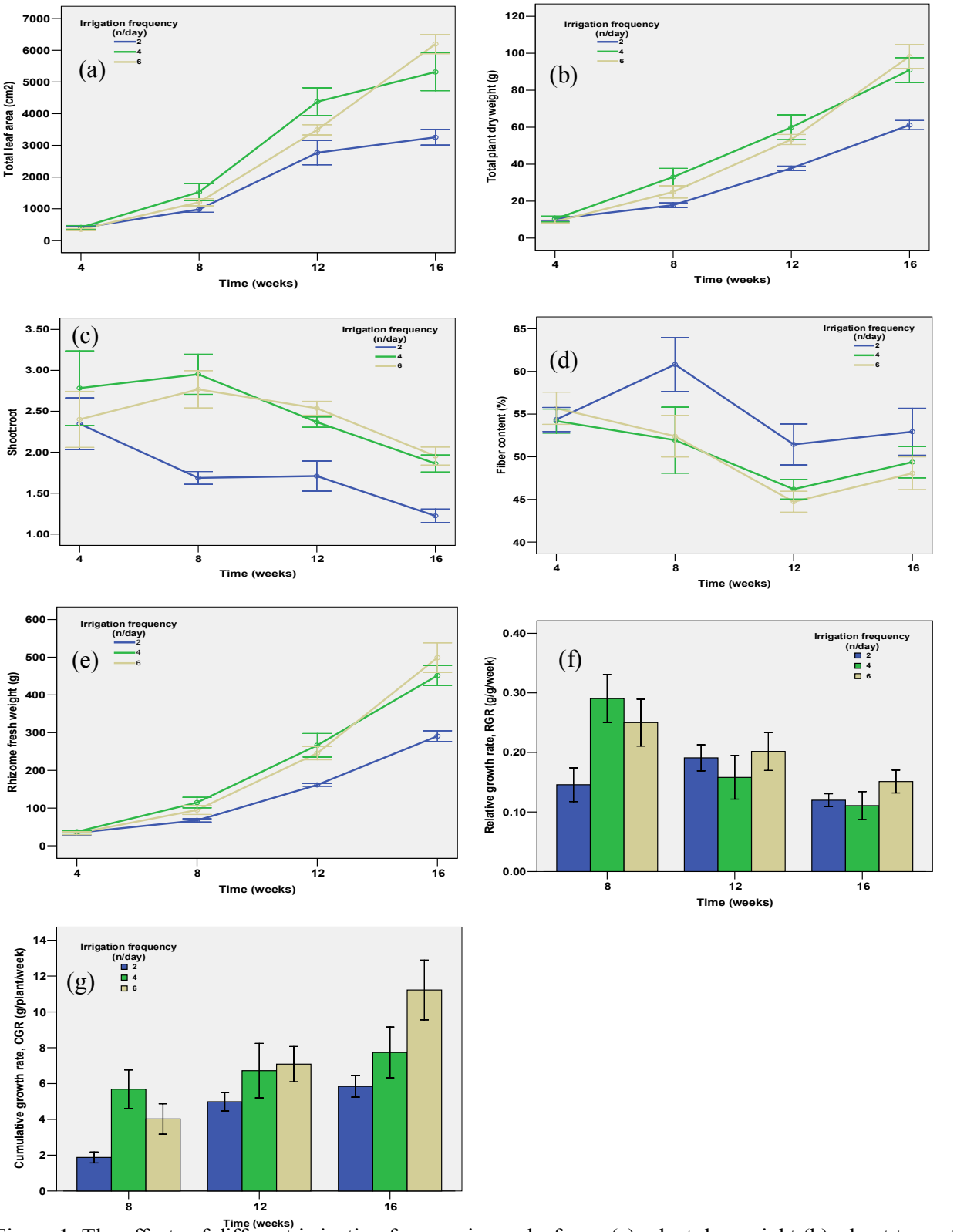

Figure 1. The effects of different irrigation frequencies on leaf area (a), plant dry weight (b), shoot to root (c), fiber content (d), rhizome fresh weight (e) relative growth rate (f) and cumulative growth rate (g) of *Zingiber officianale* at different weeks after treatment. Bars represent standard error of differences between means (SEM) at $P \leq 0.05$. n = 4

Conclusions

It was observed that ginger has the potential to be grown on lowland. It was shown that irrigation frequency of more than 4x can enhance growth of ginger grown in lowland. This was due to better water availability which enhances the hydraulic conductivity in the growth medium that contributes to the growth enhancement.

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Phenolics, Flavonoids and Antioxidant Activity of Cassava under Influenced of Organic and Chemical Fertilizers

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Introduction

Nutrients for plant growth must be efficiently managed to ensure continuous productivity without causing any potential threat to consumers and environment (Guerrero et al., 2001). Besides for plant growth and development, research is uncovering the fact that availability of plant nutrients and water can be important factors in determining secondary metabolism synthesis within plants (Aires et al., 2006; Li et al., 2008; Mohebbi and Maleki, 2010). Reports indicate that high nutrient availability leads to an increase in plant growth and development but a decrease in allocation of resources towards the production of secondary metabolite such as the phenolics, while the conventional cultivation that utilizes mineral fertilizers had also been reported to result in disruption of the natural production of phenolics in the plant.

Cassava (*Manihot esculenta*) has long been grown in Malaysia as a major source of starch for food and non-food industries (Tan, 2007). Starch is used mainly in the manufacture of monosodium glutamate, glucose and paper products. Today, the tuber is also popularly used for production of tapioca chip and also processed as feed for livestock. The leaves are rich in iron, zinc, manganese, magnesium and calcium, vitamins; B1, B2 and C and carotenoids as well as other phytochemicals that are important antioxidant components of plant food products (Wobeto et al., 2006). Cassava leaves also contain moderate level of phytochemicals that are important antioxidant components of plant food products. New food uses of cassava include as flour in gluten free or gluten-reduced products (Falade and Akingbala, 2011).

In the past, agricultural production was focusing on maximizing the quantity of crop produced for commercial market. Hence in common agricultural practices, compound fertilizer has been used in field grown cassava. Nowadays however, health conscious consumers are interested in optimizing the nutritional composition with minimal chemical residual of foods that produced through environmentally friendly agricultural practices. Substituting chemicals with organic fertilizers is one of the common principles in this production system. Compost and vermicompost have been widely applied as source of nutrient.

The nutritional quality of organically and conventionally grown plants has been compared mainly in terms of macronutrients, vitamins, and minerals. Organically produced vegetables have higher levels of vitamin C, iron, magnesium and phosphorus and less nitrates and lower amounts of some heavy metal (Worthington, 2001). There are very few studies that have compared the levels of antioxidant compounds in organically and conventionally grown products. Asami et al. (2003) reported that there was significantly higher of total phenolics in organically grown marrionberries (620 mg/100 g fresh weight) as compared to conventional method (412 mg/100 g). Whereas Antonio et al. (2007) reported that organic farming had a significant effect on nutritional content in peppers, increasing the vitamin C activity, total of phenolic compounds and carotenoid contents. Beside fertilization, genotypic differences are also one of the factors in causing a large variation in vitamin content (AVRDC, 2003), antioxidant capacity and phenolic content (Rekika et al., 2005). Therefore, the objective of this study was to determine the effect of inorganic and organic fertilizer sources on phenolic, flavonoids and antioxidant activity in the tubers of two cassava varieties.

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Materials and Methods

The experiment was conducted on sandy clay loam soil with the pH of 5.7 under open field condition. The treatments were arranged in split plot design with three replications. The main plot was fertilizer sources consisting of vegetable waste vermicompost (N:2.32%; P:1.54%; K:1.06%) empty fruit bunch compost (N:1.46%; P:1.47%; K:2.58%) and inorganic fertilizer (N:15%; P:15%; K:15%). The sub plot was cassava Medan and Sri Pontian varieties. Stem cuttings of 20-25 cm length were planted at 1 m x 1 m. There were 2 m gaps between treatments and 1 m alleys between plots. The amount of fertilizer applied was calculated based on 180 kgh^{-1} of K_2O. The soil was thoroughly plowed and mixed with organic compost during planting while inorganic fertilizer was applied equally at two and twenty weeks after planting. The soil was covered with organic mulch and sprinkler irrigated. The tubers were harvested after nine months and analyzed for total phenolic acids and total flavonoids content, DPPH free radical scavenging assay and FRAP scavenging assay.

Extraction of total phenolic acid and total flavonoid assay was conducted using modified method of (Marinova et al., 2005). Cassava tuber weighing 0.5 g was homogenized with 50 ml distilled water and transferred into covered flask. The mixture was centrifuged for 5 min at 14000 rpm and the supernatant was collected and used for total phenolic acids and total flavonoids quantification. Determination of total phenolics and total flavonoid compounds were conducted as described by Marinova et al. (2005) using the Folin-Ciocalteu assay and Aluminum Chloride Colorimetric method, respectively. For total phenolics, the absorbance was monitored using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan) at 750nm and the content was expressed as mg gallic acid equivalents (GAE)/g samples. The absorbance of the solution for total flavonoids was measured at 510 nm and the content expressed as mg catechin equivalents (CE)/g samples.

Extraction of antioxidant compounds, DPPH free radical test and total antioxidant activity using FRAP assay was conducted employing the method modified by Wong et al. (2006). 0.5 g tuber was cut into small pieces, mixed with 25 ml of distilled water in 150 ml Al foil covered conical flask. The conical flasks containing the samples were placed in orbital shaker for 1 h in the dark at room temperature. Then the samples were filtered using Whatman No. 1 paper and the extracts were stored at 0-4 °C before analysis. The initial absorbance of DPPH in methanol was measured at 515 nm until the absorbance remains constant. 40µl of extracts was added to 3 ml alcohol solution of DPPH (0.1 mM). The samples were first kept in a dark place at room temperature and after 30 minutes the absorbance was measured at 515 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc, Tokyo, Japan). The percent of inhibition was determined using the formula, percent of inhibition (%) = [(A515 of control-A515 of sample)/A515 of control] x 100.

The determination of the total antioxidant activity using FRAP assay in the extract followed after a modified method reported by Wong et al. (2006). 200 µl of extract was added to 3ml of FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM $FeCl.6H_20$ solution) and the reaction mixture was incubated in a water bath at 37 ^oC for 30 min. The increase in absorbance was measured at 593 nm using a spectrophotometer. The percent of antioxidant was calculated using the formula, percent of antioxidant (%) = [(A593 of sample-A593 of control)/A593 of sample] x 100. The data were analyzed using analysis of variance and significant differences between means was done by Least significant difference test ($p<0.05$).

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Results and Discussion

Phenolic compounds are great of importance in terms of nutritional, commercial properties of agricultural products, through their contribution to sensory properties such as colour and flavor (Antonio et al., 2007). The total phenolic content was measured by Folin Ciocalteu reagents in terms of gallic acid equivalent (standard curve equation: y= 1.961 – 0.001x, R^2= 0.553). Total phenolic and flavonoid content were found to be significantly higher in organic treatments compared to inorganic fertilizer (Table 1). The highest total phenolic content was observed with using vermicompost (10.88 mg GAE/g fw) compared to inorganic fertilizer (8.35 mg GAE/g fw). Difference in flavonoid content of Medan and Pontian was found only in vermicompost (Figure 1).

The significant positive correlation shown in Table 2 (r = 0.62) between total phenolic and flavonoid compounds indicates that an increase in phenolic was followed by an increase in total flavonoid. Both were found to be highly correlated with antioxidant activity. Since organically fertilized plants had high phenolics and flavonoids, higher DPPH scavenging activity and FRAP scavenging activity were observed. Fertilization has been reported to have influence on phyto-nutritional quality of crops. Inorganic fertilizer was found to reduce the antioxidant while organic fertilizer was proven to enhance the antioxidant content in plant (Dumas et al., 2003). In tomato, organic fertilizers had increased the content of ascorbic acid and total phenolic (Toor et al., 2006). In our study, results significantly indicated that sources of fertilizer had influenced the level of phytochemical compound in cassava.

The antioxidant activity of plant extracts were assessed by DPPH free radical scavenging method. The DPPH method has been widely applied for estimating antioxidant activity in recent years (Klimczak et al., 2007). Behbahani et al. (2007) also suggested the model of scavenging DPPH radical is a widely used method to evaluate antioxidant activities in relatively short period compared with other methods. The highest value of DPPH scavenging activity (67.30%) was observed from the treatment of vermicompost (Table 1). Based on the effect of antioxidants activity, the percentage of inhibition was increased by using vermicompost as fertilizer sources. Among two varieties, the highest percent on DPPH inhibition was found in Medan variety (47.58%). It was observed however that percentage of inhibition was found to be dependent on the source of fertilizer and the cassava varieties (Figure 2). Pontian variety would respond better than Medan when it was treated with vermicompost. There were significant positive correlation ($P \leq 0.01$) between TPC and DPPH assay, (r =0.83), (Table 2). These results showed that cassava possesses strong antioxidant activity and can be used as a good source of natural antioxidants.

The FRAP assay is widely used in the evaluation of antioxidant component in dietary polyphenols (Luximon-Ramma et al., 2005). When the samples reacted with FRAP solution, dark blue color of solution will appear which refers to the ferrous tripyridyltriazine complex. The extracts which exhibit antioxidant such as in Medan treated with vermicompost produced more ferrous tripyridyltriazine complexes compared to Pontian. Ferrous tripyridyltriazine complexes were produced as product form reaction in which the samples had the ability to reduce Fe^{3+} to Fe^{2+}. The greater Fe^{3+} reduced to Fe^{2+} the higher total antioxidant content observed. In all treatments, the percentage of antioxidant increased with increasing concentrations of the plant extracts (Alizadeh et al., 2010). Similarly, the percentage of antioxidant was found to be dependent on the source of fertilizer and the cassava varieties. Among three nutrient sources applied, plant with the application of vermicompost had significantly greater antioxidant activities followed by EFB compost and inorganic fertilizer (Table 1). Both varieties showed no significant differences in FRAP method. However, under influenced of vermicompost and inorganic source, Medan had greater total antioxidant content than Pontian (Figure 3). Variation in antioxidant compounds and phenolic content between genotypes was also reported in other crops (Rekika et al., 2005; Bok et al., 2006). FRAP assay also showed significant ($P \leq 0.01$) positive correlation (r =0.74), with total

phenolic compounds (Table 2). The relationship between the phenolic constituents and antioxidant activities in various plants was reported by several researchers. According to Antonio et al. (2007), organic farming had positive effect on the nutritional quality content of sweet peppers (*C. annuum* cv. Almuden), increasing the vitamin C activity and the level of phenolic compounds.

Our results demonstrate that vermicompost showed significantly better effects on phenolics, flavonoids and antioxidant activities than EFB compost. Their influence on physiological activities could be due to fundamental differences between the conventional composting and vermicomposting processes. In addition, enzymatic activity of worms in vermicompost as well as the presence of beneficial microorganism maybe affected the physiological activities (Atiyeh et al., 2000).

Results indicate that application of organic fertilizers can enhance antioxidant activities of field grown cassava. Medan variety with application of vermicompost showed the most promising nutritional quality. Phytochemical composition was significantly improved with application of organic fertilizer and vermicompost showed surplus effect than EFB compost. Organic fertilization could be a useful tool to minimize soil contamination while improving tuber quality.

Table1. Phytochemical compounds in tuber of cassava varieties as affected by fertilizer sources

Source	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	DPPH Scavenging assay (%)	FRAP scavenging assay (%)
Fertilizer source				
VWV	10.88a[z]	2.71a	67.30a	68.11a
EFBC	9.44b	2.32b	54.70b	54.45b
Inorganic	8.35c	2.18c	44.37c	50.08c
Variety				
Medan	9.83a	2.47a	47.58a	37.50a
Sri Pontian	9.20b	2.30b	37.11b	37.21a
Fertilizer x Variety	*	**	**	*

[z]Means with same letter are not significantly different by LSD, at 5%.
*** * significant at P≤0.01, P≤0.05, respectively*

Table 2. Correlation coefficients between total phenolics, total flavonoid and antioxidant activity determination assays (DPPH and FRAP) of cassava.

	TPC	TFC	DPPH	FRAP
TPC	-	0.62*	0.83**	0.82**
TFC	-	-	0.61ns	0.74**
DPPH	-	-	-	0.79**
FRAP	-	-	-	-

For correlation coefficients, n=18
*ns, *, ** Non significant or significant at P≤0.05 and P≤0.01, respectively.*

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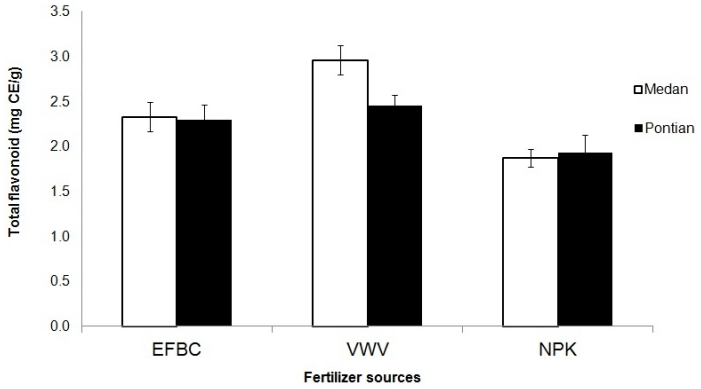

Figure 1. Flavonoids content in tubers of cassava treated with different fertilizer sources

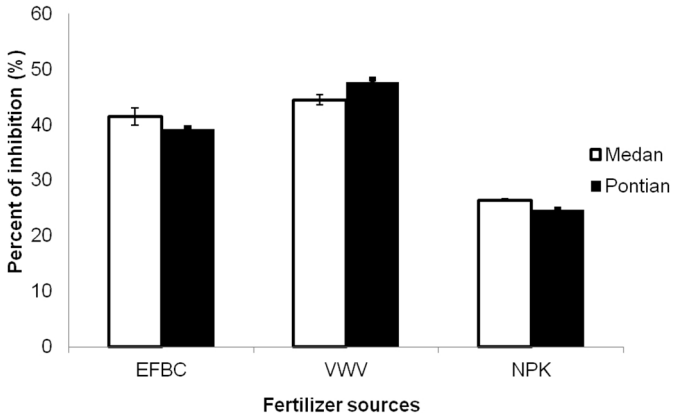

Figure 2. DPPH scavenging activities of cassava tubers treated with different fertilizer sources

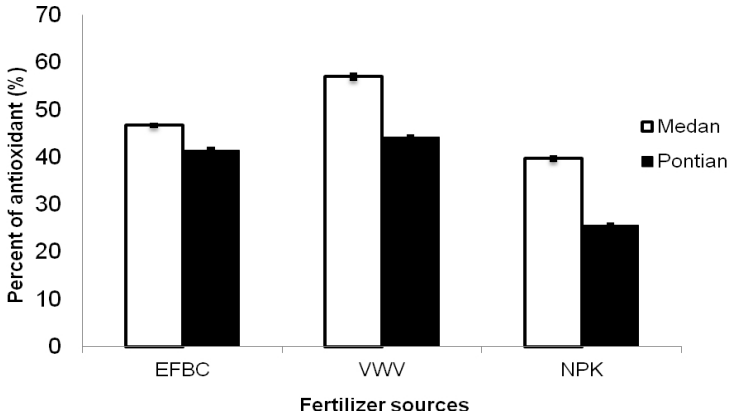

Figure 3. FRAP scavenging activities of cassava tubers treated with different fertilizer sources

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Plant Physiological and Root Profile Assessments of Potential Slope Plants

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Introduction

Vegetation has been widely used as a tool to improve slope stability. The relationship between vegetation and slope stability are complex as it involved, inter alia, the combination of soil type, plant coverage, and the steepness of slope. In addition, each species has its own physiological mechanism including root topology and geometry, surviving capacity in different soil nutrient levels and conditions. Therefore, screening of plant species in terms of observing potential slope plant characteristics (e.g. higher growth rate and extensive root system for soil reinforcement) is crucial. A set of criteria was formulated for selecting plant species for plantation by a lot of researchers (Stokes et al., 2009). For example, based on the previous potential plant species selection, slope plants should process high growth rate, photosynthetic rate, leaf area index (LAI) value, and extensive root system leading to removed water through transpiration, enhanced water uptake, reinforced soil, and increased shear strength by binding soil particles (Normaniza et al., 2008; Normaniza and Barakabah, 2011).

Mafian et al. (2009) showed that the reinforcement of soil by vegetation is highly promising solution and this approach would be more beneficial if the species acutely possess the mechanical (through reinforcement of soils by plant root), hydrological (through reduction in runoff and by keeping the slope relatively dry) and environmental (through the increase in carbon sequestration to counter the rising carbon dioxide level in atmosphere) aspects (Syed and Iqbal, 2007). Poorter and Bongers (2006) compared the leaf traits and plant performance of 53 co-occurring tree species in a semi-evergreen tropical moist forest community and demonstrated that the leaf traits are good indicators of plant performance. In relation to this, the development of shoot and root can also be considered which is influenced by soil types. It is also reported that soil density, hydraulic conductivity and soil water relation affects the root growth (Laboski et al., 1998). For that, root profiles and soil water relation are referred as vital parameters to predict slope stability and soil erosion (Normaniza and Barakabah, 2006).

Therefore, an experiment was carried out to assess the plant physiological and root properties of four selected species in different soil types, to deduce some correlations amongst the parameters studied and to determine the two best potential slope species.

Materials and Methods

Experimental site, soil and plant materials

Three types of soil (clay, sand and slope soil) and four native legume tree species namely *Leucaena leucocephylla* (LL)*, Adenanthera pavonina* (AP)*, Peltophorum pterocarpum* (PP)*, and Pterocarpus indicus* (PI) were selected for this experiment. Seeds were collected from Forest Research Institute of Malaysia (FRIM) and grown in an open-ended 30 cm PVC pipe. Individually, each type of soil was filled into PVC pipe with ten replications and in the total of 120 seedlings [3 (types of soil) ×10 (replication) × 4 (species)]. The experiment was carried out for six months under prevailing glasshouse conditions (temperature 21-32 °C, maximum PAR 2100 µE m^{-2} s^{-1} and relative humidity of 60-90%), Plant Physiology Garden, University of Malaya. The plants were arranged in a RCBD having 25 cm row to row

distance and 25 cm plant to plant distance. The plants were irrigated once in two days to avoid the water stress condition.

Plant height and biomass

Plant height was measured at 2-month interval by measuring tape. The shoot fresh weight (SFW), and root fresh weight (RFW) and dry biomass (oven-dried at 80 °C for 12 hours) were determined at the three and six month of growth.

Measurements of photosynthesis and chlorophyll fluorescence

The photosynthetic rate was measured using the portable Photosynthesis System (Model LI-6400XT, USA) at 2-month interval. The chlorophyll fluorescence was measured by portable Plant Efficiency Analyser (Model LH36/2R, Hansatech Instrument Ltd., England) at 2-month interval.

Leaf area index (LAI) and soil moisture content

Leaf area index and soil moisture content were measured using leaf area instrument (AccuPAR-LP80, UK) and portable Delta-T soil moisture meter (HH2 Moisture Meter, England), respectively, at 2-month interval.

Root profiles

The root length of all the different species was determined by scanning and using the WinRHIZO Pro Software after three and six month. This software was also used to find nodulation frequency, total root length, fine roots and the average volume of the root.

Statistical analysis

Statistical analysis was performed using SPSS software. LSD ($p=0.05$) was calculated using the error mean squares of the analysis of variance. The correlation test between the parameters studied was analyzed using Microsoft Excel.

Results and Discussion

Total photosynthesis and chlorophyll fluorescence were significantly higher in *Leucaena leucocephylla* (LL) grown in three types of soil throughout the growing period. For both *Adenanthera pavonina* (AP) and *Peltophorum pterocarpum* (PP), photosynthesis and chlorophyll fluorescence were significantly lower in sandy soil condition (Figure 1). Varietal differences of four species in photosynthesis and chlorophyll fluorescence were due to variation in plant species and type of soils. AP species in each types of soil showed overall lower photosynthesis and chlorophyll fluorescence. This chlorophyll fluorescence can also indicate an imbalance condition between the assimilation of light energy by leaf and the use of energy in photosynthesis (Rong-hua, 2006). A value about 0.82 discovered in LL grown in all soil types represented that this species had better photosynthetic ability than the other three species studied (Calatayud et al., 2002). In addition, plant height and LAI are, arguably, the most important parameters for assimilation of the species, which is the highest in LL (Figure 2).

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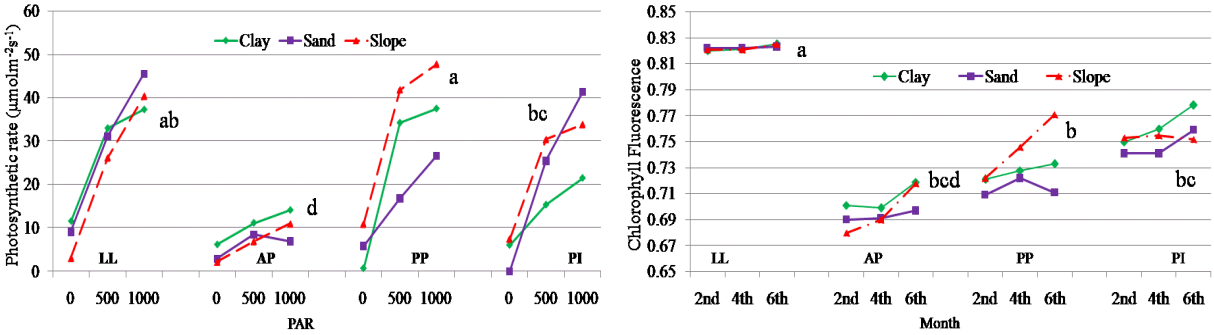
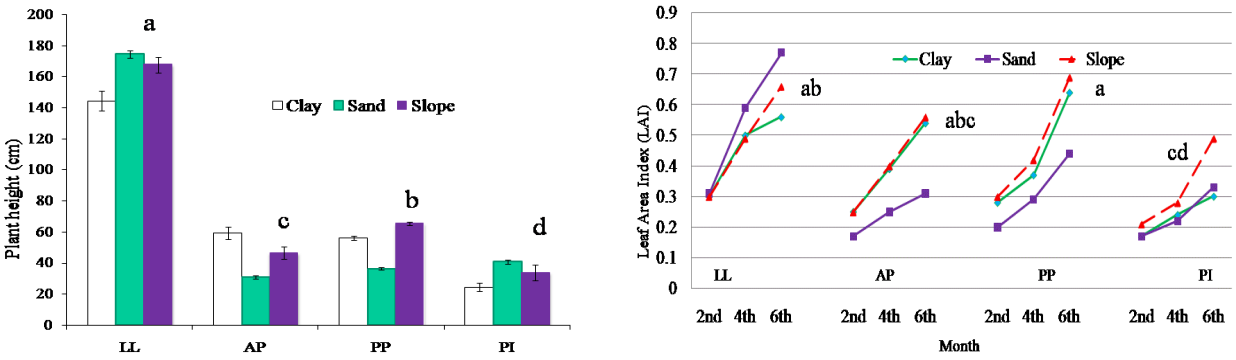


(a) (b)

Figure 1. (a) Photosynthesis and (b) Chlorophyll florescence in three types of soil. Different letters indicate significant difference among treatments.



(a) (b)

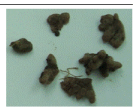
Figure 2. (a) Plant height and (b) Leaf Area Index (LAI), in three types of soil. Different letters indicate significant difference among treatments.

The differences in plant biomass production and nodules formation among the different types of soil were exhibited in Table 1. Higher shoot fresh weight (SFW) were observed in LL and PP which is presumably attributed to a higher root fresh weight (RFW) (Table 1a). Therefore, these findings have led to the proposal of root-shoot relation and that was, root growth promoted the shoot growth or LAI (Figure 4). It can be assumed that LL and PI had a strong symbiotic relationship that allowed these species to produce more nodules along with plant age and growth (Table 1b).

Table 1. (a) Plant biomass at three and sixth months of growth (b) Nodulation in different soils.

Species		3 Month Biomass			6 Month Biomass		
		SFW	RFW	Ratio	SFW	RFW	Ratio
LL	Clay	28.4	11.29	2.51	52.66	16.6	3.17
	Sand	44.7	18.96	2.35	73.33	29.96	2.44
	Slope	27	15.19	1.77	44	15.63	2.81
AP	Clay	22.26	5.9	3.77	48.7	15.31	3.18
	Sand	10.19	3.5	2.91	13.44	4.84	2.77
	Slope	14.12	2.2	6.41	31.23	11.81	2.64
PP	Clay	15.82	3.01	5.25	40	16.74	2.38
	Sand	8.7	2.46	3.53	14.66	9.93	1.47
	Slope	25.35	5.26	4.81	53.33	23.06	2.31
PI	Clay	3.27	0.89	3.67	7.96	2.84	2.8
	Sand	3.26	0.57	5.71	15.5	4.27	3.62
	Slope	4.34	0.94	4.61	23.5	5.79	4.05

(a)

Species	Nodulation	Nodule number in different soils Clay, Sand, Slope ↓ ↓ ↓	Shape	Color	Nodules
LL	Yes, at 3 month & 6 month	28.3, 68.3, 11.6 94 182 63	Triangle	Brown	
AP	No				
PP	No				
PI	Yes, at 3 month & 6 month	4.0, 6.6, 1.0 7.3 80 20	Circular	Brown	

(b)

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LL had the highest root volume and length (Figure 3). Consequently, LL also had a higher root biomass (Table 1a). Roots with high root length and volume maximize the root-soil interface and show a higher uptake rate of water (Normaniza and Barakabah, 2006). Therefore, soil grown with LL showed lower soil moisture than the other species (Figure 3a). In the present study, most of the species possessed a higher quantity of fine root length in the range of 0.5-1.0 mm (Figure 3c). It is well documented that fine roots increased the efficiency of soil binding between the soil particles and improved the cohesion as well. It is also suggested that fine roots increased the hydrological properties via its capability to absorb sufficient water, in turn lowering the risk of landslides and erosion (Shaozhong et al., 2002).

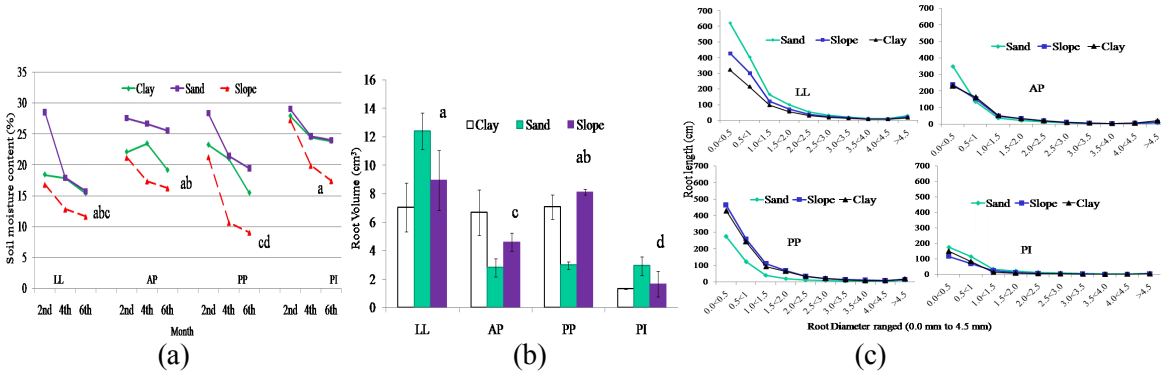

Figure 3. (a) Moisture content (b) Root volume and (c) Root diameter ranged 0.0 mm to 4.5 mm. Different letters indicate significant difference among treatments.

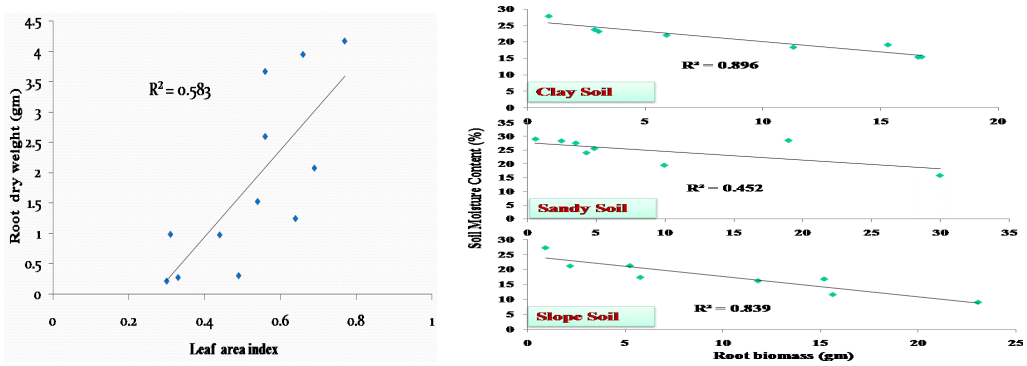

Figure 4. Correlation between leaf area index and root dry weight; soil moisture content and root biomass.

There is a positive correlation between plant LAI and root biomass (Figure 4), implying that the underground biomass would be higher if the above ground biomass higher. Whereas, soil moisture content (%) and root biomass are negatively correlated. The higher the underground biomass refers the lower soil moisture content (%). Therefore, an increase root biomass e.g. fine roots (0 to 2 mm) is greatly beneficial in absorbing the excess soil water and removed them out to the atmosphere via transpiration (Normaniza and Barakabah, 2006). As a result, removed excessive water would lead in drying the slope and stability of the slope. In addition, cumulative ranking is done to obtain the potential species according to total plant physiological performances and root profiles (Table 2).

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Table 2. Cumulative ranking (screening) analysis of parameters and species studied in different soil types (Normaniza, 2004). Ranking: Best → 4, Good → 3, Fair → 2, Poor → 1

Parameters	LL			AP			PP			PI		
↓ Species →	Clay	Sand	Slope	Clay	Sand	Slope	Clay	Sand	Slope	Clay	Sand	Slope
Plant height	3	4	2	3	1	2	2	1	3	1	1	1
Root biomass	2	4	3	3	2	1	2	1	3	2	1	2
Chlorophyll Flu.	4	3	3	2	1	2	2	1	3	3	2	1
Photosynthesis	2	4	3	3	1	2	2	1	3	3	4	2
LAI	2	4	3	3	1	3	3	2	4	2	2	3
Soil moisture	3	3	4	2	1	3	3	2	4	1	1	3
Nodulation	2	4	1	0	0	0	0	0	0	1	3	2
Root length	2	4	3	3	2	3	3	2	4	1	2	1
Root volume	2	4	3	3	1	2	3	1	4	1	2	1
Fine roots (0.0-2.0 mm)	2	4	3	2	3	2	3	2	3	1	2	1
*Total	24	38	28	24	13	20	23	13	31	16	20	17
*		1st							2nd			

Conclusions

In conclusion, based on the plant physiological and root profiles screenings, *L. leucocephala* grown in sandy soil exhibited the best performance followed by *P. pterocarpum* in slope soil. Root biomass is negatively correlated with soil moisture content and positively related to LAI. However, more stringent screening will be conducted on LL and PP to examine further their potential as slope plants.

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Greenhouse Gas Emissions from Land Use Change in Malaysia

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Introduction

The tropical forest plays an important role in the global carbon cycle of the terrestrial ecosystems. Considerable amount of biomass is stored and released from the standing and woody biomass of the forest. Forest biomass represents the potential amount of carbon as greenhouse gas emission takes place when timber harvesting, shift cultivation and forest conversion activities occur. In the current inventory these practices is viewed from the changes of forest area and the associated rate of emission is estimated using the IPCC Revised 1996 Guidelines.

The inventory also determines the relationship of deforestation with the increase in emissions of greenhouse gases (GHGs) and the reduction of carbon sequestration potential, between a time periods of 1990 to 2008. A balanced consideration of the forest conditions and activities in the past and present would therefore provide a better assessment of the situation in the country.

Materials and Methods

In this paper, the IPCC Revised 1996 Guidelines is used. The data requirements for IPCC method deal with land use and land management changes over time. The primary data required are total land area based on key categories, annual growth rates or IPCC default, annual harvest and annual land conversion. For the current reporting, detailed data is available through the National Forest Inventories, Annual Statistics and Annual Reports published by the Forestry Department Peninsular Malaysia, Forestry Departments of Sabah and Sarawak.

Information is organized in a series of worksheets each related to a different source of carbon flux. The worksheets contain the formulas necessary to compute area of each land use category. For most basic application, area under each land use category is estimated at the beginning and end of inventory period as illustrated in Table 1 and 2. If more detailed information is available, native carbon values and change can be adjusted to make the inventory as accurate as possible.

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Table 1: Illustration of Revised 1996 IPCC Guidelines-Sheet 5-1s1

MODULE	LAND USE CHANGE AND FORESTRY				
SUBMODULE	CHANGES IN FOREST AND OTHER WOODY BIOMASS STOCKS				
WORKSHEET	5-1				
SHEET	1 OF 3				
STEP 1					
	A Area of Forest/Biomass Stocks (kha)	B Annual Growth Rate (t dm/ha)	C Annual Biomass Increment (kt dm)	D Carbon Fraction of Dry Matter	E Total Carbon Uptake Increment (kt C)
			C=(A x B)		E=(C x D)

(Source: IPCC 1997)

Table 2: Illustration of Revised 1996 IPCC Guidelines-Sheet 5-2s2

MODULE	LAND-USE CHANGE AND FORESTRY				
SUBMODULE	FOREST AND GRASSLAND CONVERSION - CO_2 FROM BIOMASS				
WORKSHEET	5-2				
SHEET	1 OF 5 BIOMASS CLEARED				
STEP 1					
Vegetation types	A Area Converted Annually (kha)	B Biomass Before Conversion (t dm/ha)	C Biomass After Conversion (t dm/ha)	D Net Change in Biomass Density (t dm/ha)	E Annual Loss of Biomass (kt dm)
				D = (B - C)	E = (A x D)

(Source: IPCC 1997)

Estimation of rates of emission and sequestration from two main activities which could be considered as an anthropogenic source or sink are as follow:

a. Changes in forest and other woody biomass stocks

The inventory included all natural forest considered having some form of human intervention. Estimates are derived from annual growth rates and total areas of each forest category. For plantation crops, only oil palm and rubber have been considered. National figures for annual growth rates were used where possible.

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b. Forest and Grassland Conversion

The inventory accounts for natural forest converted for development and agricultural purposes. Forest conversion is estimated based on a 10 year average based on the initial loss of standing biomass reported in this section. Wood removed through licensed logging for commercial harvest has also been accounted for the purpose.

Results and Discussion

Forested areas

The change in total forested areas over the last 18 years has been minimal, and stabilized with a change of about 0.4% in total land use area in Malaysia. The decline was due to large forest areas converted to agriculture and plantations; where oil palm cultivation areas showed an increase of over 100% as forest plantation for timber or bio-fuel has become increasingly important in the future.

In comparison, Malaysia's 'best practices' approach has been able to conserve the biological resources and carbon stocks by avoiding deforestation. Unlike the situation in many developing countries, where harvesting is followed by burning and a gradual conversion to agricultural or grazing land, Malaysian forests under the PRFs do not undergo a change in land use. This is illustrated by the fact that the area of Malaysian forests under the PRFs has not changed substantially with a slight 14% increase in the last 18 years. However, forest areas under State land has decreased by over 50% as these areas have been long earmarked for development and designated for eventual conversion to meet demands for additional lands for agricultural, urban or other non-forest purposes (Table 3).

Table 3: Changes in Forest Areas between year 1990 and 2008

Land Use	1990 (million ha)	2008 (million ha)	% Change
Permanent Reserved Forest	12.6	14.3	+14
Totally Protected Areas	1.12	1.9	+70
State Land	6.8	3.25	-52
Total Forest	20.54	18.17	-12
Rubber	1.837	1.25	-32
Oil Palm	2.03	4.49	+121
Cocoa	0.4	0.03	-93

Source: FAO, 2010

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Aggregated CO_2 source and sink

The CO_2 flux trend over the last 18 years suggests a significant downward trend in the emissions and upward trend in removal (Figure 1). The change in trends could be attributed to significant increase in forest plantation establishments especially in Sarawak from 1998 onwards. Our current inventory results showed that greenhouse gas removal between these years was about 220 Mt CO_2e/yr while emission from the land use change is 4 Mt CO_2e/yr (Table 4).

Table 4: CO_2 Source and Sink from Land Use, Land-Use Change and Forestry in Malaysia year 1990-2008

GHG Source & Sink	CO_2 Emissions (million t CO_2 e)	Average CO_2 Removal (million t CO_2 e)
Changes in forest and other woody biomass		220
Forest and grassland conversion	4	

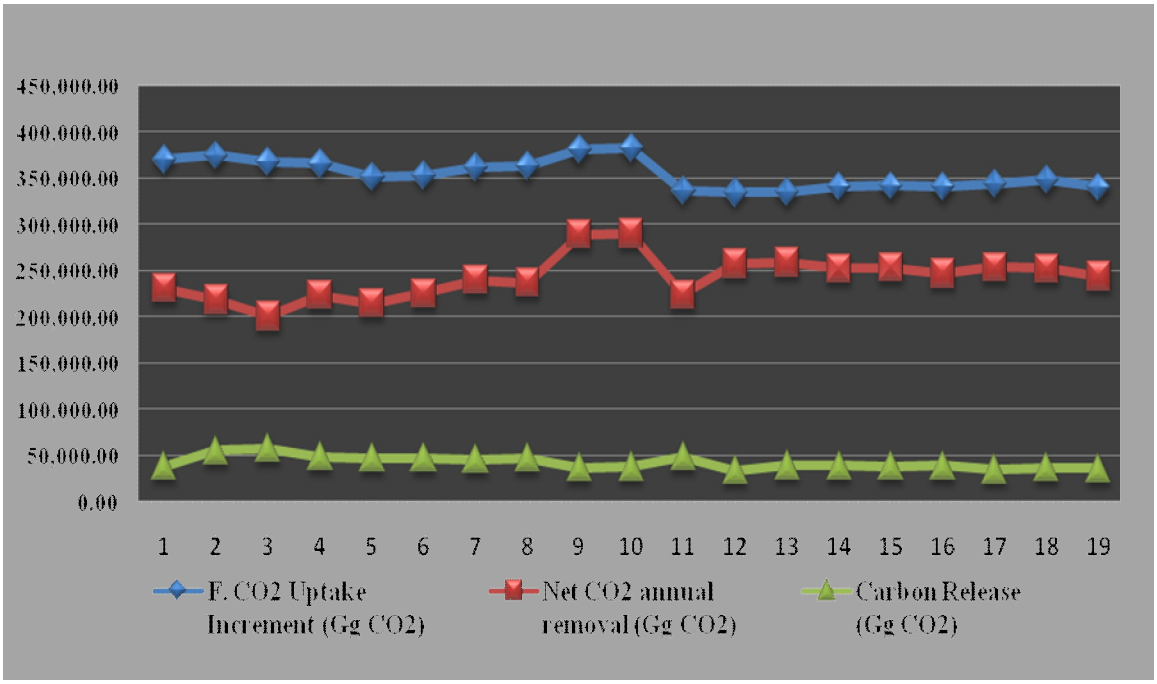

Figure 1: Time series emissions and removals between year 1990 and 2008

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Conclusions

As a conclusion, annual emission from land use change is reported for about 4 million ton CO_2 e /yr for the last 18 years. This is mainly due to the decline in forest cover from 19.45 million ha to 18.21 million ha between year 1990 and 2008.

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CHAPTER 4

BEST PRACTICES AND CURRENT TECHNIQUES

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Improved Rooting Transgenic Malaysian Eksotika Papaya: An Improved Method to Ensure Quality Roots Production and Higher Survival Rates of Transgenics

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Introduction

Critical pre-requisites in the development of transgenic papaya include efficient and reproducible plant regeneration and transformation system, and a successful acclimatization process of the transgenic plants for transfer into the field. Currently, the two major problems faced in developing transgenic Malaysian Eksotika papaya plants are low rooting efficiency of regenerated shoots after transferred into rooting medium and low acclimatization rate of rooted transgenic papaya plants. These problems could probably be due to poor root quality such as thickened, callused, and no lateral roots and root hairs formation, which affects the uptake of nutrients by rooted transgenic papaya plants after transferred into soil. Therefore, rooting efficiency and quality roots formation are needed to ensure successful and continuous production of transgenic Malaysian Eksotika papaya.

The aim of the study reported here was to improve the rooting efficiency and quality of tissue-cultured papaya shoots regenerated from ACC oxidase 1 and 2-transformed embryogenic calli. The effects of two different rooting materials and two different culture conditions (sterile versus non-sterile) on the number of roots produced, average root length obtained and the survival rate of the rooted shoots during acclimatization process were studied. The positive transgenic plantlets produced from both transformation events using RNAi and antisense constructs were used for the rooting studies. Histological analysis was also carried out to assess the morphology of the roots produced. The improved rooting method presented here will be useful for the development of transgenic Malaysian Eksotika papaya with improved commercial traits.

Materials and Methods

Induction of embryogenic callus

Embryogenic callus of papaya (*Carica papaya* L.) cultures were initiated from immature zygotic embryos obtained from Malaysian Eksotika papaya fruit of 90 days after pollination. The immature embryos were cultured on the induction medium consisting of half-strength Murashige and Skoog (MS) basal salts medium (Murashige and Skoog, 1962) supplemented with 50 mg/L myo-inositol, full-strength MS vitamin, 6% (w/v) sucrose, 45.2 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.35% (w/v) phytagel. The pH of the medium was adjusted to 5.8 prior autoclaving. The callus cultures were grown at 25±2°C in the dark for one month.

Papaya transformation and shoot regeneration

One-month-old Eksotika embryogenic cultures were transformed separately with antisense ACC oxidase 2 construct (pASACO2E1) and RNAi constructs (pRNAi *ACO1*, pRNAi *ACO2* and pRNAi CACO) using

a previously established *Agrobacterium*-mediated transformation method for Eksotika (Vilasini et al., 2000). To select for putative transformed tissues, the transformed calli were transferred onto half-strength MS medium supplemented with kanamycin. The selection process was carried out for a total of four months. The first selection was carried out with 75 mg/L kanamycin for one month, followed by 150 mg/L kanamycin for the remaining 3 months. Surviving calli on the selection media were transferred onto the De Fossard regeneration medium supplemented with 0.89 µM 6-benzyladenine (BA), 1.1 µM 1-napthaleneacetic acids (NAA) and 150 mL coconut water for shoot regeneration.

Polymerase Chain Reaction (PCR) analysis of putative transformants

The presence of the transgene(s) in transformed regenerated papaya shoots were verified using PCR. For putative transgenic plants harbouring the antisense transgene, PCR was carried out to verify the presence of *npt*II and antisense ACC oxidase 2 genes. While for putative transgenic plants harbouring the RNAi transgene, PCR was carried out to verify *npt*II, *gus*A, pyruvate dehydrogenase kinase (PDK) intron and ACC oxidase 1 and 2 genes in the plant genome.

Effects of different rooting substrates on roots development of regenerated putative transgenic papaya shoots

Positive transgenic shoots that reached 4 cm in height were first cultured on full-strength MS medium supplemented with 9.8 µM IBA for 4 days before they were transferred into different sterile rooting materials consisting of either vermiculite or perlite. The rooting materials were supplemented with either half-strength MS medium or just water, and left for a month. For half-strength MS medium only sterile condition was applied due to contamination of cultured plants if transferred under non-sterile condition. For treatment in water medium, two different culture conditions were applied: sterile and non-sterile conditions. Sterile condition was where all the transferring process was carried out in a laminar flow hood in which the shoot was placed in a closed sterile jam jar containing vermiculite, wetted with sterile distilled water. For non-sterile condition, the shoot was placed in a sterile jam jar containing vermiculite, wetted with sterile distilled water and the mouth of the jam jar was covered with a clear plastic sheet punched with 22 holes, each hole measuring 5 mm diameter. The cultures were kept at 25±2 °C for 4 weeks under 16-hour photoperiod of white fluorescence light supplied at 25 mMol photon/m^2/s. The number and length of roots formed, and the quality of the roots were recorded after 4 weeks of culture. The experiments were repeated thrice with 20 explants used per treatment.

Acclimatization and field planting of rooted putative transgenic papaya plantlets

Rooted transgenic plantlets with finer root system, with at least 4 lateral branches and abundant root hairs were individually transferred into 9 cm x 15 cm size polybags containing vermiculite, sand and mixed soil (soil and coconut husk) at a ratio of 1:1:1. Each polybag-plantlet was then covered with a clear plastic sheet punched with 22 holes, and subsequently left in a temperature controlled growth chamber at 25±2 °C for 2 weeks. After two weeks, the plantlets were transferred into soil contained in bigger polybags measuring 14 cm x 26 cm, and grown in a temperature controlled chamber at 28 °C for 3 weeks. Following which the acclimatized plantlets were moved to the transgenic glasshouse without temperature control for further hardening.

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Histological studies on roots produced from vermiculite and perlite-grown plantlets

Two samples each of roots produced from agar-, vermiculite- and perlite-grown plantlets were subjected to histological analyses to determine the morphological differences between the types of root formed. For the histological analysis, sample fixation, processing and staining were performed at the Microscopy Laboratory, Advance Biotechnology and Breeding Centre, Malaysian Palm Oil Board.

Results and Discussions

Agrobacterium-mediated transformation of antisense and RNAi constructs

A total of 15,000 calli and 6000 calli were transformed with RNAi and antisense constructs, respectively. The PCR results showed that out of 60 putative calli transformed with antisense *ACO2* construct, 46 were positive for the presence of the *ACO2* gene. For RNAi construct, 160 out of 176 lines tested were positive for the presence of *npt*II, *gus*A, PDK intron and specific *ACO1* and *ACO2* genes. All these PCR-positive shoots were used for the rooting experiment.

Effects of different rooting substrates on root development of regenerated transgenic papaya shoots

Two different rooting media (vermiculite and perlite) supplemented in half-strength MS or just wetted with water were tested and the results were as summarized in Table 1. Overall, the results showed that plantlets transferred into rooting medium consisting of sterile half-strength MS were healthier and grew more vigorously with green leaves compared to those grown in just water. Vermiculite with half-strength MS seems to be a more suitable medium for rooting of regenerated transgenic Eksotika papaya shoots. In this treatment, 92.5% of the transferred shoots rooted and exhibited better roots quality comprising of many lateral roots and root hairs. This finding was consistent with Panjaitan et al. (2007) who reported 95% rooting efficiency of non-transgenic shoot tips of hermaphrodite field-grown Eksotika papaya with a mean number of 1.4 roots per shoot and mean root length of 2.0 cm. However, better results were obtained in this study with mean root number per shoot and average root length of 4.8 and 4.2 cm, respectively. The roots initiated were observed after 10 days of transfer with less percentage of leaves abscised. With perlite, lower percentage of rooting efficiency (77.5%) was observed. However, the roots produced were harder and compact with fewer number of lateral roots and root hairs. In this rooting material, rooting was delayed to after 15 days of transfer. Plantlets transferred under sterile condition produced healthier and greener shoots and exhibiting less leaf abscission compared to plantlets transferred under non-sterile condition.

Under non-sterile condition, most of the leaves abscised after 5 days of transfer particularly those shoots grown in perlite rooting medium. In this condition, more than 75% of the leaves turned yellowish after 10 days of transfer and leaves abscission followed subsequently, and the shoots also required longer time to produce roots. In the water medium, insufficient nutrients supply might have resulted in leaves turning yellow and abscised. More than 94% of the rooted plantlets transferred into soil mixture medium in polybags survived after 4 weeks during the hardening process in the transgenic glasshouse. After transferred into soil in the net house, more than 92% survived with healthy green leaves.

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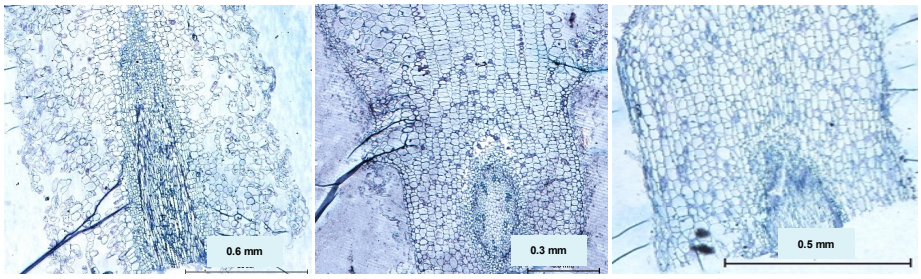
Table 1. Effects of vermiculite and perlite on roots development of regenerated transgenic papaya shoots

Rooting medium	Rooting percentage (%)	Days to visible root	Root number[1]	Root length (cm)	Leaf abscised
Vermiculite + ½ MS (Sterile)	92.5	10	4.8[a]	4.16[a]	1.2[d]
Vermiculite + water (Sterile)	50.0	23	2.1[c]	1.61[c]	4.7[a]
Perlite + ½ MS (Sterile)	77.5	15	3.1[b]	2.56[b]	3.2[c]
Perlite + water (Sterile)	47.5	22	1.2[d]	1.31[c]	5.1[a]
Vermiculite + water (Non-sterile)	35.0	25	0.9[d]	1.09[d]	4.3[b]
Perlite + water (Non-sterile)	23.8	26	0.8[d]	0.89[e]	5.2[a]

[1]*Number of primary root*
Means with the same letters are not significantly different at p<0 .05 by Duncan test.

Histological analysis of roots produced

Although both perlite and vermiculite were able to increase the transgenic papaya rooting efficiency, different root morphologies were observed. The roots produced in perlite medium were more compact with fewer lateral roots while the opposite was observed with vermiculite medium. In perlite, the cortex cells are more compactly arranged compared to vermiculite. In the agar medium, the root produced was thicker and vitrified compared to the roots produced in perlite and vermiculite. From histological analysis, the cortex cells are loosely arranged.



(a) (b) (c)

Figure 1. Histological analysis of roots of transgenic papaya plants grown in different rooting media. a: Agar, b: Vermiculite and c: Perlite

Conclusions

Based on these results, the rooting efficiency of transgenic Eksotika papaya considerably improved with good root quality and high survival rate after transfer into soil.

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Dualex 4® Reading Values as Indicator of Total Flavonoid and Chlorophyll Content of Three Varieties of *Labisia pumila* Benth. (Kacip Fatimah) Seedlings under Greenhouse Condition

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Introduction

Flavonoids have the property of being UV absorbers, and therefore screen the chlorophyll absorption. Recently, Dualex 4® uses this role of natural UV filters to identify and quantify flavonol content in the leaf (Figure 1). Results from a CNRS (National Center for Scientific Research) research team and the University of Paris Sud Orsay indicated that this new leaf-clip can simultaneously and accurately monitor the chlorophyll and flavonol content from the leaf epidermis (Cartelat et al., 2005). The optical properties of chlorophyll allow an accurate and rapid measurement by transmittance. A first wavelength very close to red quantifies the chlorophyll and a second wavelength in near infrared can take into account the effects of leaf structure. The wavelength in the UV is compared to a second wavelength in the red. Both wavelengths excite the fluorescence of chlorophyll, but only UV is affected by the presence of flavonols. The difference in chlorophyll fluorescence measured in the infrared is thus directly proportional to the amount of flavonols present in the epidermis of the leaf (Cerovic et al., 2002; Demotes et al., 2008). Being versatile, it is dedicated to plant science and agriculture research. Used both on cereals, vine or perennials, this tool is simple to use. Measurements are instantaneous and non-destructive. It requires no prior adjustment, and no sample preparation. Therefore, measurements can either be done in the laboratory or in the field, in all conditions of temperature and ambient light. This equipment has just been introduced in the market and there were no studies being conducted on the correlation between actual measurement with this equipment and laboratory analysis of total flavonoids and chlorophyll content. In this study, a comparison was made between measurement of total flavonoid and chlorophyll content using Dualex 4® and laboratory analysis to compare the relationship between Dualex 4® and laboratory analysis in the three varieties of *Labisia pumila* Benth (var. alata, pumila and lanceolata).

Materials and Methods

The experiment was carried out in growth chambers at Field 2, Faculty of Agriculture Glasshouse Complex, Universiti Putra Malaysia (longitude 101°44′N and latitude 2°58′S, 68 m above sea level) with a mean atmospheric pressure of 1.013 kPa. Three-month old *L. pumila* seedlings of var. alata, var. pumila and var. lanceolata were left for a month to acclimatize in a nursery until ready for the treatments. The seedlings were planted in soilless medium containing coco-peat, burnt paddy husk and well composted chicken manure in 5:5:1 (v/v) ratio in 25 cm diameter polyethylene bags. Day and night temperatures in the greenhouse were maintained at 27-30 °C and 18-21 °C, respectively, and relative humidity ranged from 50 to 60%. All the seedlings were irrigated using overhead mist irrigation at a frequency of four times a day or when necessary. Each irrigation session lasted for 7 min. This factorial experiment was arranged in split plot using a randomized complete block design with varieties being the main plot, and methods of analysis as the sub-plot, and replicated three times. Each treatment consisted of ten seedlings.

The method of extraction and quantification for total flavonoid contents followed after Jaafar et al. (2010). An amount of ground tissue sample (0.1 g) was extracted with 80% ethanol (10 mL) on an orbital shaker for 120 min at 50 °C. The mixture was subsequently filtered (Whatman™ No.1), and the filtrate was used for the quantification of total flavonoids. For total flavonoid determination, a sample (1 mL)

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was mixed with $NaNO_3$ (0.3 mL) in a test tube covered with aluminum foil, and left for 5 min. Then 10% $AlCl_3$ (0.3 mL) was added followed by addition of 1 M NaOH (2 mL). Later, the absorbance was measured at 510 nm using a spectrophotometer with rutin as a standard (results expressed as mg g^{-1} rutin dry sample).

Total chlorophyll content was measured by method from Ibrahim and Jaafar (2011a) by fresh weight basis. Prior to each destructive harvest, each seedling was analyzed for the leaf chlorophyll relative reading. The leaves of *L. pumila* with different greenness (yellow, light green and dark green) were selected for analysis and total leaf chlorophyll content was analyzed. For each type of leaf greenness, the relative DUALEX-4 value was recorded (five points/leaf) and the same leaves were sampled for chlorophyll content determination. Leaf disk of 3 mm in diameter was obtained from leaf sample using a hole puncher. For each seedling the measurement was conducted on the youngest fully expanded leaves on each plant. Generally, the second or third leaf from the tip of the stem was used. The leaf disks were immediately immersed in acetone (20 mL) in an aluminum foil-covered glass bottle for approximately 24 h at 0 °C until all the green colour had bleached out. Finally, the solution (3.5 mL) was transferred to be measured at absorbances of 664 and 647 nm using a spectrometer (UV-3101P, Labomed Inc., USA).

After that, the least squares regression was used to develop predictive relation between DUALEX -4 meter of flavonols and chlorophyll reading and total flavonoid and chlorophyll concentrations obtained from the laboratory analysis.

Figure 1. Dualex-4® flavonols and chlorophyll meter that was used in the study

Results and Discussion

Analysis of variance (ANOVA) showed that there were no significant differences of total chlorophyll and flavonol content between varieties of *L. pumila* and methods of measurement (Dualex 4® and Laboratory analysis). Figure 2 showed that Dualex 4® measurement and destructive cholorophyll measurement had a positive relationship (R^2= 0.987) that indicated that the Dualex 4® index for chlorophyll content can be used to estimate total chlorophyll content in *L. pumila* by using the regression coefficent (y = 0.85x - 1.54). However, the Dualex 4® flavonol content showed a positive log regression with the destructive measurement (Figure 3; y = Ln 1.11x + 1.31; R^2 = 0.973). Both measurements showed that there was a possibility to use both equations to estimate total chlorophyll content and total flavonoid content in leaves of *L. pumila* without conducting the destructive analysis in the Laboratory. This information was beneficial for early identification of the phyto-medicinal properties of *L. pumila*, especially total flavonoid content that describes the phyto-estrogenic properties of this plant (Ibrahim and Jaafar, 2011b).

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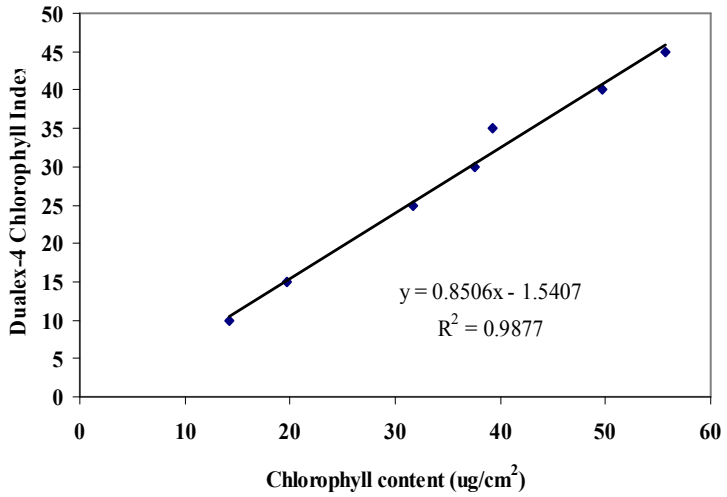

Figure 2. The relationship between Dualex 4® chlorophyll index with destructive total chlorophyll content analysis

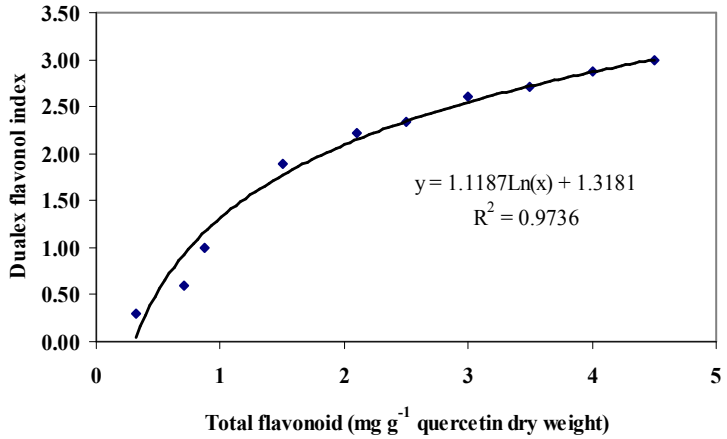

Figure 3. The relationship between Dualex 4 ®flavonol index with destructive total flavonol content analysis

Conclusions

The results indicate that Dualex 4® can be used as total chlorophyll and total flavonoid content indicator in *L. pumila* plants. The regression analysis can be used to determine actual total chlorophyll and flavonoid content in the plants and showed a possible potential for fast chlorophyll and flavonoid analysis of *L. pumila* plants.

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Flowering Enhancement of Chok Anan Mango through Application of Potassium Nitrate

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Introduction

Total world mango *(Mangifera indica*) production has reached over 30 million tones in 2009 making mango one of the five most important fruit species worldwide together with banana, orange, grape and apple. This is followed by other countries such as China, Thailand, Indonesia, Pakistan, Mexico, Brazil and Bangladesh with India, being the largest producer, accounting for more than three-fifth (with over 13 million tonnes) of world production (FAOSTAT, 2009).

The popular mango clones planted in Malaysia are Harumanis (MA128), Chok Anan (MA 224), Masmuda (MA 204), Sala, Siam Panjang (MA 205), Nam Dorkmai (MA 223) and Laris (MA 154). However, mango growers in Malaysia face a problem of inconsistency in fruit production mainly due to inconsistency flowering behavior. The mango production in Malaysia is generally better at area in the northern region such as Perlis, Kedah and Northern of Perak, which has a distinct dry period and becomes very suitable for mango tree to initiate flowering (Rukayah, 1989).

Good flowering is necessary to obtain high fruit set and yield. Flowering is unreliable because of the environmental signals for flower initiation is often inconsistent, subtle or poorly defined. Knowledge on the factors that control the flower initiation in mango trees is very limited. Although chemical inducing substances have been tested for promoting/inhibiting flower production in mango in different countries, their effects have been limited to certain variety and geographical location. Therefore, the chemical inducing substances and application rates that can promote or induce flowering were tested under local conditions for the locally available mango varieties. Hence, the aim of this study was to determine the influence of different application rates of chemical inducing substance, potassium nitrate (KNO_3), on enhancement of flowering in mango clone "Chok Anan" (MA 224) and ultimately, the fruit production.

Materials and Methods

A field experiment was conducted at Ladang Pertanian 2, UPM Serdang, Selangor. The mango trees clone "Chok Anan" (MA 224), aged about twelve months and five-year-old, were used as test materials. The plants were subjected to three treatments of spray application with 1% KNO_3, 2% KNO_3, 5% KNO_3 onto mango shoots at two weeks intervals until flower initiation. Sapol was used in the spray solution as wetting agent. The experiment was arranged in a Randomized Complete Block Design (RCBD). Treatments were started in early May 2011.

The parameters recorded were panicle length (cm), flowering intensity, and fruit number. Flowering intensity in terms of the percentage of shoots that flowered on canopy was assessed on selected branches. Panicle length was measured after full bloom with a few panicles taken randomly. Number of fruits produced per tree was recorded. Then, the collected data were analyzed by using SPSS Statistical Package Version 16.

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Results and Discussion

In this experiment, flowering was evident in five-year-old mango tree within 4th weeks after spraying with 2% (on 23 May 2011) and 5% KNO_3 (on 28 May 2011), while treatment with 1% of KNO_3 (on 16 Jun 2011) gave late response of flowering initiation. Trees were highly responsive with 2% KNO_3 where over 60% of the shoots flowered on the canopy (Figure 1). However, there was slight decrease in panicle length when high rate of KNO_3 was applied (Table 1). On the other hand, more fruit set was formed with 2% and 5% KNO_3 application, thus increased the number of fruits produced (Figure 2).

Young mango tree treated with 1% KNO_3 initiated little flowering with vegetative flushes (Figure 3) at five weeks after treatment. Flowering induction was not observed on terminal bud treated with 2% and 5% KNO_3, only new leaf flushes were formed at two months after treatment (Figure 4). Due to the prevailing humid rainy weather conditions, most of the flowers dropped as a result of severe anthracnose and this brought to very low fruit set (Table 2).

Table 1. Effect of KNO_3 sprays application on panicle length.

Treatment	Average panicle length (cm)
Control (12 months)	No flower produced
*KNO3 (12 months)	
1%	18
2%	Formed new leaves flushes
5%	Formed new leaves flushes
Control (5 year-old)	23.64
*KNO3 (5 year-old)	
1%	28.6
2%	33
5%	24.57
* Foliar spray at every 2 weeks intervals.	

Table 2. Effect of KNO_3 sprays application on flowering intensity and fruit number

Treatment	Average percentage of inflorescence/ tree	Average no of fruit/ tree
Control (12 months)	No flower produced	No fruit produced
*KNO_3 (12 months		
1%	14.29%	10
2%	No flower produced	No fruit produced
5%	No flower produced	No fruit produced
Control (5 year-old)	28.98%	20
*KNO_3 (5 year-old))		
1%*	31.75%	25
2%	80.00%	64
5%	70.21%	47
* Foliar spray at every 2 weeks intervals		

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Figure 1. Flowering initiation in 5 year-old tree treated with 2% KNO_3

Figure 2. Fruit formation

Figure 3. Flowering initiation in young mango tree treated with 1% KNO_3.

Figure 4. Formation of new leaves in young mango tree treated with 2% and 5% KNO_3.

"Juvenility" refers to the period between planting and first flowering. Juvenile trees attain "Phase changes" before flowering and still undergo transition period from young to mature tree. In this study, young mango tree size was smaller and had shorter shoots. The tree height was about 160 cm and tree circumference was 18 cm. Their leaves were retained at the last 2-3 internodes. Early flowering induction on young shoots may result in leaf flush instead or no flowering response. Young trees responded occasionally and were limited to certain period of induction. The shoots also only responded to flowering induction at lower concentration of KNO_3 due to its capability to uptake the molecules that may be involved in the plant metabolism. Astudillo and Bondad (1978) found that the results for KNO_3 sprays were influenced by the physiological age since aged vegetative flushes (5-8 months old) responded better to KNO_3 application than young flushes.

The entire fruit industry in Malaysia is relatively small and unable to fulfill consumer demand. As a consequence, Malaysia imports substantial quantity of fruits. KNO_3 is one of chemical inducing substances which have shown some potential for inducing flowering of mango beside enhances fruit production. It was first reported to induce off season mango flowering (Barba, 1974). The use of KNO_3 was spread to the other countries like India (Pal et al., 1979), Mexico (Nunez-elisea, 1986), Unites States of America (Hawaii) and also Malaysia (Omran, 1999).

The mechanism of action of KNO_3 involves a biochemical process where the reduction of nitrate to ammonia. Ammonia is used in the nitrogen metabolism of plants to form amino acids and one of them is methionine. Methionine is converted to S- adenosylmethionine (SAM) and then to 1-aminocyclo-propane- 1- carboxylic acid (ACC), and finally converted to ethylene. Ethylene might be an important second messenger in plant development and also capable of promoting flowering (Burg and Burg, 1966).

Some previous studies suggested that KNO_3 may be a stimulus for flower initiation. KNO_3 does not induce flowering but helped in sensititizing buds to the floral stimulus when KNO_3 is sprayed to the terminal bud of mango shoots. (Kulkarni, 1988) stated that the floral stimulus was already present in shoots at the time that buds were forced to response to KNO_3. Lauchli et al. (2006) explained KNO_3 might be involved in inductive process where active component of KNO_3 might transform shoots from vegetative phase (dormant terminal bud) to reproductive phase (axillary spearshaped protruberances), then grow into panicles and primary branches of panicles, and lastly bear flowers.

Flowering response to KNO_3 spray is dependent on tree condition, shoot age, environmental condition and time of application, which may influence the endogenous GA levels (possible flowering inhibitor) in the shoot tips**.** Flower initiation in mango is inhibited or delayed at high GA level and flowering will ensue if GA is absent or present at low levels.

Conclusions

It could be concluded that the age of tree and shoot maturity may influence the flowering response to KNO_3 application. Treatment with 2% KNO_3 spray was identified to be the best rate of application to induce early flowering in five-year-old mango tree clone “Chok Anan” (MA 224). More detailed experiments are required to explore the mode of action of KNO_3 and the mechanism for its effect during flowering induction and this may increase the income of mango growers.

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Physiological and Mechanical Properties of Selected Plants for Slope Protection

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Introduction

The use of vegetation in slope bioengineering application has increasingly accepted as a cost effective method and proven to solve the instability problem of natural and man-made slopes (Petrone and Preti, 2010). However, vegetation establishment are limited to certain factors such as geography and local climate (Burylo et al., 2007; Florineth et al., 2002). Other than that, different species have different reinforcing trend based on the properties of the species. Plant root, the most important part that provides reinforcement varied between species. Root architecture and root profiles are the main aspects that become interest by researchers to understand how vegetation provides reinforcement (Fan and Chen, 2010). Thus, species selection is crucial to ensure the success of this method. In line with the important of species selection, a series of sites and laboratory studies were conducted to assess the capability of local species to fulfill the needs of slope bioengineering in Malaysia.

These series of test carried out at both field and in laboratory were aimed to evaluate the pull out, tensile and shear strength of the selected species. Hence, it was also aimed to observe the relationship between the plant root architecture and profiles towards the reinforcing trend.

Materials and Methods

Plant materials

Two tropical species namely *Acacia mangium* and *Leucaena leucocephala* were chosen. Site selected was located within University of Malaya campus (3° 07' 51" N and 101° 39' 25.9" E) with abundant numbers of these species grown naturally on flat base land.

Test methods

a) *Pull-out Test*. The total of nine samples for each species was chosen, three for each stem diameter treatment (0-20 mm, 20-40 mm and 40-60 mm). Prior to the equipments set up, surrounding ground was cleared. Plant physical properties were measured before the stem was cut into 10 cm from the root crown.
b) *Shear box Test*. A customized shear box was fabricated to suit the site test. Soil contained the roots were cut to fit the steel made shear box size of 300 mm x 300 mm x 160 mm. The soil blocks were applied with normal load of 13.3 kPa and shearing rate at 1.5 mm/minute.
c) *Tensile Test.* Test were carried out in the laboratory with total of 21 and 36 root segments of *L. leucocephala* and *A. mangium* root segments were cleaned, washed and cut into 200 mm in length. Test was conducted using Universal Testing Machine, attributed to the tap and lateral roots breakage, respectively (Instron, Model 5582, United Kingdom).

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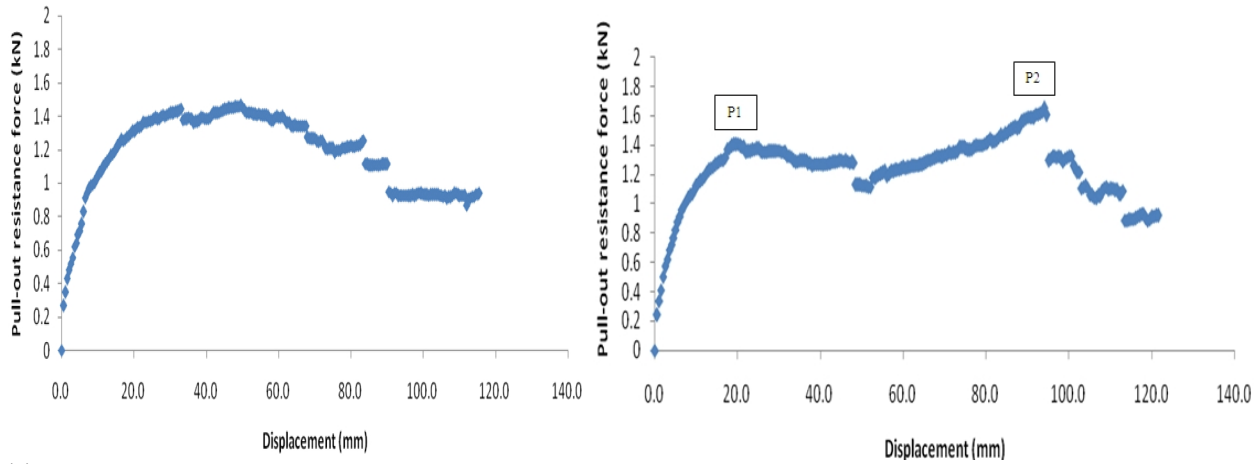
Results and Discussions

The results from pull-out test indicated that, for *A. mangium* the resistance increased with displacement before falling down when the maximum force was achieved (Figure 1a). However, a slight different trend was observed for *L. leucocephala* (Figure 1b). Two peak values were observed, P1 and P2. In both species, the pull-out resistance was contributed by the lateral roots. *L. leucocephala* had a taproot that penetrates deep to the soil as compared to *A. mangium*. It was observed that *L. leucocephala* had a rooting depth of 37.02 ± 3.57 cm which was deeper than the rooting depth of *A. mangium*, 26.09 ± 4.54 cm. This is the features of the VH-type roots (Figure 2). The lateral and the tap root provide greater reinforcement towards the soil.

The shear box test indicated that, the shear strength also increased with displacement and in stem diameter. Both species shows the same trend, increased in displacement before falling after the failure of roots (Figure 3). *A. mangium* had the highest value of shear strength as compared to *L. leucocephala*. Fan and Chen (2010) discovered that the VH-type root gave greater reinforcement. It was also found that H-type root had less reinforcement impact in shearing. In contrast, different result was occurred in this field test. *A. mangium* with H-type root have greater shearing resistance compared to, *L. leucocephala* with VH-type root. The contrast results may be due to the varying properties of soil including soil type and water holding capacity.

In terms of cohesion factor, *L. leucocephala* had the highest as compared to the control and *A. mangium* (Figure 4a). Cohesion is important in erosion control especially on top surface where soils are exposed to erosion agents such as wind and rainfall. The plant roots hold the soil particles, lessen the movement and thus reduce soil particles detachment (Huat et al., 2005). Nilaweera and Nutalaya (1999) also carried out a similar pull-out and tensile test. However, they did not incorporate the pull-out and tensile strength. While the result of relationship analysis between these pull-out and tensile strength showed positive results (Figure 4b), where R^2=0.77.



(a) (b)

Figure 1. Average pull-out resistance force-displacement curve for a pull-out test on five replicates of *A. mangium* (a) and *L. leucocephala* (b).

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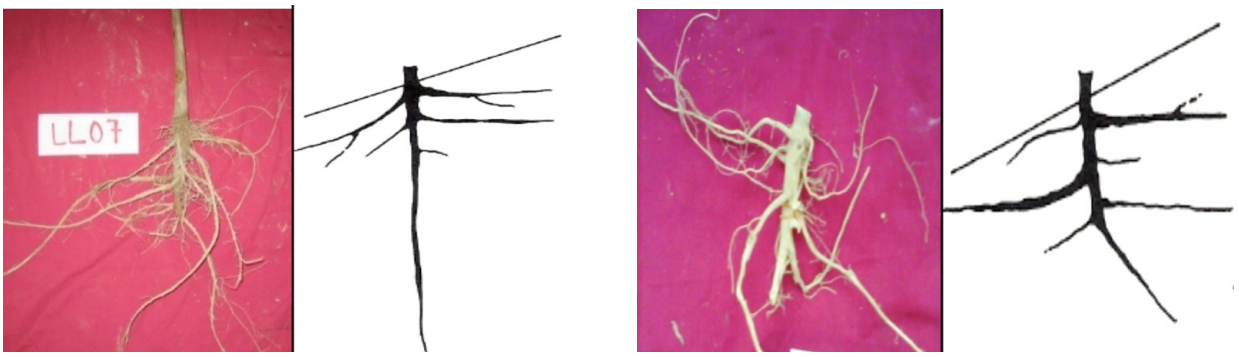
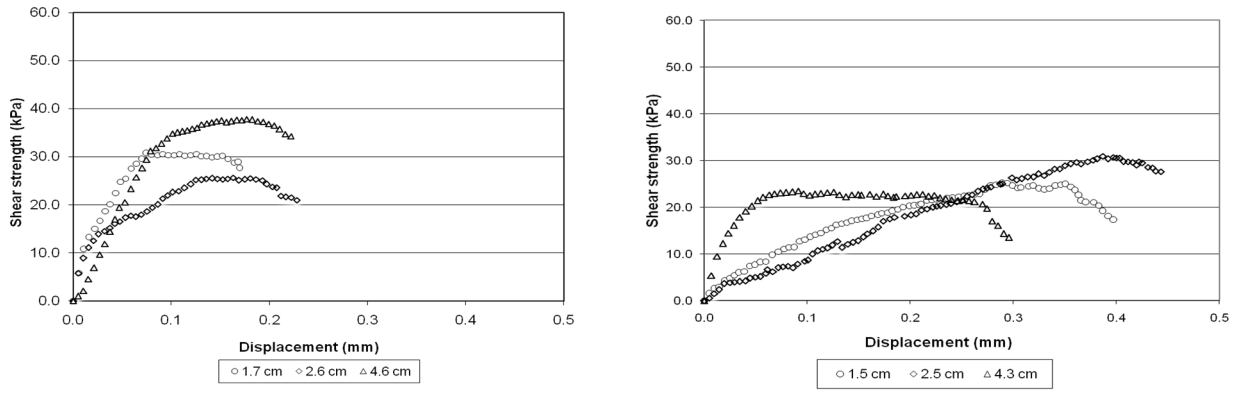


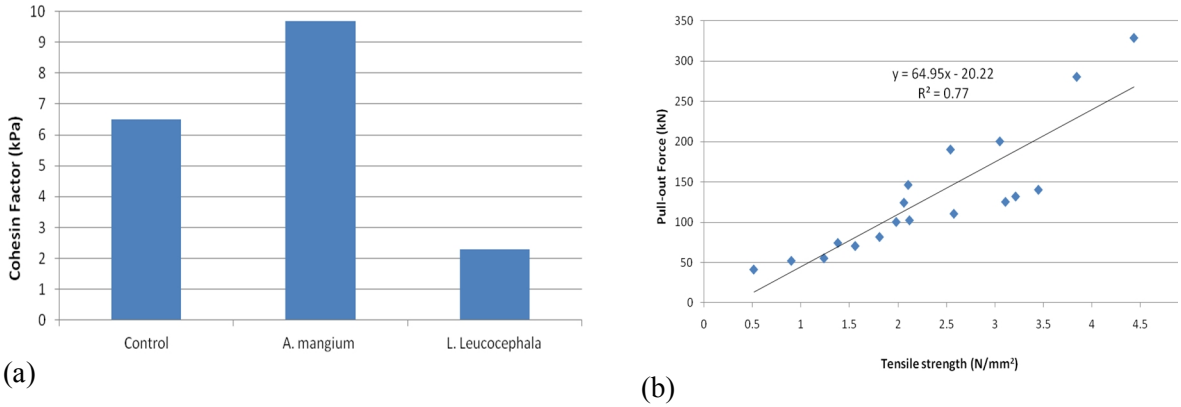
(a) (b)

Figure 2. Root growth pattern of *L. leucocephala* (a) and *A. mangium* (b). (after Yen, 1972).



(a) (b)

Figure 3. Shear strength of *A. mangium* (a) and *L. leucocephala* (b) versus displacement with different stem diameter at 13.3 kPa load.



(a) (b)

Figure 4. Cohesion Factor of species studied (a) and Relationship between pull-out forces versus root tensile strength (b).

Conclusions

In conclusions, the shear strength of the species studied was gradually increased with increasing in stem diameter. The pull-out test shows that it was much affected by the root properties. The root tensile strength determines the pull-out resistances of each species. It is emphasized that root properties are the main part that influences the reinforcing trend of each species studied.

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Analyses of Some Phenolic and Flavonoid Compounds using HPLC in Microwave Obtained Extracts of Three Varieties of *Labisia pumila* Benth.

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Introduction

Extraction using microwaves is a new extraction method for scientists in order to study the biological activities of medicinal plant. This extraction method results in an increased yield in shorter time and at the same temperature using less solvent compared to other extraction method. For microwave extraction, the choice of physical parameters is very important which include solubility, dielectric constant, and the dissipation factor. Most of the higher plants have been used in traditional medicine for a long time (Proestos and Komaitis, 2007) and many of these plant species have been used as drugs throughout the world. Plant secondary metabolites are important sources of various fine chemicals (phytochemicals) that are used directly or as intermediates for the production of pharmaceuticals (Balandrin et al., 1985). Plants provide us with rich sources of natural antioxidants. Nutrients with antioxidant properties such as phenolics, flavonoids, carotenoids as well as vitamins C and E and selenium, and possibly other nutrients and food components help to protect the proteins, lipids, and DNA in cells from damage by oxygen. Flavonoids and phenolics are the most diverse groups of phytochemicals that protect the body against reactive oxygen species. Free radicals and reactive oxygen can damage and ruin the body cells and tissue structure, which are produced during normal oxygen metabolism or are induced by exogenous damage (Galindo et al., 2010).

Labisia pumila Benth. (Myrsinaceae family) locally known in Malaysia as Kacip Fatimah is a woody, small sub herbaceous plant with creeping stems (Jaafar et al., 2008). Stone (1998) had categorized three varieties of this herb in Malaysia, namely *L. pumila* var. alata, *L. pumila* var. pumila and *L. pumila* var. lanceolata. Each of these varieties has different uses. Recently, it was reported that the bioactive compounds of *L. pumila* consisted mainly of resorcinols, flavonoids and phenolic acids (Ibrahim et al., 2010). These compounds have been implicated as natural antioxidants, which can safely interact with free radicals and terminate their chain reactions before vital molecules could be damaged. This research was performed to investigate the accumulation of bioactive compounds such as flavonoids, isoflavonoids and phenolics in the leaves of three varieties of *L. pumila* using extracts obtained by microwave-assisted extraction.

Methods and Materials

Plant materials

Seedlings of *L. pumila* varieties of alata and pumila were collected from Kota Tinggi, Johore, and raised under glasshouse for 18 months. Leave parts of plant were separated, frozen dried and kept for further analysis.

Plant extraction

Samples were extracted using methanol as a solvent and the extraction techniques used microwave method (Xiao et al. 2008) with slight modification.

Determination of total phenolic compound

Total phenolic content of the extract was determined colorimetrically using the Folin-Ciocalteu method as illustrated by Ismail et al. (2010). The extract was measured at absorbance 765 nm and the result expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter.

Determination of total flavonoid compound

Total flavonoid content was determined using standard flavonoid rutin as described by Ismail et al. (2010). The extract was measured using absorbance at 510 nm and the result was expressed as milligrams of rutin equivalents per gram of dry matter.

Analyses of phenolic and flavonoid compounds by RP-HPLC

The phenolic and flavonoid compounds of leaf, stem and root of three varieties of *L. pumila* were quantitatively measured by reversed-phase HPLC (Crozier et al., 1997) with some modification. Phenolic standards were gallic acid, pyrogallol, syringic acid, vanillic acid, salicylic acid and caffeic acid. Flavonoid standards were quercetin, rutin, myricetin, kaempferol, naringin and apigenin.

Results and Discussion

Phenolic and flavonoid compounds, as important phytochemicals, are present in vegetables, fruits and cereal grains. These secondary metabolites are natural antioxidants that have multiple biological effects and play an important role in the defense against cardiovascular disease, aging and cancer (Karimi et al., 2010). The overall results demonstrated that *L. pumila* var. pumila leaves had a higher total flavonoids content (2.77 mg rutin equivalent/g DW) than var. alata leaves (2.49 mg rutin equivalent/g DW), but the leaves of var. *alata* contained higher total phenolics (3.48 mg gallic acid equivalent/g DW) than var. pumila (3.37 mg gallic acid equivalent/g DW) .The HPLC analysis results also indicated that *L. pumila* var. pumila contained various types of flavonoids such as quercetin which had not been observed in *L. pumila* var. alata, instead phenolic pyrogallol was detected in L. pumila var. alata. Apigenin, kaempferol, rutin and myricetin were the main flavonoid compounds presented in the two varieties. This research also revealed that gallic acid and caffeic acid were the major phenolic compounds in all leaves extracts of *L. pumila* var. alata and pumila.

Conclusions

Microwave extraction method was used to extract the flavonoids and phenolics compound from the leaves of three varieties of *L. pumila* Benth. The results demonstrate that *L. pumila* extracts contain variable patterns of flavonoids and phenolic compounds. Plant secondary metabolites are far more restricted than plant primary metabolites and often accumulate in small quantities (Balandrin et al., 1985). They are known as flavonoids, phenolic compounds, essential oils, curcuminoids and others. They posses anti-oxidant, anti-inflammatory, anti-aging and a lot more functions (Bernal et al., 2011). The overall result obtained from this research suggests that all varieties of *L. pumila* Benth. are sources of bioactive compounds.

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Improvements in Oil Palm Liquid Culture System

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Introduction

The production of oil palm suspension cultures using the individual shake flask system has been established (Wong et al., 1999; Tarmizi et al., 1999; Tarmizi, 2002). The protocols were developed to produce a reliable supply of regenerable plant tissues. The liquid culture system was also developed to address the inefficiency issues in micropropagation. In addition to the high production cost, a large number of culture vessels are required for propagating liquid cultures using the shake flask system (Takayama and Akita, 2005). Therefore, there is a need to improve the efficiency of the liquid culture system. To address these issues, various innovative technologies have been developed to further improve the efficiency of the liquid culture system.

Materials and Methods

MPOB Fast Transfer Technique (MoFaTT) in liquid culture system

Approximately 0.5 g of embryogenic suspension cultures was inoculated in 20 ml MS medium in the MoFaTT system. For comparison, suspensions from the same cultures were transferred into individual 100 ml flasks with an inoculation of 0.5 g per 20 ml of the same medium and both systems were incubated in darkness at 100 rpm on an orbital shaker.

Two-in-One MPOB Simple Impeller (2-in-1 MoSLIM) and Simple Impeller with Fast Transfer Technique (SLIM-FaTT) in liquid culture system

Approximately 1.6 g embryogenic suspension cultures were inoculated into 400 ml MS medium (Murashige and Skoog) in both the 2-in-1 MoSLIM and SLIM-FaTT systems and were incubated in darkness. For controls, suspensions from the same cultures were transferred to individual 100 ml flasks with an inoculation of 0.5 g per 20 ml of the same medium and incubated in darkness at 100 rpm on an orbital shaker.

MPOB Modified Vessel (MPOB-MoVess) for liquid culture system

This vessel consists of an agitation shaft, a magnetic stirring bar, a plastic impeller for agitation and perforated tubing for aeration process in liquid culture system. The whole vessel is then placed on a magnetic stirrer for agitation. Approximately 4 g of embryogenic suspension cultures were inoculated in 1.8 L MS medium in the MoVess.

MPOB Motorized Vessel (MPOB-MotoVess) for liquid culture system

This vessel contains an agitation shaft and an impeller for agitation, and perforated tubing for aeration in the liquid cultures. The impeller was driven by a special motor. Approximately 5.5 g of embryogenic suspension cultures were inoculated in 4.3 L MS medium in the MotoVess.

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Results and Discussion

MPOB Fast Transfer Technique (MoFaTT) in liquid culture

This is a rapid and convenient method for liquid medium replenishment during maintenance and maturation of oil palm suspension cultures (Figure 1). The cultures can be maintained for a few months (e.g. four months) with medium replenishment done on the shaker at any desired interval, e.g. monthly. The fresh weight increment is better or comparable with the normal performance of the cultures in the individual shake flask system. The improvement observed using MoFaTT compared to shake flask system was 3 to 10-fold. The cultures regenerated normally upon transfer to solid medium.

The advantages of using MoFaTT are that: i) it simplifies medium replenishment from 10 steps (in the shake flask system) to only two steps; ii) no movement of cultures from culture room to laminar flow required; iii) replenishment could be done on-site or on the shaker itself; iv) it reduces the risk of contamination due to less movement and handling; and v) it has the potential for automation upgrade (Tarmizi and Zaiton, 2004).

Figure 1. One possible arrangement in MoFaTT

Two-in-One MPOB Simple Impeller (2-in-1 MoSLIM) in liquid culture

This economical system uses commonly available culture bottles in the laboratory for liquid culture instead of using bioreactors or special commercial flasks (Tarmizi, 2002; Tarmizi et al., 2003). It also provides simultaneous aeration and agitation (2-in-1) in the form of a single device for liquid culture propagation (Figure 2). This new vessel could produce culture aggregates with fresh weight increments of two- to six-fold over a 30- to 40-day period.

Figure 2. Two-in-One MPOB Simple Impeller (2-in-1 MoSLIM)

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Simple Impeller with Fast Transfer Technique (SLIM-FaTT) in liquid culture

The synergistic integration of MoSLIM and MoFaTT resulted in the development of the SLIM-FaTT. Experimental observations revealed that oil palm cultures could be multiplied more effectively in the SLIM-FaTT system. The cultures could be maintained for three to four months with the medium replenishment performed in the culture room at any desired time interval, e.g. monthly. A fresh weight increment of about three- to 16-fold was obtained after about four months maintenace in the system. The fresh weight increment varied among the clones tested. Clone PL 139 in this study showed the highest fresh weight increment of about 16-fold. Cultures regenerated normally upon transfer to solid media. The advantages of the SLIM-FaTT are similar to the MoFaTT system with the added advantage that higher culture volumes are applicable in the SLIM-FaTT.

MPOB Modified Vessel (MoVess) for liquid tissue culture system

This modified vessel was designed mainly to reduce cost and time during culture proliferation in bioreactors. Propagation experiments of three clones using this vessel demonstrated a fresh weight increment in cultures of five- to 35-fold was obtained after 30 to 60 days. The advantages of using the MoVess system are that: i) it is a simple and economical procedure to scale up the culture volume; ii) it uses a simple inoculation method; iii) there is no need to move the cultures as media could be replenished on-site and this reduces the contamination risk; iv) it could be applied to liquid cultures of other crops or even animal cultures; and v) it has the potential for automation.

MPOB Motorized Vessel (MPOB-MotoVess) for liquid tissue culture

MotoVess is an improved version of the MoVess. This system omits the use of the magnetic stirrer used in the MoVess. The magnetic stirrer limits the culturing of volumes to 1 to 2 L and the omission will thus allow culturing of larger volumes between 2 to 9 L (Figure 3). For agitation, the MotoVess consists of a motor with a stand and a shaft with impeller, made of perforated stainless steel to aerate the medium as well. Data recorded demonstrated that a two- to six-fold increment in fresh weight cultures could be obtained after 40 days using the MotoVess.

Figure 3. MPOB Motorized Vessel (MPOB-MotoVess).

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Conclusions

The basic protocol using the shake flask system has been established and the bioreactor technique was also previously developed to further improve the liquid culture system. MoFaTT, which was developed earlier, was a rapid and convenient method for liquid media replenishment during maintenance and maturation of cultures in the shake flask system. However, this technique could only be conducted with the shaker system at the time. The 2-in-1 MoSLIM was then developed as an alternative to this shake flask system whereby the cultures could be maintained on a magnetic stirrer rather than a shaker. Subsequently, the SLIM-FaTT in Liquid Culture System was then developed as a rapid and convenient method for liquid media maintenance and maturation of cultures in the bottle (MoSLIM) system together with efficient medium replenishment. This further improves the overall efficiency of the liquid culture system. The system can also be applied to any fluidic system. Various experimental approaches could be designed using the SLIM-FaTT system, such as intermittent medium replenishment and on-site application of various exogenous treatments to cultures. The more recently developed MoVess and MotoVess systems could be used for a larger scale production of cell aggregates and their performance was found to be comparable with commercial bioreactors of the same working volume. Furthermore, all technologies developed so far can be exploited for further enhancements through updating to a semi or fully-automated process for oil palm clonal production.

Experiments are in progress to establish more clones in the shake flask, bioreactor, MoFaTT, MoSLIM, SLIM-FaTT, MoVess and MotoVess systems to monitor their regeneration capability on solid culture media. These cultures would then be established in the soil for further evaluation of their clonal fidelity. The protocols for these systems will be further optimized.

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Antioxidant and Free Radical Scavenging Properties of Oil Palm *Elaeis guineensis* Cell Suspension Culture

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Introduction

Recently, much attention has been focused on plant rich in antioxidant, because of their functions in reducing oxidative damage and preserving biological functions of cells. Plant antioxidants are composed of a broad variety of different substances like ascorbic acid, tocopherols, polyphenolic compounds and terpenoids. Antioxidants help to neutralize free radicals which are unstable molecules that are linked to the development of a number of degenerative diseases and conditions including cancer, cardiovascular disease, cognitive impairment and immune dysfunction (D'Abrosca et al., 2007). A number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimutagenic, anticarcinogenic, anti-inflammotory, antimicrobial and other biological properties (Robbins, 2003). The antioxidant activities of plant phenolics are mainly due to their redox properties which allow them to act as reducing agents and hydrogen donors (Huda-Faujan et al., 2009).

Oil palm has been known as the most productive oil-producing tree in many parts of tropical country in the world. Apart from its oil, there are several beneficial uses that oil palm or *Elaeis guineensis* can produce. Studies have shown that there are carotenes, coenzyme Q10, water soluable antioxidants and phenolic compounds found in palm oil fruit as well as leaves which exhibit beneficial properties such as antioxidative and anticancer (Sundram et al., 2003; Han and May, 2010). Nevertheless, there are no studies on these activities that had been done on cell suspension culture of oil palm. Thus, this research is to determine the antioxidants of the oil palm cell suspension culture grown by tissue culture technique.

The search for new uses of oil palm derived biotechnology currently has been a priority towards complete utilization of oil palm (Sasidharan et al., 2010). Plant tissue culture has played a vital role in search of alternatives to production of desirable compounds from plants (Rao and Ravishankar, 2002). In this study, two types of cell suspension culture clones were screened for their total phenolic and total flavonoid contents. The radical scavenging activity of cell suspension culture clones was also analyzed.

Materials and Methods

Sample collection

Oil palm calli were collected from MPOB tissue culture laboratory, Bangi. There are two types of clone namely clones R169 and R160. All procedures in the laboratory were carried out under aseptic conditions under laminar flow.

Initiation of cell suspension and maintenance

Suspension cultures were initiated by transferring 1 g callus in 50 ml medium of the same composition except agar was used for callus culture. The calli were inoculated into flasks with a liquid medium which

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were then placed on orbital shaker at 100 rpm in the dark. The oil palm cell suspension cultures were subcultured every 1 month period to ensure fresh medium.

Preparation for the extract

The oil palm cell suspension cultures were oven dried at 50 °C for 48 hours and ground using mortar and pestle. Dried sample (approximately 100 g) was added to methanol (300 mL) and soaked for 4 days at room temperature (30 ± 2 °C). The cell suspension was stirred from time to time to allow the leaf powder to fully dissolve in the methanol. Removal of the sample from the solvents was done by filtration through cheesecloth followed by filter paper (Whatman No. 1). The filtrate was concentrated under vacuum to one-fifth its volume using a rotary evaporator at 60 °C.

Total flavonoid content (TFC)

Total flavonoid was measured with an aluminum chloride colorimetric assay where catechin was used as a standard solution developed by Dewanto et al. (2002) with some modifications. Stock solution of catechin was prepared at 1000 µg/mL. A 1 mL aliquot of appropriately diluted standard solution of catechin (0, 200, 400, 600, 800 and 1000 µg/mL) was prepared. Five percent $NaNO_2$ at 0.3 mL and 0.3 mL 10% $AlCl_3$ were added to the flask. At the sixth minute, 2 mL 1 M NaOH was added to the mixture. Absorbance of the pink colour mixture was determined at 510 nm using UV-Vis spectrophotometer. Total flavonoid of extract was expressed on fresh weight basis as mg/g catechin equivalents (CE). Samples were analyzed in three replications.

Total phenolic content (TPC)

Total phenol was determined according to the method by Singleton and Rossi (1965) with some modifications. The total phenolic content of plant extracts was determined using Folin-Ciocalteu's reagent (FCR) and calculated using gallic acid calibration curve as a stardard. Stock solution of gallic acid was prepared at 0.1 mg/mL. About 0.1 mL of diluted extract or standard solution (0, 0.5, 1.0, 2.0, 3.0 and 5.0 µg/mL) was prepared and 20% Na_2CO_3 was added to the mixture. The mixture was shaken thoroughly and made up to 10 mL using distilled water. The absorbance at 750 nm was determined using UV-Vis spectrophotometer. The absorbance of extracts was compared to gallic acid calibration curves. The total phenolic content of each extract was expressed on fresh weight basis as mg/g gallic acid equivalents (GAE). All determinations were carried out in triplicates.

Radical scavenging activity by DPPH

The DPPH (2,2-diphenyl-2-picrylhydrazyl) assay with some modification was performed. The stock solution of crude extracts or standard solution of quercetin was prepared as 1 mg/mL in methanol. The solutions were diluted to different concentrations (7.82, 15.63, 31.25, 62.5, 125, 250 and 500 µg/mL in methanol) in a 96-well microliter plate. Then, 5 µL of DPPH solution (prepared as 2.5 mg/mL in methanol) was added to each well. A blank solution that served as control was prepared containing the same amount of methanol and DPPH. The plate was shaken gently and placed in the dark for 30 minutes at 37 ^{o}C. The absorbance was measured at 515 nm using microplate reader. The experiment was performed in triplicates and the graph was plotted with the means values. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ Radical scavenging activity} = 1 - [A_{sample} / A_{control}] \times 100$$

whereby A_{sample} and $A_{control}$ are absorbances of sample and control. Decreasing DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity.

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Statistical analysis

The experimental results were expressed as mean ± standard deviation (SD) of triplicate measurements. The results were processed using Microsoft Excel 2003.

Results and Discussion

The total flavonoid and phenolic contents of the cell suspension culture clone were measured. The antioxidative effects are mainly due to phenolic components such as flavonoids (phenolic acids and phenolic diterpenes (Ozsoy et al., 2008). The majority of the free phenolic are flavanols while the bound phenols are phenolic acids. Several hydrolytic procedures are used to quantify the phenols. Bonoli et al. (2004) indicated that different groups of phenolic compounds can be quantified by measuring absorbance at different wavelengths.

Total flavonoid content (TFC)

Aluminum chloride colorimetric is an assay specificity to explore typical structure of flavonoids. The flavonoids content were determined by extrapolation of catechin calibration curve ($y = 0.002x – 0.027$, $R^2 = 0.989$) and expressed in milligrams of catechin (CE)/gram. The total flavonoid content of cell suspension culture clone R160 was 28.0± 0.24 mg CE/100g of sample while clone R69 only showed 22.0±0.4mg CE/100g of sample .

Table 1. Total flavonoid content of samples expressed in mg CE/g and phenolic content of samples expressed in mg GAE/g

Clone	Total flavonoid Content (CE/g)	Total phenolic content (mg GAE/g)
R160	28.0± 0.24	52.3±0.34
R169	22.0±0.4	32.5±0.21

Note: Values are expressed in means ± SD (n=3)

Total phenolic content (TPC)

TPC of the oil palm cell suspension culture clone were determined by extrapolation of gallic acid calibration curve ($y = 0.086x - 0.010$, $R^2 = 0.986$) and expressed in milligrams of gallic acid (GAE)/gram. The total phenolic content of the oil palm cell suspension culture clone varied where clone R160 showed total phenolic content of 52.3±0.34 mg GAE/100g of sample and clone R169 showed 32.5±0.21 mg GAE/100g of sample. The Folin Ciocalteau procedure has been proposed to rapidly estimate the level of total phenolics in food and supplement (Prior et al., 2005). The Folin Ciocalteau method is based on the reduction of the reagent where the product of reduction exhibits a blue colour with maximum absoption at 765 nm (Singleton and Rossi, 1965). Thus, TPC could be used as an indicator of the amount of the total phenolic compounds present in oil palm cell suspension culture clone.

Radical scavenging activity by DPPH

In this study, the radical scavenging activity of the cell suspension cultures were estimated using DPPH radical assay. This method depends on the reduction of the purple DPPH by accepting electron or hydrogen radical from antioxidant (A–H) to a stable diamagnetic molecule (yellow-coloured diphenyl picrylhydrazine).

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$$\underset{\text{(purple colour)}}{DPPH\cdot + A\text{-}H} \longrightarrow \underset{\text{(yellow colour)}}{DPPH\text{-}H + A\cdot}$$

The degree of discoloration indicated the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (Blois,1958).

The results obtained from the aqueous extracts of cell suspension were compared with quercetin as standard reference. The reduction in DPPH radical was determined by the decrease of its absorbance at 515 nm (in methanol) induced by antioxidants. Cell suspension culture clone R160 has decreased the DPPH solution absorbance by half (50% radical scavenging activity) at lower concentration (250 µg/mL) than clone R169 which was at 320 µg/mL. Based on the results obtained, it was shown that clone R169 had the highest antioxidant activity at 500 µg/mL.

Conclusions

Cell suspension culture of oil palm clone has been shown to contain flavonoid and total phenolic compounds which may contribute to the antioxidant and radical scavenging activities.

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CHAPTER 5

BIOTECHNOLOGY

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Characterization of an F-box Family Gene from Kesum (*Polygonum minus* H.)

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Introduction

F-box is a component of protein ubiquitin ligase complex known as SCF complex which stands for its SKP1, Cullin and F-box subunit. This complex plays role as an E3 ligase in the ubiquitin mediated protein degradation pathway. In this pathway, SCF complex transfers ubiquitin chains from E2 to the target proteins to be degraded by 26S proteasome. There are several types of F-box protein have been identified based on different motif combination in the F-box proteins. The first motif which exists in all F-box protein is the F-box motif. Downstream the F-box motif could be WD repeat, leucine rich repeat (LRR), kelch repeat or other motifs. One of the largest F-box family is Kelch Repeat Containing F-box protein (KFB). Among approximately 700 F-box gene identified in *Arabidopsis thaliana*, around 100 of them are believed to encode KFB, and four KFB proteins have been well studied including ATTENUATED FAR-RED RESPONSE (AFR) which involved in light signalling (Harmon and Kay, 2003), ZEITLUPE (ZTL), FLAVIN BINDING KELCH REPEAT F-BOX1 (FKF1) and LOV KELCH PROTEIN2 (LKP2) which are involved in regulation of circadian oscillator and photomorphogenesis (Sawa et al., 2007; Fukamatsu et al., 2005; Somers et al., 2004).

Kesum (*Polygonum minus*) is very famous among Malaysians as a plant that exerts sweet and pleasant aroma. The leaves are commonly used in preparing malay dishes and normally used in making gravy for laksa. Recently, kesum also found to have high antioxidant activities (Qader et al., 2011; Izzreen and Noriham, 2011), cytoprotective activities (Wasman et al., 2010), antimicrobial and also anti tumor activities (Radu and Kqueen, 2002). In previous study in our lab, an F-box gene was found differentially expressed in kesum after the plant treated with jasmonic acid (Gor et al., 2010). This discovery provides chances to study more detail about the F-box gene named *PmF-box1* (for *Polygonum minus* F-box gene 1) and its deduced protein. Hence, the first objective of present study is to obtain the full length sequence of the *PmF-box1* cDNA. The second aim is to characterise the putative PmF-box1 protein and finally to analyse the expression pattern of the gene after different elicitor treatment.

Materials and methods

Cloning of PmF-box1 full-length cDNA by RACE

The EST in the cDNA library was found to have a segment of a putative F-box gene and used as template to amplify *PmF-box1* by rapid amplification of 5' cDNA end and 3' cDNA end (RACE) technique.

Sequencing and sequence analysis

The full length sequence *PmF-box1* cDNA, deduced amino acid sequence and open reading frame (ORF) were analyzed, and the sequence comparison was conducted through database search using BLAST program (NCBI, National Center for Biotechnology Services, http://www.ncbi.nlm.nih.gov). Online software MyHits (http://myhits.isb-sib.ch/cgi-bin/motif_scan) was used for motifs and domains

annotation in the deduced protein. PmF-box1 was aligned with F-box protein from other plant species using DNAMAN version 7 (Lynnon Biosoft, Vaudreuil, QC, Canada) with default parameters.

Hormone treatment and expression analysis

For jasmonic acid and salicylic acid treatment, the areal parts of the plants were sprayed with 5 mL of 150 µM of each phytohormone. For the treatment with mixture of both phytohormone, the areal parts of the plant were sprayed with 5 mL solution containing 150 µM jasmonic acid 150 µM salicylic acid. Then the leaves were harvested after 3, 6, 12, 24, 48 and 72 hours. Untreated plants were used as control. The leaves were directly frozen in liquid nitrogen and stored in -80 ^{o}C. Semi-quantitative reverse transcription PCR (RT-PCR) was performed for expression analysis. For the quantification of the PCR products, P. minus cyclophylin was used as control. The ratio of gene specific expression to cyclophylin signal was measured using ImageJ software and defined as relative expression.

Results and Discussion

In present study, we obtained the full length cDNA sequence of *PmF-box1* based on expressed sequence tag (est) sequence and the amino acid sequence was deduced. *PmF-box1* has an ORF encoding a polypeptide of 487 amino acid long and share moderate sequence homology with other F-box proteins from other species. The putative PmF-box1 contains glutamine rich region and F-box motifs in the N-terminal and Kelch repeat motif in the C-terminal (Figure 1). The F-box and kelch repeat motifs show high homology identity with other F-box proteins from other plant species. This data indicates that PmF-box1 belongs to the Kelch repeat containing F-box (KFB) protein family. KFB is one of the largest F-box family since there are approximately 100 FBK genes found in Arabidopsis thaliana (Schumann et al., 2011), and four of them have been functionally characterized which are AFR which involved in light signaling, ZTL, FKF1 and LKP2 which are involved in regulation of circadian oscillator and photomorphogenesis. ZTL, FKF1 and LKP2 have LOV domain at the N-terminal to the F-box protein acts as the light censoring domain for the F-box protein. Even though PmF-box1 lack the LOV motif, it still has the possibility to play roles in regulating light signalling in kesum since most of the characterized KFB proteins involved in light signalling. Instead of LOV motif, putative PmF-box1 have glutamine rich region in the N-terminal. This domain is rarely found in other KFB from other plant since there is low homology identity in this region compare to the other KFB. Glutamine rich region is an important domain in transcription factors and usually functions in activating gene expression by binding to the TATA box binding protein (TBP) or RNA Polymerase II Subunit III to start transcription process (Dai et al., 2003; Ding et al., 2006; Xiao and Jeang, 1998). This data suggest that PmF-box1 also activating gene expression directly instead of activating through ubiquitin mediated protein degradation pathway.

Based on previous study by (Gor et al., 2010), *PmF-box1* expression was found increased in kesum after phytohormone jasmonic acid treatment. To study the expression pattern in more detail, we evaluate the expression of *PmF-box1* at different time point after elicitation with jasmonic acid and salicylic acid. As we can see in Figure 2, after treated with 150µM jasmonic acid, the expression of *PmF-box1* was increased but not continuous. The transcript level was increased 3.7 fold compared to control after 3 hours. However, the transcript level back to basal level after 6 hours and keep increasing until 24 hours at 3.9 fold and maintained to 48 hours. For 150 µM salicylic acid treatment, the transcript level remains at low level until 72 hours which the level increased to 3 fold compared to control. When kesum treated with both jasmonic acid and salicylic acid at the same concentration, the transcript level increased to 2.7 fold after 3 hours and then declined at the sixth hour before it climb to 7.2 fold at 48th hour before declined to 5.7 fold at 72 hour. Recent study by (Paquis et al., 2010) also showed that a KFB gene from grapevain upregulated by methyl jasmonate and salicylic acid treatment with the expression level is

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higher for methyl jasmonate treatment. This findings support present study which shows that the expression of *PmF-box1* increased in both phytohormone treatments.

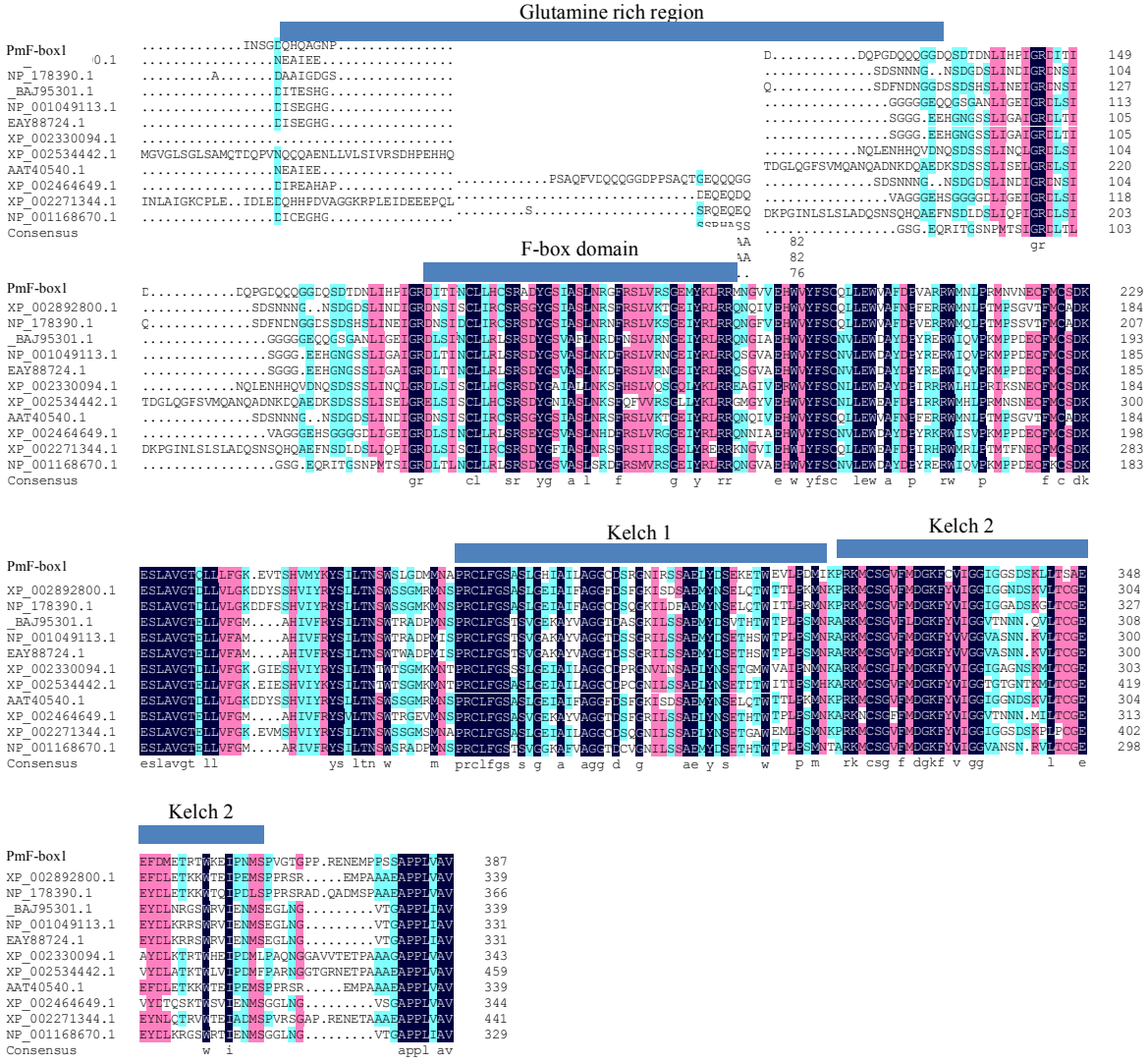

Figure 1. Comparison of the putative amino acid sequences of *PmF-box1*. The specific site for glutamine rich repeats, F-box, and kelch repeats were presented. The aligned F-box subunit sequences were from *Arabidopsis lyrata* XP002892800, *Arabidopsis thaliana* NP178390, *Hordium vulgare* BAJ95301, *Oryza sativa* group Japonica NP001049113, *Oryza sativa* Indica group EAY88724, *Populus trichocarpa* XP002330094, *Ricinus communis* XP002534442, *Solanum demissum* AAT40540, *Sorghum bicolour* XP002464649, *Vitis vinifera* XP002271344, and *Zea mays* NP_001168670.

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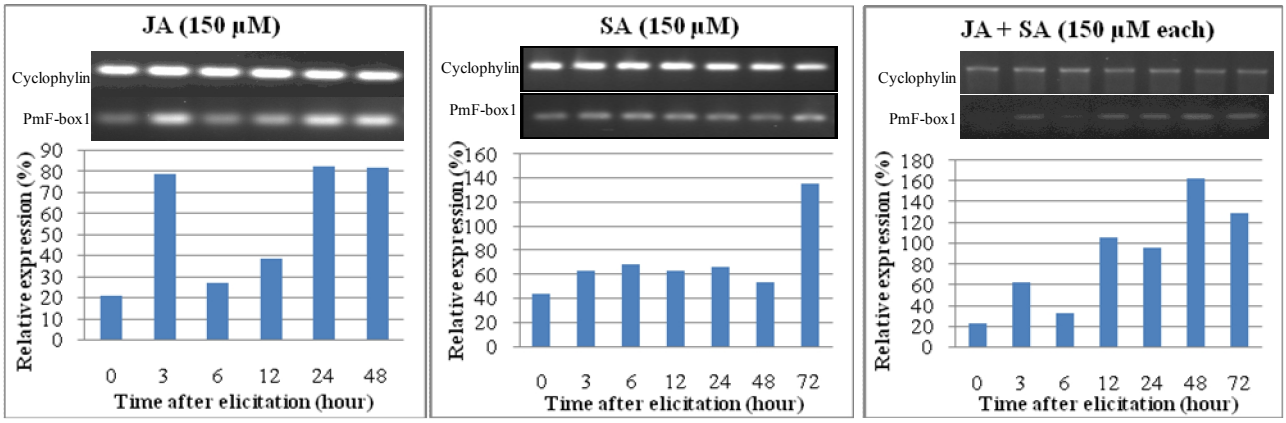

Figure 2. Expression patterns of the *PmF-box1* gene after treated with phytohormone jasmonic acid (JA), salicylic acid (SA) and mixture of both phytohormone with the same condentration (JA+ SA) The relative expression was normalized to cyclophylin.

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In Vitro Regeneration of *Pogostemon cablin* using Nodal Segment Explant

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Introduction

Pogostemon cablin or patchouli, famously known by its commercial name nilam, is an aromatic plant. It is also the main source of patchouli oil obtained in their essential oil through extraction process. It has benefits to perfumery industry for many years. Patchouli oil has fixative properties which make the smell last longer on the skin (Sugimura et al., 2005; Kongkathip et al., 2009). Since this substance has high market demand, its continuous supply to the industry has become a major concern. It has been claimed that patchouli has non-synchronous flowering system and there are plants that never flower (Sjamsudin et al., 1997). As a result, conventional vegetative propagation by stem cutting (Swamy et al., 2010) has been alternatively practiced by the planters but it only results in limited stock for this plant. It is due to the presence of potential diseases resulted from viral infection, fungal and also bacterial infection as well as insect pests (Misra, 1996). These constraints lead to a bad impact to the industry. As an alternative method of propagating the plant, plant tissue culture technique can give advantage in multiplying million fold of a desired plant (Warar et al., 2008).

Thus, this study was done to regenerate shoots using nodal segment explants of *P. cablin* on full strength MS (Murashige and Skoog) medium with different concentrations of BAP and NAA. Nodal segment is a part of stem which new growth can occur naturally. It may produce new leaves, flowers, and cones and even stems itself thus make it a perfect explant to be used for growing new desired plants.

Materials and Methods

Sample collection and sterilization

Patchouli or nilam was collected from Ayer Keroh, Melaka. The plant description, natural habitat, growth, distribution, scientific studies and uses were documented. Voucher specimen was deposited at the Herbarium Institute of Bioscience, Universiti Putra Malaysia. In the tissue culture laboratory, plant parts were cleaned and sterilized using bleach (Clorox 20%) for 15 minutes prior to culture. Aseptic techniques were applied to each step in the laboratory.

Plant regeneration

Nodal segment explants were cultured on full strength MS medium. The explants were placed on MS medium with different concentrations of NAA hormone (0, 0.25, 0.5, 1.0, 2.0 mg/L) and BAP hormone (0, 0.25, 0.5, 1.0, 2.0 mg/L). The cultures were incubated in growth room at adjusted temperature of 25 °C and under initial photoperiod of 16 hours light and 8 hours dark period. The number of shoots and percentage of regeneration were observed and recorded.

Subculture

After 4 weeks of initiation, the explants were subcultured onto fresh medium to ensure that the nutrients were enough to eliminate cell growth exhaustion. The clumps of shoots were separated and cultured onto the five best concentrations, i.e. 0.25 mg/L BAP, 0.5 mg/L BAP, 0.25 mg/L BAP/0.25 mg/L NAA, 0.5

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mg/L BAP /0.25 mg/L NAA and 1.0 mg/L BAP /0.25 mg/L NAA. Observation and measurement on plant regeneration, number and length of shoots were done and all the data were recorded.

Statistical analysis

The results were expressed as mean values ± standard error of mean. One-way analysis of variance (ANOVA) at 95% confidence interval of probability was used in data analysis using Microsoft Excel 2007.

Results and Discussion

Plant growth was monitored by the measurement on number of shoots in each explant. There were 25 concentrations of hormone involving BAP and NAA alone and in combinations supplemented in full strength medium.

Based on Figure 1, a significant number of shoots was produced in full strength of MS medium within 4 weeks after initiation. One -way ANOVA analysis showed that the concentration of hormones in a medium had significant effect on the number of shoots formed ($P<0.0001$). The highest number of shoots (32.93 ± 3.93 shoots per explant) was obtained with MS medium supplemented with 0.25 mg/L BAP followed by MS medium supplemented with 1.0 mg/L BAP and 0.25 mg/L NAA (30.7 ± 0.5 shoots per explant). However, NAA had a very small effect on shoot differentiation. This finding is similar to that obtained in the study done by Misra (1996) where indicated that the presence of auxin alone would not give any formation of shoots within 4 weeks.

Increasing the level of BAP resulted in declined number of shoots. The elevation of BAP level also contributed to the decline in the formation of multiple shoots thus reduced the number of shoots obtained in the study carried out by Paul et al. (2010). On the other hand, plant growth regulator cytokinin is vital for shoot regeneration using leaf explants (Paul et al., 2010). In this case the use of low concentration of cytokinin (0.25 mg/L BAP) gave highest number of shoot number.

Since *P. cablin* cultures obtained in clumps after 4 weeks of initiation, the experiment was tested further in five selected concentrations (0.25 mg/L BAP, 0.5 mg/L BAP, 0.25 mg/L BAP/ 0.25 mg/L NAA, 0.5 mg/L BAP/ 0.25 mg/L NAA, 1.0 mg/L BAP/ 0.25 mg/L NAA) that gave high number of shoots. The clumps of shoots (5 shoots) were separated and subcultured into the fresh MS medium containing the five selected concentrations. The medium that produced maximum number of shoots also gave the highest length of shoots (3.80 ± 0.27) (Figure 2). This proved that low level of BAP was sufficient to obtain the optimal shoot number of *P. cablin* using nodal explants.

According to Paul et al. (2010), high concentrations of BAP combined with low concentration of NAA tested on leaf explants resulted in the formation of multiple shoots. In the present study, MS medium supplemented with of 1.0 mg/L BAP and 0.25 mg/L NAA resulted in considerable multiple shoot formation using nodal segment explants. However, low concentration of BAP alone (0.25 mg/L BAP) was good enough for the elongation of shoots.

It was found that the same concentration of cytokinin and auxin (0.25 mg/L BAP + 0.25 mg/L NAA) and high concentration of cytokinin (1.0 mg/L BAP/ + 0.25 mg/L NAA) caused the formation of callus. As a result, the frequency of multiple shoots formation was reduced.

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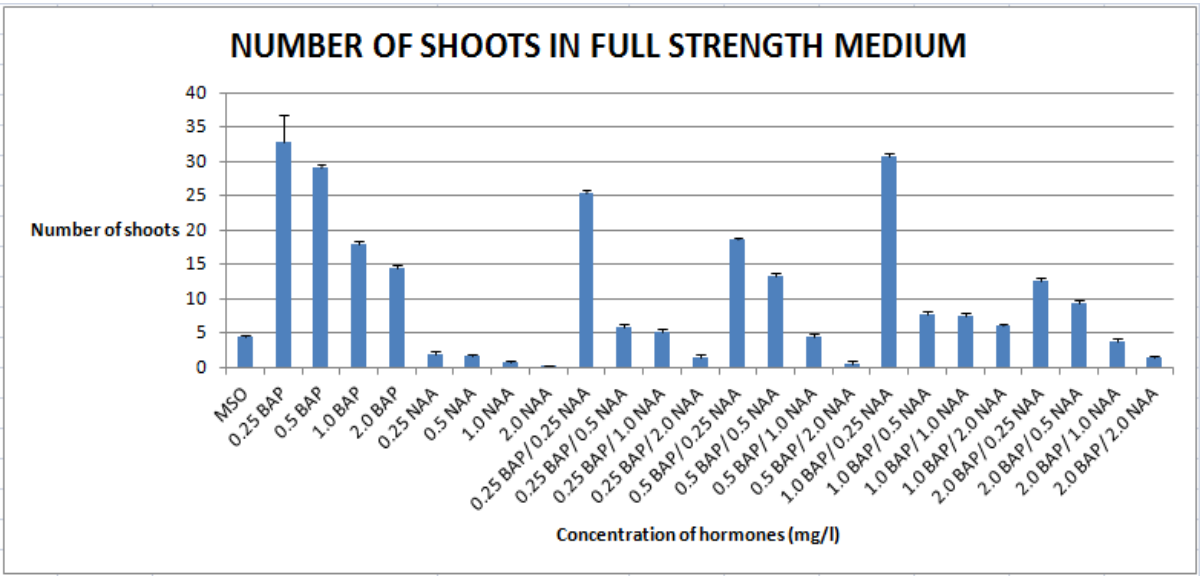

Figure 1. The number of shoots regenerated in different concentrations of hormones supplemented in full strength MS medium after 4 weeks culture.

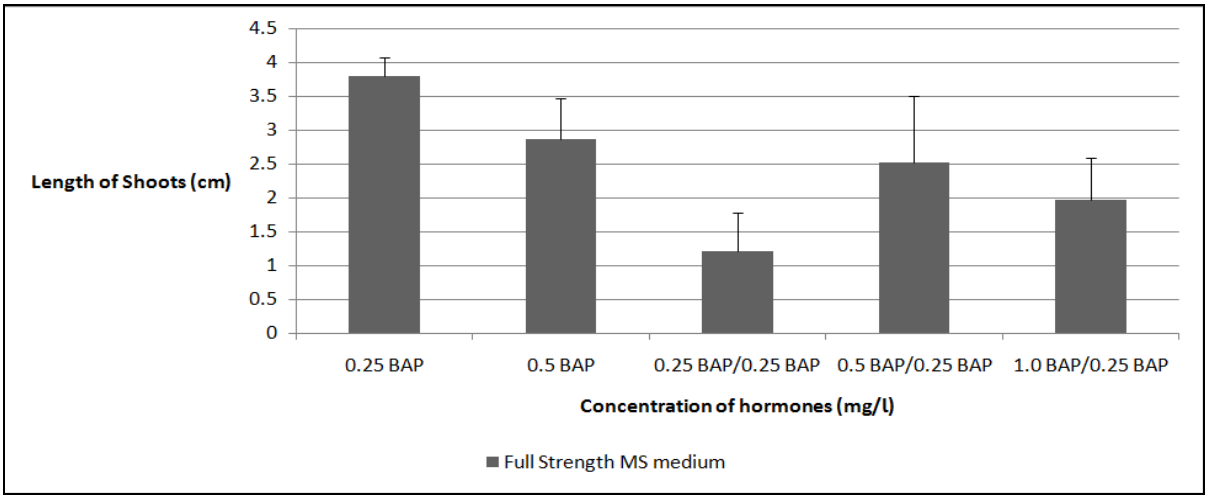

Figure 2. The length of shoots regenerated in five best concentrations of hormones supplemented in full strength MS medium after 4 weeks culture.

Conclusions

In conclusion, an efficient method was established for direct shoot regeneration from the nodal segment explants of *P. cablin* on full strength MS media. This plant can be regenerated via *in vitro* cultures with a very low concentration of hormone (0.25 mg/L BAP) which gave highest number of shoots (32.93 ± 3.93 shoots per explant) and highest length of shoots (3.80 ± 0.27 cm). In vitro regeneration of *P. cablin* from nodal explants in MS medium supplemented with BAP or NAA was successfully done. The healthy propagules obtained in this study can be used for further procedure of acclimatization.

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Effect of Explant Source on *In Vitro* Regeneration of *Capsicum annuum* L. var CB4

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Introduction

Capsicum annuum L. is belonging to the family Solanaceae and is an important vegetable and spice crop around the world. However, tissue culture techniques in chilli lag behind compared to most other vegetable crops, mainly due to its recalcitrance to regeneration (Liu et al., 1990). Though various reports on organogenesis in *C. annuum* varieties are available (Ochoa-Alejo and Ramirez-Malagon, 2001), researchers around the world faced the same problem in regenerating capsicum; formation of ill defined buds or shoot like structures either resisting elongation or producing rosettes of distorted leaves which generally do not produce normal shoots (Franck-Duchenne et al., 1998; Steinitz et al., 1999; Ochoa-Alejo and Ramirez-Malagon, 2001). Several of these reports suggest a strong influence of source of explants on the regeneration process (Ramirez-Malagon and Ochoa-Alejo, 1996). We investigated the effects of different sources of explants on the regeneration of *Capsicum annuum* L.var CB4.

Materials and Methods

Hypocotyl, petiole and cotyledon of *in vitro* germinated seedlings were cultured on MS medium containing 5 mg/L BAP, 1 mg/L IAA, 25g/L DJ nutrient (chilli seedling extract) to induce shoot bud. Shoot buds were transferred on MS medium containing 3 mg/L BAP, 1 mg/L IAA, 15g/L DJ, 2 mg/L GA_3, 10 mg/L $AgNO_3$, 15 g/L DJ nutrient for elongation. Rooting was induced on MS basal medium containing IAA.

Results and Discussion

Explants obtained from 10-14 days old seedling of *Capsicum annuum* L. var CB4 were cultured on induction medium. Buds were produced directly from the explants within 1 week of culturing on MS basal medium supplemented with 5 mg/L BAP, 1 mg/L IAA, 25g/L DJ nutrient and 30% sucrose.

Results showed that cotyledons produced the highest buds (78.3%) compared to hypcotyl (74.2%) and petiole (70.7%). However, buds produced by cotyledons were ill defined buds that looked like crown. Hypocotyls produced 98% leafy structure shoots and buds initiated from petioles were combination of both (76.4% leafy structures). Crown structures (Figures 1a and b) were actually the ill defined buds and these structures failed to develop and leafy shoot buds (Figure 1c) frequently developed into leafy structures (rosette) (Figure 1d) or normal shoots (Figures 1e and 1f).

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Figure 1. Type of bud: (a and b) crown like structure or ill defined buds, (c) leafy shoot buds, (d) rosette structures, (e and f) normal shoots produced from explant of *Capsicum annum* cultured on induction medium

The formation of rosettes or blind leaf structures without shoot elongation has been observed in *Capsicum* and reported as the major problem in establishing tissue culture regeneration (Szasz et al., 1995; Franck-Duchenne et al., 1998). This phenomenon is common in *Capsicum* (Ochoa-Alejo and Ramırez-Malagon, 2001) and may be associated with fasciated and degenerative meristems (Mezghani et al., 2007). Meanwhile, Ochoa-Alejo and Ramirez-Malagon (2001) suggested a problem in auxin perception and/or signal transduction leading to malformed meristem.

This result indicated that regeneration of CB 4 was highly influenced by source of explants. The best response was obtained from hypocotyls and petioles. Cotyledons were the most inefficient material for shoot elongation. Shoots from hypocotyls and petioles were better formed and easier to elongate. These buds proliferated further into numerous shoot buds when they were subcultured on elongation medium. In an earlier study by Jayashankar (1998) and Hyde and Phillips (1996), it was shown that high frequency of buds could be induced in cotyledons, but with low frequency of shoot elongation. A similar result was obtained by Dabauza and Peña (2001). They concluded that the percentage of explants producing buds and shoots depended on the explant type.

Although the protocol has limited applications in mass propagation *in vitro*, it can be utilized for genetic transformation studies of chilli. Further studies on increasing the percentage of shoot elongation could be useful in local chilli.

Conclusion

In conclusion, we have demonstrated that shoot formation primarily depends on the source of explants used and we have established a promising protocol for regeneration of *Capsicum annuum* var CB4.

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In Vitro Multiple Shoot Regeneration from Nodal Explants of *Gynura procumbens* (Lour.) Merr.

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Introduction

Sambung nyawa is widely distributed in South East Asian countries such as Malaysia, Indonesia and Thailand. This plant traditionally been used for the treatment of fevers, rashes, kidney disease, migraine, constipation, hypertension, diabetes mellitus and cancer. Recently pharmacologic studies reported that *G. procumbens* also has anti-Herpes virus (Nawawi et al., 1999), anti-hyperglycemic (Hassan et al., 2010), anti-inflammatory (Iskander et al., 2004), antiulcer (Mahmood et al., 2010), anti- hyperlipidemic (Zhang and Tan 2000) and blood pressure reduction capabilities (Kim et al., 2006).

Due to medicinal values, there is a great potential to develop various products from this plant. For a sustainable raw material supply in manufacturing of *G. procumbens* products, propagation of this plant on a large scale will be a key step. The *in vitro* culture techniques can be used as the alternative for the superior planting material and continuous provision of plantlet stocks for large scale field cultivation. We therefore investigated the suitable regeneration of *G. procumbens* using nodal segments derived from adult plants. This protocol can be used at a large scale for clonal propagation of this species.

Materials and Methods

The nodal segments of *G. procumbens* were washed with detergent and rinsed under running tap water. They were immersed in 95% ethanol for 30 s and then surfaced sterilized with 20% Clorox® for 15 min, and rinsed three times with sterile distilled water. They were again surface sterilized second time with 5% Clorox® and rinsed again three times with sterile distilled water. The nodal segments were cut to obtain 0.5-0.6 cm nodal segment explants and were inoculated onto MS (Murashige and Skoog, 1962) medium containing 30 g/L sucrose and 2.8 g/L gelrite for 5 days before transferred onto MS medium supplemented with 0 – 10 mg/L BAP.

Results and Discussion

The nodal segments were used as initial explants for establishment of *in vitro* culture system of *G. procumbens*. High aseptic nodal segments (90%) were successfully established using double sterilized technique (20% and 5% Clorox). The nodal segments were then cultured on Murashige & Skoog medium supplemented with 1-10 mg/L BAP to determine the best concentration for production of multiple shoots.

Initially one shoot bud per explant emerged after 5-8 days of inoculation and gradually the number of shoot buds per explant increased up depended on BAP concentration. These shoots appeared to proliferate from the node via axillary branching of buds from the explants. After 6 weeks, results showed 100% of the explants produced shoots on induction medium. An average of 3 to 8 shoots was formed from each nodal segment. MS supplemented with 3mg/L BAP induced the most number of shoots, 8.2 shoots per explant (Figure 1). Nodal segments cultured on MS medium without any growth regulator were differentiated into single shoot per explant.

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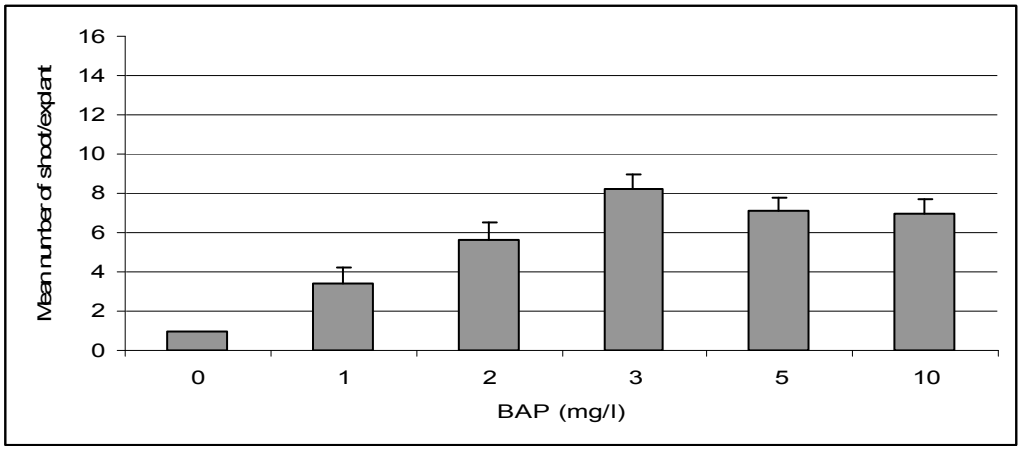

Figure 1: Effect of different concentration of BAP on shoot induction of *G. procumbens*

These results showed that BAP was a very effective in induction of multiple shoot from nodal segment. BAP is considered as the most useful cytokinin in bud breaking in plants and has been widely used in plant micropropagation (Safdari and Kazemitabar, 2010; Alam et al., 2010). The enhanced rate of multiple shoot induction in cultures supplemented with BAP may be largely ascribed due to increased rates of cell division induced by cytokinin (BAP) in the terminal and axillary meristematic zone of explant tissues. Cells in this zone divide with the faster pace and thus, produced large number of shoots (Niranjan et al., 2010).

However, the number of shoots was reduced when cultured at higher concentrations of BAP. It also resulted in stunted growth of the shoots. Similar results were reported by Chan et al., (2009) and Subash and Vaishana (2010).

The regenerated shoots were excised and placed on the MS medium without any growth regulator. Shoot elongation was simultaneously observed along with root induction. Root initiation occurred directly from the cut ends of microshoots after 2 weeks of culture. This result showed that, basic MS medium without the addition of any auxin was sufficient for the establishment of *in vitro* rooting of the micro-shoots of *G. procumbens*. Rooted plantlets were removed from agar, washed thoroughly and placed in a mixture of sterilized vermiculite and sterilized soil (1:1) before acclimatized in greenhouse.

Conclusions

Here, we report an efficient protocol for clonal multiplication of this plant species using nodal segments as explants. The MS medium with 3 mg/l BAP gave the highest number of shoots per explant. All the micro-shoots produced normal roots within two weeks of culture on the basic MS medium without any plant growth regulators. The plantlets are growing well without any phenotypic aberrations. This protocol can be suitably exploited for the mass multiplication on a large scale for commercial.

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Molecular Characterization and Inflorescence Description for selected *Curcuma* species

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Introduction

Curcuma sp. is a perennial rhizomatous herb, medium in size of about 0.5 to 1.5 meters tall. It is easily distinguished amongst other Zingiberaceae genera by their peculiar inflorescence. The inflorescence consists of bracts that fuse at the sides of neighboring bracts to form pouches. Bracts at the basal part of the inflorescence are known as fertile bracts because they enclose 1 to 8 flowers which open one after the other. The sterile bracts at the upper part of inflorescence, known as coma bracts are usually bigger and brightly colored. The showy inflorescence plays an important role in morphological characterization because the most popular morphological character to be identified is the color of the inflorescences (Lema-Rumińska et al., 2004). The stem is usually a pseudostem formed by closely embracing leaf sheaths. *Curcuma* sp.has gained recognition in the floriculture industry as pot flowers, landscape and cut flowers. The Siam Tulip (*C. alismatifolia*) for instance is the most utilized ornamental species of this genus (Nahar and Sarkar, 2007). *Curcuma* sp. has the potential to become commercially cultivated floriculture crop and contribute towards Malaysia's economic development. Although it has high potential as one of the major crops it is currently unexploited especially species native to Malaysia (Chin, 2007). In spite of its importance the genus is poorly understood in terms of biology and chemical characteristic (Singh et al., 2003). Many species of *Curcuma* sp. are similar looking and in addition they hybridize naturally. They exhibit large morphological variation and wide diversity of hybrids but similarities between them lead to confusion of identity (Apavatjrut et al., 1999). Accurate identification of cultivar and varieties is also essential for breeding, propagating and marketing of *Curcuma* (Anuntalabhochai, 2006). Seven species of *Curcuma* which pose promising potential as ornamentals are *C. alismatifolia* Chiang Mai Pink, *C. alismatifolia* Chiang Mai White, *C. cordata* and *C. sparganifolia*. Included in this study were three unknown species designated as *Curcuma* sp.1*, Curcuma* sp. 2, and *Curcuma* sp. 3. Firstly in order to introduce *Curcuma* as a new ornamental plant, accurate identification of these selected species is essential which can be achieved through morphological and molecular characterization. The objectives of this study are to study the inflorescence morphology of selected Curcuma species and to identify the relatedness among selected species using RAPD markers and to estimate the similarities among selected Curcuma species.

Materials and Methods

Plant materials

Seven types of ornamental *Curcuma* sp. from Field 2, University Putra Malaysia, were used in this experiment namely; *C. alismatifolia* var. 'Chiang Mai Pink', *C. alismatifolia* var. 'Chiang Mai White'*, C. cordata, C. sparganifolia,* and three unknown species assigned as *Curcuma* sp. 1, *Curcuma* sp. 2 and *Curcuma* sp. 3.

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Inflorescence morphology

Inflorescences of *Curcuma* spp. were harvested at maturity stage (when 50% of inflorescences are at full bloom stage). Measurements for quantitative data and qualitative data for each inflorescence were measured and recorded. Quantitative data included stalk length, length and width of inflorescence, bract and true flower, number of bracts and true flowers, while qualitative data included inflorescences color by tiers, inflorescence shape, color and shape of bract and true flower. Seventeen morphological characteristics of *Curcuma* inflorescence were observed, measured and given scores. The data and scores were analyzed with NTSYSpc® version 2.10 software (Numerical Taxonomy and Multivariate Analysis System) (Rohlf, 1993) using Simple Matching coefficient (SM) to create a dendrogram. Botanical description for each inflorescence was also formed based on morphological data.

Molecular characterization

DNA Extraction was done using 100 mg of fresh leaf sample and a commercial DNA extraction kit, GENEALL Plant SV Mini Kit. Seven oligonucleotide primers were purchased in lyophilized form from 1st Base (Asia). OPC01, OPC05, OPC10, OPD07, OPK19, OPL14 and OPX03 were used in this research (Anuntalabhochai, 2007; Hussain et al., 2008). Polymerase Chain Reaction (PCR) Amplification was done using a master mix containing 5.5 µl sterilized distilled water, 5 µl Taq buffer ,4 µl dNTP mix 0.5 µl Taq polymerase, 5 µl HQ Buffer, 3 µl DNA Template and 2 µl of selected Primer. 25 µl volume of reaction mixture containing 6.5 µl of master mix, 2.5 µl PCR buffer, 9 µl of sterilized distilled water, 3 µl of Template DNA, and 4 µl of selected PCR primer was prepared for each PCR tube. Amplification conditions consisted of pre-denaturation at 94°C for 3min, denaturation at 94°C for 45sec with 40 cycles, annealing at 39.5 or 43.6°C for 1min, extension at 72°C for 1min, and final extension at 72 °C for 5min. PCR products from the RAPD reaction were then separated on agarose gel of 2% and visualized by ethidium bromide staining (Weising et al., 2005). Gel documentation and band scoring were conducted using UVIDoc software. RAPD bands that were discerned from the agarose gel ware recorded as present (1) or absent (0) and assembled into a data matrix. The dendrogram following the NTSYS (Rohlf, 1993)-UPGMA algorithm was generated with the Jaccard co-efficient based on all the markers generated. Additional data for number of polymorphic loci was obtained using POPGENE program (Yeh and Boyle, 1997).

Results and Discussion

Morphological characterization

Three different groups were derived from clustering and dendrogram based on 17 inflorescence morphological characteristics of selected *Curcuma* spp. The similarity coefficients ranged from 24% to 68%. A high coefficient of similarity of 68% indicated the lowest amount of variation amongst *C. alismatifolia* var Chiang Mai Pink and *C. alismatifolia var* Chiang Mai White while the lowest of 24% indicated high variation between *C. cordata* compared to *C. alismatifolia* var Chiang Mai Pink and *C. alismatifolia var* Chiang Mai White. *Curcuma sparganifolia* had a coefficient of similarity 50% and 47% in comparison with *C. alismatifolia var* Chiang Mai Pink and *C. alismatifolia var* Chiang Mai White, respectively. It had a lower coefficient of similarity of 29% when compared to *C. cordata* indicating a high variation amongst both species. There was no 100% coefficient indicating that all the selected species were different in terms of inflorescence morphology. Based on the dendrogram (Figure 1), the selected *Curcuma* species can be grouped into three clusters. *Curcuma alismatifolia* var Chiang mai pink and *C. alismatifolia var* Chiang Mai White can be grouped together as group I. Group II consist of *C. sparganifolia* while group III comprises of *C. cordata.*

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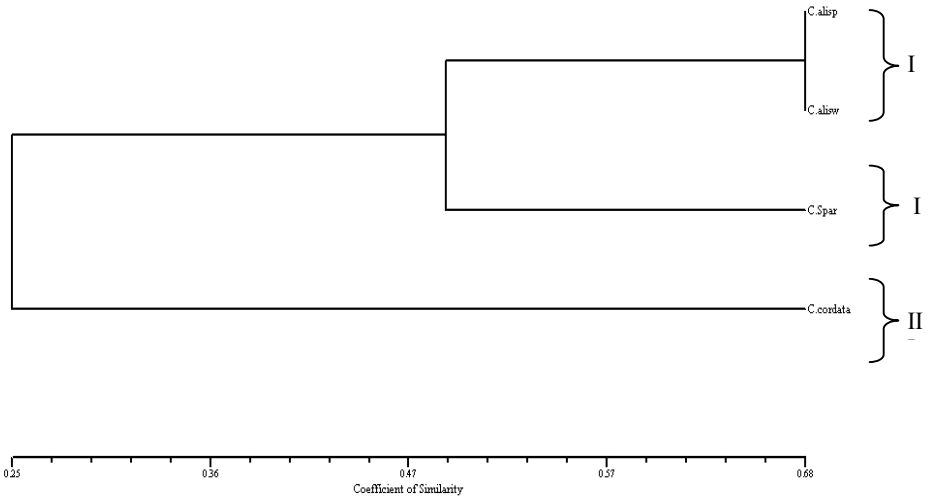

Figure 1: UPGMA dendrogram shows a relationship of selected *Curcumas* based on morphological characterization. *C. alisw, C. alismatifolia* 'Chiang Mai White'; C.alisp, *C. alismatifolia* 'Chiang Mai Pink'; C. spar, *C. sparganifolia*; C. cordata, *Curcuma cordata.*

RAPD analysis

Seven polymorphic RAPD primers showed positive amplification and were considered for the analysis of selected *Curcuma* plants. A total of 95 RAPD alleles were obtained from the seven selected primers with 99% polymorhphism. The similarity coefficients between selected *Curcuma* species ranged from 2.5% to 12.1%. The low coefficient of similarity between the selected *Curcuma* species indicates there was a high variability amongst the species. The similarity matrix was used to establish the level of genetic relatedness amongst selected *Curcuma* species. Based on the dendrogram (Figure 2), the selected *Curcuma* species can be grouped into three clusters. Group I consists of *C. alismatifolia* 'Chiang Mai White', *C. alismatifolia* 'Chiang Mai Pink' and *Curcuma* sp. 1. The second group (II) consists of two plants namely *C. sparganifolia*, *Curcuma* sp. 2 and *Curcuma* sp. 3 while *C. cordata* was grouped into a third group (III). Other than that, the results of cluster analysis by using UPGMA method showed that all the selected *Curcuma* sp. can be differentiated by RAPD markers. The results indicated that *C. alismatifolia* 'Chiang Mai White', *C. alismatifolia* 'Chiang Mai Pink' and *Curcuma* sp. 1 were closely related while *C. sparganifolia, Curcuma* sp. 2 and *Curcuma* sp. 3 were closely related to each other. However, *C. cordata* was dissimilar compared to the other six *Curcuma* and was grouped separately.

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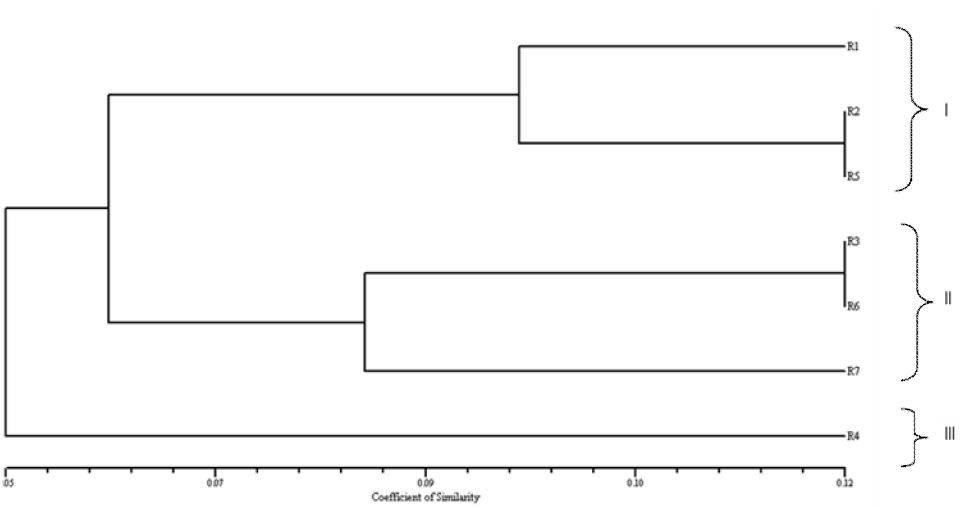

Figure 2: UPGMA dendrogram showing relationship of selected *Curcumas* based on molecular characterization. R1*, C. alismatifolia* 'Chiang Mai White'; R2, *C. alismatifolia* 'Chiang Mai Pink'; R3, *C. sparganifolia*; R4, *C. cordata*; R5, *Curcuma* sp. 1; R6, *Curcuma* sp. 2; R7, *Curcuma* sp. 3

Conclusions

In conclusion, four morphological characteristics namely, inflorescence color and shape, and variation in bract shape and number yielded the most variation from the four inflorescences of *C.alismatifolia* Chiang Mai Pink*, C. alismatifolia* Chiang Mai White*, C. sparganifolia* and *C. cordata.* The overall size of inflorescence also gave distinctive characteristics since *C. cordata* had a large inflorescence, while *C. alismatifolia* Chiang Mai Pink and Chiang Mai White were moderately sized and *C. sparganifolia* was rather miniature in size compared to the other three flowers. There was no 100% coefficient indicating that all the selected species were different in terms of inflorescence morphology. It is known that the most popular morphological character to be identified is the color of the inflorescences (Lemma- Ruminska et al., 2004). The data obtained clearly shows the similarities and dissimilarities amongst selected *Curcuma* spp. based on its inflorescence morphology.This study also confirmed that polymorphism based on RAPDs was useful in revealing the genetic relatedness among seven selected ornamental *Curcuma* species, namely *C. alismatifolia* Chiang Mai Pink, *C. alismatifolia* Chiang Mai White, *C. cordata, C. sparganifolia, Cucurma* sp. 1*, Cucurma* sp. 2 and *Cucurma* sp. Molecular characterization revealed a wide genetic diversity in intraspecific and interspecific levels. Although results from this study will be supportive in future breeding programs for the production of new and attractive varieties, more specific molecular marker such as ISSR markers may yield a more accurate result and also overcome the weakness of RAPDs low reproducibility.

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Embryogenesis of Orchid Hybrids, *Aerides odorata* × *Aerides quinquevulnera* var Calyana and its Reciprocal Cross

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Introduction

Orchids comprise a unique group of plants. Taxonomically they represent the most highly evolved family, Orchidaceae. They exhibit an incredible range of diversity in size, shape and colour of their flowers. Malaysia is one of the countries, properous with these jewels. *Aerides;* a genus of epiphytic orchids native to tropical Asia, vastly distributed in Malaysia. *Aerides odorata* and *Aerides quinquevulnera* var *calyana* are two fragrant species in this genus and have not been domesticated**.** Hybridization in orchids introduces new dimension in floriculture industry with constant production of better breeds. The ease with which free gene flow is permitted across the taxonomic limits and hybrid embryos rescued have added to new dimensions in orchid breeding (Vij, 1998). Orchid pollination provides more than adequate seeds to produce great amount of seedlings. Since the pattern of the adult plant is formed during the course of embryo development from seed, the study of embryogenesis provides an "essential information on the inception of the varied form and structure of plants" (Wardlaw, 1955). Compared with the majority of flowering plants, orchids have an atypical pattern of embryo development (Arditti, 1992; Dressler, 1993). Previous studies as mentioned by Withner showed large number of variations in the development of the embryo in Orchidaceae. This high degree of variation indicates that there may be many different patterns, as yet undescribed, in huge number of unstudied species. Development stages of orchid embryos from seeds have not been studied extensively. Besides, accumulation of all-inclusive data on seed morphology and embryogenesis is a daunting task which may never be completed in a taxon of innumerable varieties. However, it is necessary, to implant some footing upon which to explicate. Therefore, in this study the pattern of embryo development of *A.odorata* × *A.quinquevulnera* var calyana and its reciprocal cross from seed to leaf primordia was investigated.

Materials and Methods

Plant materials, *A. odorata* were obtained from the collection of wild orchid Shade House, Horticulture Unit, MARDI, Serdang while *A. quinquevulnera* var calyana were collected from United Malaysian Orchid Farm, Rawang, Selangor, Malaysia. Pollination was performed in October 2009 when *A.odorata* and *A.quinquevulnera* var calyana were flowering synchronously. After pollination, the capsules were allowed to develop by maintaining the plants in the orchid shade house until it reaches its matuarity. The mature seeds were freshly collected from the naturally dehiscing capsules. Seeds of these hybrids were cultured in MS medium. Samples were taken from the developing embryos showed different or new structure from previous stage. Observation on embryogenesis was made by using light microscope (100X). This study was carried out at Botany and Anotomy Laboratory, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia.

Results and Discussion

Orchid seeds have often been claimed not to contain endosperm or sufficient nutrients to enable them to grow as an autotrophic plantlet. However, it has been proved that embryo cells contain lipid, protein

bodies and starch grains, to the almost entire exclusion of other organelles (Manning and van Staden, 1987; Rasmussen, 1995; Richardson et al., 1992). These reserve nutrients help the zygote to enlarge in size and turn into an ovoid body, called embryo. Thereafter they should incorporate with mychorriza or supplied nutrients from man made mediums for further developments. In this study, hybrids, AO × AQ and AQ × AO were provided with all necessary nutrients through MS media. These two hybrids are almost structurally homologous however, variation do exist comparatively. Duration of time taken by AQ × AO seeds (Fig. 2a) to change form from one to another was far more faster than the AO × AQ seeds (Figure 1a). By the absorption of nutrients from medium, a change took place in the seeds: embryo swelled, enlarged in the number of cells filling the air space inside the seed coat (Figures 1b; 2b). Decisive maturation is a process of dehydration where voculation which been observed before were abandoned with the approaching maturity (Manning and van Staden, 1987).

As the embryo developed further, the outer integument also known as seed coat or testa cell broaden and became thin string-like structure and subsequently moved to the micropyle end (Figure 1c) before it was completely detached from the embryo. In these hybrids, the seed coat remained until the embryo formed irregular shape (Figures1d; 2c). At this stage, the inner integument remained as a closely fitting film around the embryo (Figure 1e). The same phenomenon stated by Carlson (1940) and Veyret (1969) in their studies: only few cell strands connect the two integuments at maturity. The mature testa, that forms well-known reticular-foveate pattern, consists of dead cell walls from the outer integuments. The cells of the inner integument are almost completely resorbed, but a thin layer persists, the so-called carapace, which is hydrophobic and sheathes the embryo, being interrupted only at the micropyle. The carapace in AO × AQ embryos was thicker as compared to the AQ × AO embryos (Figure 1d). This observation authenticated reason for the long duration time taken by AO × AQ embryo for transfiguration. Veyret noted that seeds with the particularly well developed carapace such as in *Cephalanthera* and *Epipactis* species, germinated with difficulty. The thickness of the carapace varied with the provenance of the seeds. The carapace acts as a barrier for water and nutrient absorption. Testa colour varies upon maturation. The colour of AO × AQ seed coat during detachment was yellowish brown like sand while blackish brown in AQ × AO. The next phase took place in this embryogenesis was protocorm stage. It was observed at this stage that, embryos of AO × AQ were elongated in shape in contrast to AQ × AO which were round and odiametric like onions. They turn completely yellow at this stage. Rhizoid (Figure 2e) were started to form from raised cushions on the surface of protocorms unlike some orchid embryos has only sparse hair or even glabrous. After certain amount of growth has taken place, dome-like protuberance (Figures 1f; 2f) were visible on the apical part of the protocorm and the size increased following subsequent development. The pattern of embryogenesis was the same for both hybrids.

The final phase for the present study was shoot initiation and formation of leaf primordium. Protocorm completely turned to green over this stage. Both hybrid embryos produced ridge like structure (Figures 1g; 2g) on the shoot apex. Many apical buds were visible on the surface of the protocorm (Figure 2h). Before the protrusion of leaf primordium, the apical part of the protocorm became dorsiventral. The change in symmetry of the apical dome is associated with the formation of vascular system in the axial part of the apical bud and expansion of the mid-vein region of the leaf primordium (Kull and Arditti, 2002). Leaf primordia were arranged distichously in these buds. They give rise to more shoots, which developed monopodially. The hyrids were found spindleform in longitudinal section (Figure 1g). Shoot of AO × AQ protocorms spindled downwards while AQ × AO were pointed sharply. The growth of these protocorms in the direction of longitudinal axes is due to division of cells at the base of apical bud. True root did not form in the basal of protocorm where it appeared only after the formation of first leaf. However, there was hypophysis singled out in the basal part of protocorm. These cells are playing an important role during root meristem formation (Titova, 1997). According to Johansen (1950), the orchid embryo does not have a hypophysis. However other investigators have reported the existence of this structure in orchid embryos (Shamrov and Nikiticheva, 1992; Clements, 1999). These cells degrade,

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became dry and turn to dark brown in colour during growth (Figure 6h). The morphological features of the basal parts were the epidermal hairs (Figures 1h; 2h), also called root by Bernard (1909) and Veyret (1974).

Conclusions

Observation showed similarity in the pattern of development of both *Aerides* hybrids. However the duration time taken for the development was different. This is due to the morphology of the embryo. First phase in embryogenesis started with the development of embryo inside the seed coat. When the embryo enlarged, the testa cells expanded, moved to the microphylar end and finally detached completely from the embryo. The inner integument attached to the embryo was thicker in AO × AQ compared to AQ × AO. This makes delayed growth in AO × AQ hybrid. Embryo then transformed from globular to irregular shape. Rhizoids were formed on the surface of protocorms and also dome-like protuberance was visible on the apical part of the protocorm.

The next phase was shoot initiation and formation of leaf primordium. Hypophysis existed in the basal part of protocorm. It degraded and turned dry during growth of leaf primordium.

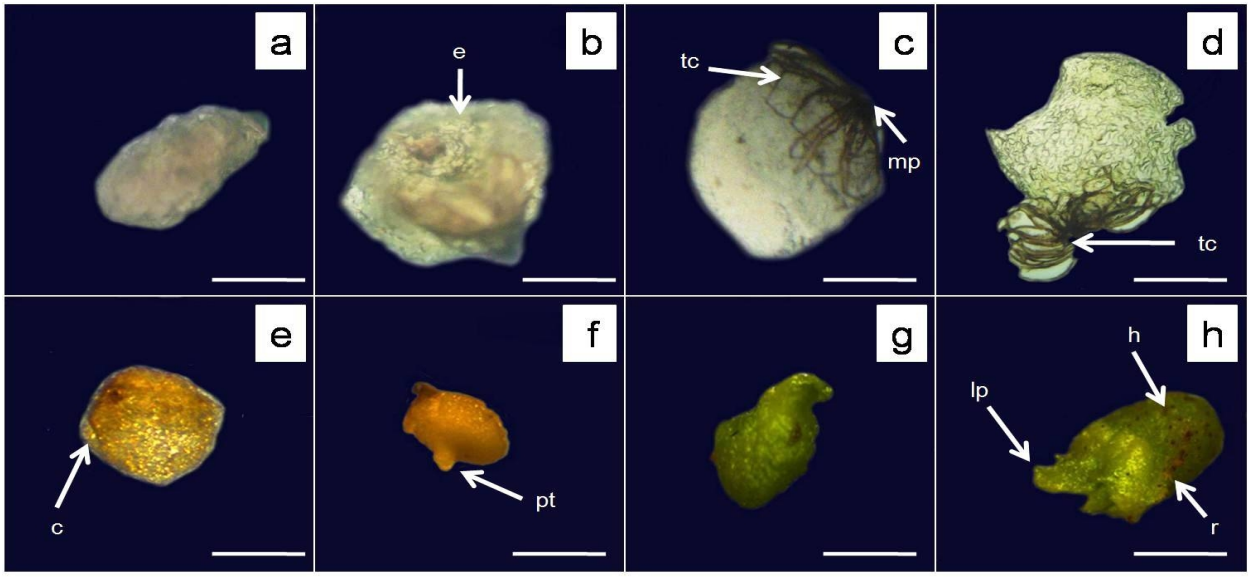

Figure 1. Development stages of AO × AQ hybrid from seed to leaf primordia in asymbiotic culture. (a)Seed fully covered with testa cell. (b)Embryo swelled filling air space within the seed coat. (c) String like testa cell. (d) Testa cell moved to the microphylar end; embryo in irregular shape. (e)Carapace layer around embryo. (f)Dome-like protuberance on protocorm. (g)Ridge like structure on shoot apex. (h)Formation of leaf primordia; root on hypophysis cells. Bar = 50µm (e=embryo; tc=testa cell; mp=microphylar end; c=carapace layer; pt=protuberance; h=hypophysis; lp=leaf primordia; r=root)

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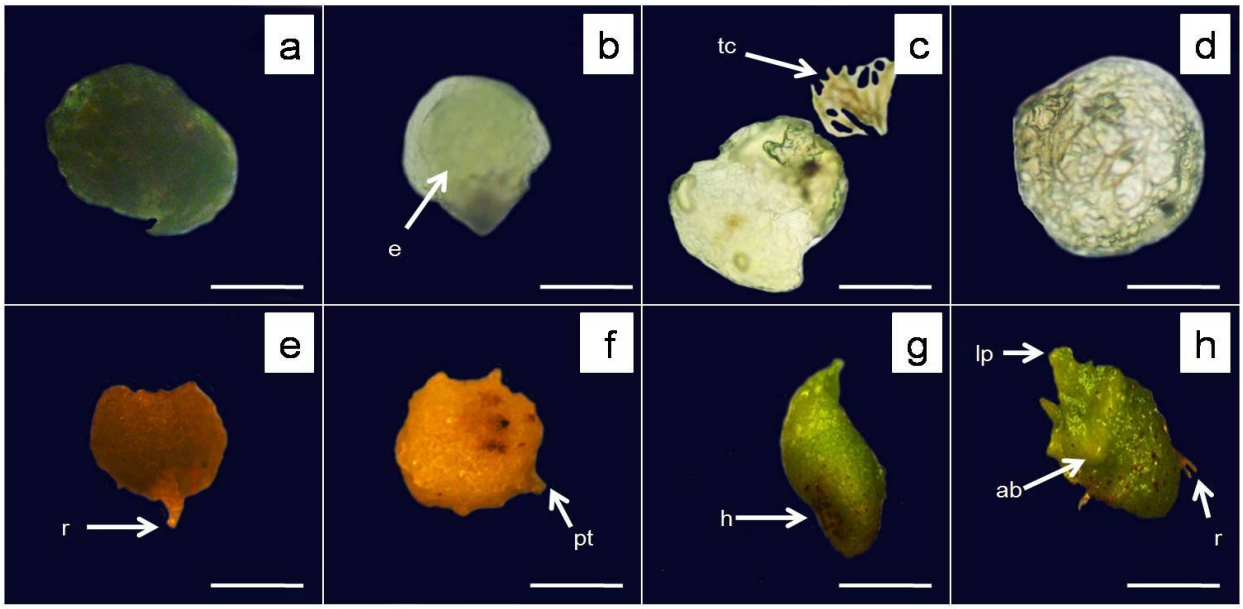

Figure 2. Development stages of AQ × AO hybrid from seed to leaf primordia in asymbiotic culture. (a) Seed fully covered with testa cell. (b) Embryo swelled filling air space within the seed coat. (c) Detachment of testa cell from irregular shaped embryo. (d) Embryo with thin layer of carapace. (e) Rhizoid on protocorm. (f) Dome-like protuberance on protocorm. (g) Ridge-like structure on shoot apex and hypophysis. (h) Apical buds on protocorm surface; formation of leaf primordial; roots on hypophysis cells. Bar = 50µm (e=embryo; tc=testa cell; r=rhizoid; pt=protuberance; h=hypophysis; ab=apical bud; lp=leaf primordia; r=root)

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Molecular Identification and Genetic Relationships of Five *Curcuma alismatifolia* Varieties using ISSR Markers

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Introduction

Curcuma alismatifolia belongs to the Zingiberaceae family. It is native to Indochina (Burma, Cambodia and Thailand) and distributed in all regions of Thailand, at altitudes up to 900 m a.s.l. It is a valuable crop for the cut flower industry and for potted plant production. Thailand exports approximately two million rhizomes per year to Japan, the European Union and the USA (Wichailux, 2005). It has become an important crop for breeding new varieties with novel or improved traits, due to its high economic value as a tropical ornamental (Prathepha, 2000). DNA-based molecular markers are a versatile tool in the fields of taxonomy, physiology, embryology, genetic engineering, etc. (Schlotterer, 2004). Conventional taxonomic techniques in conjunction with molecular biology tools may go a long way in resolving the taxonomic confusion prevailing in the genus. Though a few studies on morphological and anatomical characterization of *Curcuma* species and cultivars have been attempted, not much has been done on molecular characterization (Sasikumar, 2005). *Curcuma* molecular biology studies, so far, are confined to few isozyme-based characterization of germplasm accessions / species (Shamina et al., 1998; Apavatjrut et al., 1999; Paisooksantivatana et al., 2001). Relying much on the morphological characters alone in species delimitation has its own limitations in the genus. Molecular biology techniques like ISSR/RAPD markers thus assume significance. The present work is the first attempt in molecular characterization of 5 economically important *Curcuma* varieties and it adds relevance in the present ongoing context of the taxonomic revision of the genus.

Materials and Methods

The study was conducted at the Floriculture Laboratory, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia. Fully opened fresh tender leaves of the *curcuma* varieties were used for the isolation of DNA. The genomic DNA was isolated by Cetyltrimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). ISSR reaction was carried out in 25 µl reaction volume containing 1µl genomic DNA, 2X DreamTaq™ Green PCR Master Mix (Fermentas, International Inc.) with 1 µM oligodeoxynucleotide primer. Amplification was performed in a thermal cycler (Bio-Rad Laboratories, Inc.). Data were scored as ‘‘1’’ for presence and ‘‘0’’ for absence. The binary data matrix was entered into the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.10e).

Result and Discussion

Ten ISSR primers were detected and a total of 101 amplification fragments, varying from 3 (UBC-847) to 18 (UBC-841) fragments per primer and ranged from 180 to 2151 base pair in size. All tested primers revealed polymorphisms among five varieties ranging from 37% for primer (UBC-842) to 90.9% for primer (UBC-834). The overall polymorphism (PPB) for the 10 primers across all five varieties was 77.2% (Table 1) suggested that ISSR markers were polymorphic markers suitable to detect the genetic diversity of these varieties of *Curcuma* at the DNA level. The estimates of the genetic similarity ranged

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from 40% for the most distant varieties (Doi Tung 554 & Chiang Mai Pink) to 55% between Chiang Mai Red variety and Kimono Pink. The UPGMA dendrogram showed two main clusters (Figure 1).

Table1. ISSR polymorphic primer sequences used for analysis of 5 varieties of *C. alismatifolia* with number of bands amplified, number of polymorphic bands amplified percentage of polymorphism, product size.

Primer	Total number of markers	Number of polymorphic markers	Percentage of polymorphic bands (%)	Product size (range in bp)
UBC_811	10	8	80.00	1517-540
UBC_812	8	5	62.50	1570-348
UBC_818	6	5	83.33	1781-420
UBC_826	10	9	90.00	2019-300
UBC_834	11	10	90.91	1810-413
UBC_835	13	11	84.62	1860-295
UBC_841	18	15	83.33	1860-180
UBC_842	8	3	37.50	2151-234
UBC_847	4	2	50.00	1176-196
UBC_880	13	10	76.92	1907-300
Total	101	78	77.23	
Mean	10.1	7.8		

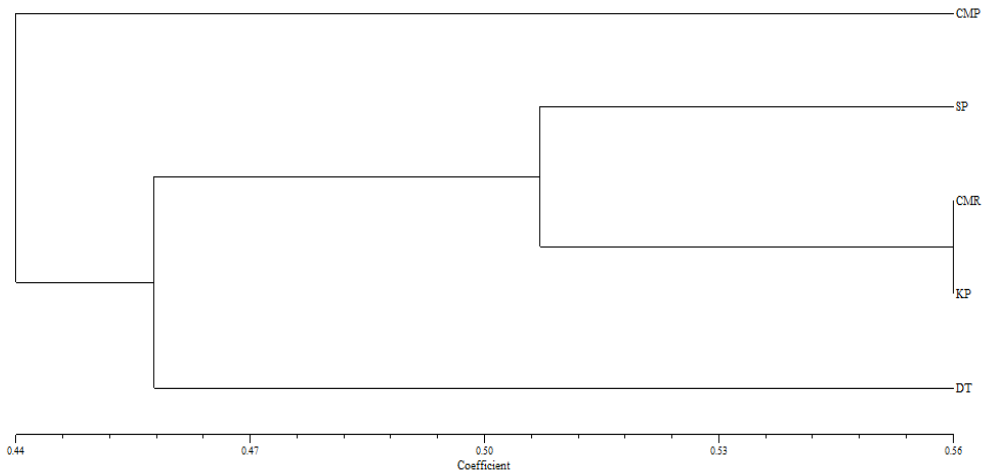

Figure 1. Dendrogram demonstrating the relationships among the five *C. alismatifolia* varieties based on ISSRs

Conclusions

The present investigation clearly demonstrated that the five *Curcuma* varieties could be distinguished by these ISSR primers and a high level of polymorphism of ISSR technique suggests that this marker amplification techniques can be a useful and potentially a powerful technique for genotypic studies in *Curcuma.*

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Characterization of α-Farnesene Synthase Gene from *Polygonum minus*

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Introduction

Polygonum minus or kesum is a herb classified into family Polygonaceae and genus Polygonum. It produces a broad range of volatile compounds such as sesquiterpenes which contribute to the characteristic fragrance of this species (Plant for the future, 1996). In plant, α-farnesene synthase will produce farnesene, which acts as the biosynthetic precursor of the cyclic secondary sesquiterpene aldehydes that carried out various biochemical functions in organisms. Sesquiterpenes including farnesene may be used as medicine, pesticides, fragrance and flavor. Sesquiterpenes are difficult to isolate in large quantities and high purity because plants usually produce low quantities in complex mixture with various structural isomers (Tan et al., 1999). The objective for this research is to study the function of AFS through recombinant protein expression.

Methods

Full length cDNA clone

Total RNA was extracted from leave tissues as a template in the first strand cDNA synthesis of RT-PCR as well as for 5'- and 3'-RACE PCR. A pair of degenerate primers was designed based on two highly conserved regions of several plant species and was used in RT-PCR to obtain a partial cDNA sequence. Upon ligation and transformation, several clones were screened and the positive transformants were sequenced. Isolation of the 5' end and 3' end of AFS cDNA clone was carried out using Clontech SMART^{TM} RACE cDNA Amplification Kit following manufacturer's instructions.

Expression of AFS in Escherichia coli

Recombinant expression of AFS protein was performed in bacterial expression system, *Escherichia coli,* using pQe-2 vector. The recombinant plasmid was transformed into *E. coli* M15.The transformants were grown in Luria Bertani (LB) medium containing 100µg/ml ampisilin and 25µg/ml kanamisin at 37°C until OD_{600} reached 0.6. Then recombinant protein was induced by addition of isopropylthio-b-D-galactoside (IPTG) to a final concentration of 0.5mM, followed by further cultivation at 25°C for 5 hours.

Assay for AFS enzyme activity

The enzyme activity was determined by adding 1 mg of desalted partially purified protein with 40 ul Tris-HCl pH 7.5, 10 µl 1 M MgCl2, 100 µl DTT (2 mM), 20 µl FPP and topped up to 2 ml with dH_2O. The mixture was incubated at 30 ^oC for 90 minutes and followed by headspace GC-MS analysis.

Results and Discussion

The full length sequence of α-farnesene synthase (AFS) cDNA clone from *P. minus* was obtained with the size of 2035 bp encoding predicted protein containing 562 amino acid residues with the expected

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molecular mass of 65 kDa. BLASTx analysis showed that the deduced amino acid sequence of the cDNA was highly similar to sesquiterpene synthase from *Santalum album* (44%) and *Santalum spicatum* (44%), E-beta-farnesene synthase from *Artemisia annua* (40%), delta-cadinene sintase from *Gossypium arboreum* (40%) and *G. hirsutum* (39%) and (E,E)-alpha-farnesene sintase from *Vitis vinifera* (41%). In addition, the AFS amino acid sequence was found to contain the conserved DDXXD and RxR motifs which are motifs for most terpene synthases believed to act as a recognition site for binding of metal diphosphate (Figure 1).

```
G.hirsutum      ASYLRVHGEDILDEAISFTSNHLSLAVASL---DHPLSEEVSHALKQSIRRGLPRVEARH 216
G.arboreum      ASYMRVHGEDILDEAISFTTAQLTLALPTL---HHPLSEQVGHALKQSIRRGLPRVEARN 217
V.vinifera      ATHLMVHGEDILEEALAFTTAHLQSVATDP---NNPLSKQVIRALKLSIHNGVTSVGARH 221
A.annua         AAFMRVEDETILDNALEFTKVHLDIIAKDPS-CDSSLRTQIHQALKQPLRRRLARIEALH 237
P.minus         ACHLRLHGEGVLDEALSSTESNLIKLVSDPNPLSEALEERVKHALHKPLNKRLVRVESVR 223
                * .: :..* :*::*:  *  :*           .*  .: :**: .... :  : : .

G.hirsutum      YLSVYQDIE-SHNKVLLEFAKIDFNMVQLLHRKELSEISRWWKDLDFQRKLPYARDRVVE 275
G.arboreum      FISIYQDLE-SHNKSLLQFAKIDFNLLQLLHRKELSEICRWWKDLDFTRKLPFARDRVVE 276
V.vinifera      YISIYQEDG-SHNESLLKLAKLDFNLLQSLHRKELSEITRWWK-VRLCHEATFARDRLVE 279
A.annua         YMPIYQQET-SHNEVLLKLAKLDFSVLQSMHKKELSHICKWWKDLDLQNKLPYVRDRVVE 296
P.minus         YMQVYEEDDPLHNEALLKFAKLDFNLLQVLHKQELSEMCRWWKKVNMTKKMELR-DRMVE 282
                :: :*::    **: **::**:** ::* :*::***.: :*** : : .:     **:**

G.hirsutum      GYFWISGVYFEPQYSLGRKMLTKVIAMASIVDDTYDSYATYEELIPYTNAIERWDIKCID 335
G.arboreum      GYFWIMGVYFEPQYSLGRKMLTKVIAMASIVDDTYDSYATYDELIPYTNAIERWDIKCMN 336
V.vinifera      IYFSALGVCFEPQYSLSLRFLTKVAIMITMVDDIYDAYGTIEELTLLTEAIERWDASSID 339
A.annua         GYFWILSIYYEPQHARTRMFLMKTCMWLVVLDDTFDNYGTYEELEIFTQAVERWSISCLD 356
P.minus         SYFWGSAVYYEPQFSLARKCNVPGCQILTTLDDLYDAYGTLEELHTFTDAVDKWDKSCLD 342
                 **   .: :***.:             :** :* *.* :**   *:*:::*. .:::
```

Figure 1: Multiple sequence alignment of *P. minus* AFS with the gene from other plants.

AFS was successfully ligated into pQe-2 expression vector and the recombinant expression of AFS protein was induced using 0.5 mM IPTG at 37 °C and 25 °C grown culture. Enzyme activity assay showed that the recombinant protein AFS *P. minus* successfully converted FPP to α-farnesene (Figure 2).

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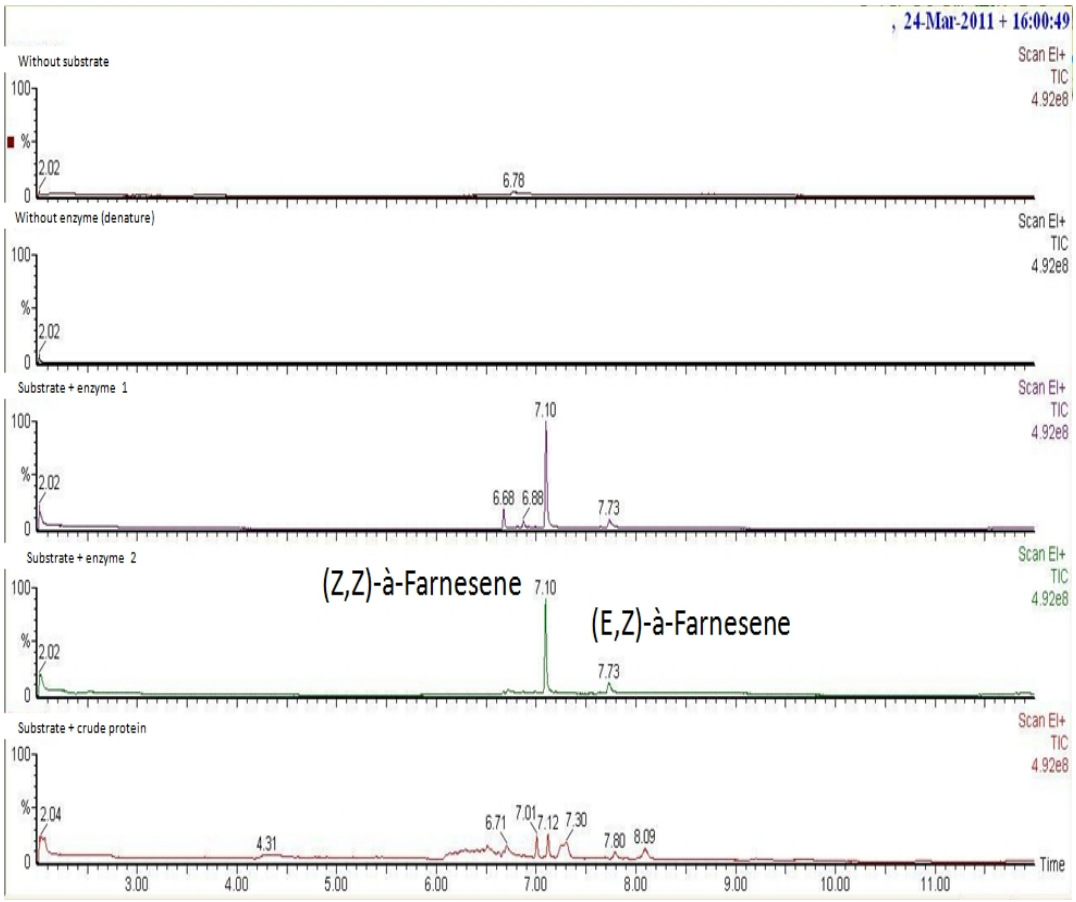

Figure 2: Headspace GC-MS analysis of the product released by the action of AFS on FPP.

Conclusions

Recombinant protein expression for *P. minus* AFS was successfully produced as soluble fraction in *E. coli* M15 cells. Enzyme activity assay showed that the recombinant AFS was able to successfully convert FPP to α-farnesene.

Acknowledgements

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CHAPTER 6

PEST AND DISEASE MANAGEMENT

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Current Status of Sheath Blight Disease in Rice and its Management in Malaysia

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Introduction

Sheath Blight Disease of rice or Penyakit Hawar Seludang Padi is a disease caused by fungal pathogen *Rhizoctonia solani*. It is world major rice disease after Rice Blast caused by *Pyricularia oryzae*. Sheath blight is more consistent but much less dramatic than rice blast, causing routine problems and losses in many fields each year. In India, sheath blight disease accounts for about 20% loss of rice yield (Grover and Pental, 2003). In Japan, the disease caused a yield loss of as high as 20% and infected about 120 000-190 000 ha. A yield loss of 25% was reported if the flag leaves are infected (Castilla et al., 1995). Before the 1970s, sheath blight was a relatively common but minor rice disease in the Midsouth rice producing area of the USA. However, sheath blight is now recognized as the most economically important rice disease in the Midsouth, USA. A yield loss of 50% was reported when susceptible cultivars were planted (Groth et al., 1992). This important disease of rice is also widespread in Arkansas. It is easily found in 50-60% of rice fields randomly surveyed in 1993 and 1994. In Asia and South East Asia, the disease is estimated to lower rice yield by 8 to 50% in the rice growing countries. Like other countries, Malaysia is also facing the same problem with sheath blight disease. It has become one of the major threats to the Malaysian rice industry. The disease is endemic in all major rice growing areas in Malaysia and gained importance with the introduction of nitrogen responsive modern varieties and direct seeding planting system practice in early 1980s. Infected areas had increased about 100-fold from 17 ha in 1985 to 1680 ha in 1994. So far, there is no specific control method for sheath blight control.

Rhizoctoni solani is a very common soilborne pathogen with a great diversity of host plants. It is not only infecting rice but also causing disease in different crops such as bean, tomato, groundnut, maize and carrot (Sneh et al., 1991). The impacts of *R. solani* infection in other crops were not as much effect as in rice infection. It usually starts at the base of the plant near the water level. Later, the symptoms are observed on the upper leaf sheath and on the leaf blade. The disease usually infects the plant at late tillering or early internode elongation growth stages. Disease may spread from one hill to another through leaf-to-leaf or leaf-to-sheath contacts. It is commonly assumed that the critical factors for disease development are relative humidity and temperature. High supply of nitrogen fertilizer (Nathan et al., 2003), and growing of high-yielding, high-tillering, nitrogen-responsive improved varieties favor the development of the disease. High leaf wetness and high frequency of tissue contacts among plants also favor the disease. The pathogen can be spread through irrigation water and by movement of soil and infected crop residues during land preparation. The sclerotia germinate and initiate infection once they get in contact with the rice plant. The fungus penetrates through the cuticle or the stomatal slit. Infection pegs are formed from each lobe of the lobate appressorium of infection cushion. The mycelium grows from the outer surface of the sheath going through the sheath edge and finally through the inner surface. Primary lesions are formed while the mycelium grows rapidly on the surface of the plant tissue and inside its tissue. It proceeds upwards and laterally to initiate formation of the secondary lesions. The disease starts during the maximum growth stage of the rice crop. Under favorable conditions, the disease increases as

the plant grows older. The damage caused by the disease depends on the infection of the plants at plant growth stages.

Initial lesions are small, ellipsoidal or ovoid, greenish-gray and water-soaked and usually develop near the water line in lowland fields. Older lesions are elliptical or ovoid with a grayish white center and light brown to dark brown margin. The lesions may reach the uppermost leaf under favorable conditions. The lesions also may coalesce forming bigger lesions with irregular outline and may cause the death of the whole leaf. Severely infected plants produced poorly filled or empty grains, especially those on the lower portion of the panicles. The disease can be identified by the presence of water soaked lesions and sclerotia. Sclerotia and mycelia may be produced on the lesions (Marzukhi et al., 1991).

Good cultural practices can help to reduce the disease. This can be done by the use of healthy seed, eliminating weed hosts, adopting optimum spacing, avoid excess doses of nitrogen fertilizer and avoid flow of irrigation water from infected fields to healthy fields. Instead of good cultural practices and varietal resistance, control of sheath blight relies heavily on the use of fungicides. Effective fungicides such as difeconazole, propiconazole and validamycin are available to manage the disease but are not considered as long-term solutions due to concerns about exposure risks, health and environmental hazards, residue persistence and development of tolerance. Since the sclerotia of the fungus is soil borne and can survive in soil for years, soil drenching with fungicide is impractical under flooded conditions. Finding a resistance variety is the best way to manage the disease. However, efforts to breed resistant varieties by conventional breeding were hampered by lack of resistant genes against *R. solani* in rice and its related species (Eizenga et al., 2002). Therefore, genetic engineering could be used to develop transgenic rice resistance to sheath blight disease. This can be done by isolating antifungal genes from soil bacteria, developing suitable gene constructs and transformation of the genes into commercial rice varieties to produce functional transgenic.

Materials and Methods

Soil samples were collected from six different locations at paddy fields in Seberang Perai. Each bacterial isolates obtained were screened for antagonistic activity against *R. solani* via dual culture test on potato dextrose agar plate. Antagonist bacteria against *R. solani* were grown in nutrient broth and incubated for 5 days. Antifungal proteins were purified using ammonium sulphate precipitation and column chromatography. Protein bands were identified using N-terminal sequencing and the genes encoding the proteins were isolated using PCR based method. Proteins were expressed in *E. coli* system, purified and tested against *R. solani* using dual culture method. Antifungal genes were transformed into MR 219 rice varieties using *Agrobacterium* mediated transformation technique.

Results and Discussions

A total of 390 bacterial isolates were successfully isolated from rhizosphere soil of low land rice in Seberang Perai area of which only 13 isolates exhibited antagonistic properties against *R. solani*. Inhibition was clearly seen as a clear-zone area surrounding the bacterial colonies with diameter range from 8-20 mm. Four bacterial isolates (SP 184, SP 289, SP 325 and SP 330) which showed the best inhibition zone were further selected for identification using 16S ribosomal RNA sequence-based identification. A BLAST analysis of the 1.5-kb 16S rRNA gene sequence showed high nucleotide identity to 16S rRNA gene of *Bacillus subtilis* and *Bacillus amyloliquefaciens*. However, phylogenetic analysis showed that the antagonist bacterial isolates were found to be closely related to *Bacillus amyloliqufaciens*.

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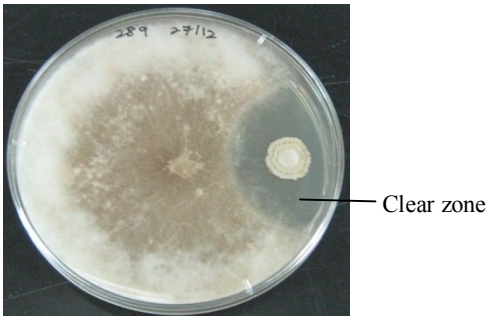

Figure 1. One of the best inhibition zone produced by isolate SP 289 against *R. solani*

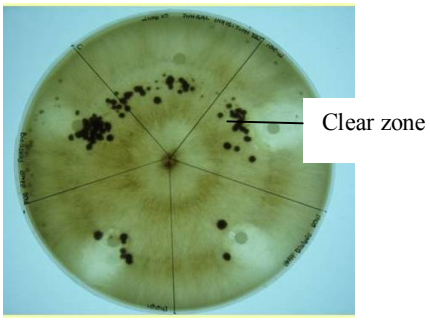

Figure 2. Antifungal assay of recombinant *ynfF* protein against *Rhizoctonia*

One of the isolates, *Bacillus* SP 289 that gave the best inhibition of *R. solani* mycelial growth (Figure 1), was selected for protein purification. Three extracellular antifungal proteins were successfully purified from culture supernatant of *Bacillus* SP 289. The three proteins were identified as *endo-β-1,3-1,4-glucanase*, chitin binding protein and ynfF protein by N-terminal sequence analysis. The genes encoding the three proteins; *β-glu*, *ChbA* and *ynfF* were successfully isolated, cloned and sequenced. The genes are 720 bp, 621 bp and 1272 bp nucleotide long (Figure 3) and encode a polypeptide of 239, 206 and 423 amino acids with a calculated molecular mass of 26.9, 22.5 and 47.8 kDa respectively. Based on protein sequence search using pfam protein families database at ExPASy proteomics server (http://br.expasy.org/tools/), the *Bacillus* SP 289 *endo-β-1,3-1,4- glucanase* hit the glycosyl hydrolase family 16 group and function in hydrolysis of β-D glucosidic linkages in β-D glucans, a major component of fungal cell wall. In addition, the *ChbA* was grouped into the chitin binding domain protein family which involves in binding of the insoluble crystalline chitin thus promote chitin degradation by chitinases. The *ynfF* was placed in the O-glycosly hydrolase family 30- protein family and involves in degradation of xylan, a complex polysaccharide found in plant cell wall including fungi.

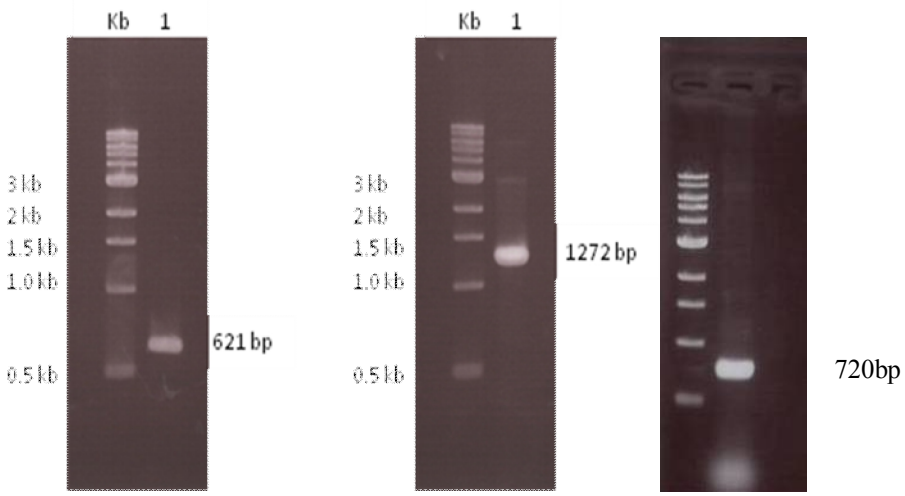

Figure 3. PCR amplification of full length of *Chb A* (621 bp), *ynfF* (1272bp) and *endo-β-1,3-1,4-glucanase* gene (720 bp) from *Bacillus* SP 289.

The three genes were successfully constructed into a plant transformation vector, pCAMBIA 1305.2. The two genes (*β-glu*&*ynfF*) in which the antifungal activity have been validated *in vitro* were transformed into Malaysian rice varieties (MR219) using A*grobacterium* mediated transformation with different parameters. A total of 112,493 of rice calli were used in the transformation. For *β-glu* gene, somatic embryos were obtained after 10-16 weeks on selection medium containing Hygromycin (Hg) whereas for *YnfF* gene, somatic embryos were obtained after 8-14 weeks. To determine the regeneration ability, the initiated green somatic embryos were transferred onto a regeneration medium and cultured for several weeks. The regenerated plantlets were then transferred onto vermiculite and planted in the Transgenic Glass House Complex. Molecular analysis and cultural management of R_0 plants for obtaining their R_1 seeds are now in progress.

Conclusions

The antifungal genes from *Bacillus* SP 289 were successfully transformed into Malaysian local rice varieties, MR 219. Future work would be performed to evaluate the transgenic R_1 plants for resistance against sheath blight disease in contained environment.

Acknowledgements

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Sequence Analysis of Partial Coat Protein Gene of Cucumber Mosaic Virus (CMV) from Chilli Leaves

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Introduction

Interest in agriculture has been intensified where it is heralded as the next (third) engine of growth in the term of Gross Domestic Product (GDP) after manufacturing industries and service sector. It is also listed in the National Key Economic Area (NKEA) of Malaysia with almost 8.44 million hectares or 25% of land area has been devoted to agriculture. On the other hand, Cucumber Mosaic Virus (CMV) is considered as one of the most important viruses infecting the horticultural crops with potential cause of virus epidemics in crops. Infection of many vegetables, fruit and ornamental plants with CMV results in economically important losses in all parts of the world. Since the disease expresses a vast array of symptoms, hence it is difficult to detect its presence by judging the symptoms appeared. CMV is a pathogenic plant virus in the genus Cucumovirus, family Bromoviridae and firstly discovered in cucumber (*Cucumis sativa*) in the USA. It has the reputation of having the widest host range of any known plant virus including monocots and dicots, herbaceous plants, shrubs, and trees (Roossinck, 2002). Palukaitis et al. (2003) reported that CMV host range is estimated to be over 1000 species in 85 families. Chilli (*Capsicum annum*) is considered as one of the most important commodity crops in Malaysia and the occurrences of virus disease on chili have been reported since the British colonial administration. Among them, CMV is one of major diseases in chilli. It was transmitted primarily by aphids and also by contaminated starting plant materials. It was the worse prevalence and account as a major limitation factors in the chili cultivation beside severe economic losses in agricultural sector. Early detection of CMV is essential for controlling the disease.

Materials and Methods

The CMV was propagated in chilli plant at cotyledon stage and grown in a glasshouse. Healthy and CMV-infected chilli leaves were grounded separately using mortar and pestle. Total RNA was obtained from both chilli leaves extracted using conventional phenol-chloroform method. RT-PCR was carried out using the designed primer set CMV168-483. The primer pair was targeted at CMV coat protein gene with expected product of approximately 317 bp in size. The purified specific amplification product was sent for sequencing to confirm the amplicon sequence. Multiple sequence alignment analysis of sequencing results was carried out using BioEdit and Basic Local Alignment Search Tool (BLAST) for verification of amplified sequences with the database. Phylogenetic tree analysis was constructed using Mega 4.1 software.

Results and Discussion

A fragment of approximately 317 base pairs was successfully amplified by conventional RT-PCR technique using total RNA extracted from leaf samples using the primer set, CMV168-483 (Figure 1). The optimum annealing temperature of the primers was found to be at 60 ^{o}C via gradient RT-PCR reaction. Upon purification and sequencing, a total of 317 nucleotides were successfully sequenced (Figure 2). The BLAST results of the sequence showed that the amplified products were 99% identical to the CMV coat protein genes isolated from pepper and cucumber leaves with accession nos. FR820446

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and FN552542, respectively (Figure 3). Both isolates were from Thailand. The coat protein-based phylogenetic analysis of CMV in chilli leaves with other CMV sequences in GenBank was constructed using MEGA v4.1 (Figure 4). It was inferred using the neighbor-joining method with 1,000 replicate bootstraps. In the phylogenetic tree, the 31 isolates were clustered to 3 groups. Group I was the largest group and consist of 15 isolates. Meanwhile Group II and group III contain 12 and 4 isolates, respectively.

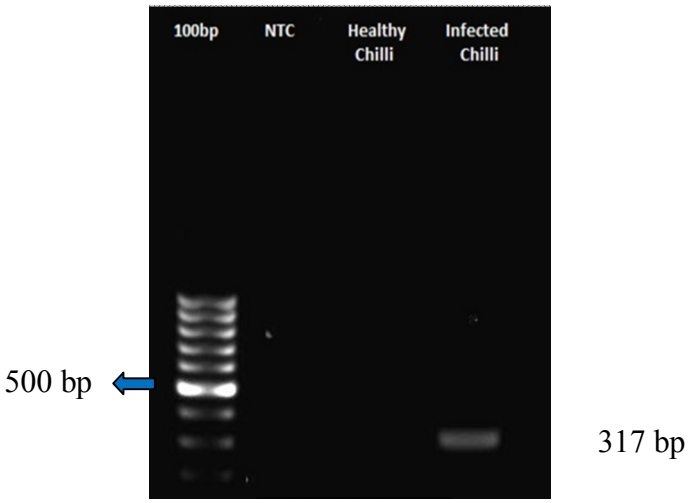

Figure 1**:** Agarose gel electrophoresis of RT-PCR amplification using primer set CMV168-483 using total RNA extracted from chilli leaves.
Lane 1: 100bp DNA Ladder, Lane 2: No-template control, Lane 3: Healthy chilli, Lane 4: Infected CMV chilli leaves.

```
                    10                  20                  30                  40                  50
                    .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |
CMV_Chilli          TAACCTTTGT GGGTAGTGAG CGTTGTAAAC CTGGGTACAC GTTCACCTCG

                    60                  70                  80                  90                  100
                    .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |
CMV_Chilli          ATTACCCTGA AGCCACCGAA AATAGACAAA GGTTCTTATT ATGGCAAAAG

                    110                 120                 130                 140                 150
                    .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |
CMV_Chilli          GTTGTTACTT CCTGATTCAG TCACTGAGTT CGATAAGAAG CTTGTTTCGC

                    160                 170                 180                 190                 200
                    .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |
CMV_Chilli          GCATTCAAAT TCGAGTTAAT CCTTTGCCGA AATTTGATTC TACCGTGTGG

                    210                 220                 230                 240                 250
                    .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |
CMV_Chilli          GTGACGGTCC GTAAAGTTCC TGCCTCCTCG GACCTGTCCG TTTCCGCCAT

                    260                 270                 280                 290                 300
                    .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |   .   .   .   .   |
CMV_Chilli          CTCTGCTATG TTCGCGGACG GAGCCTCACC GGTACTGGTT TATCAGTACG

                    310
                    .   .   .   .   |   .   .   .   .   |   .   .
CMV_Chilli          CTGCATCGGG CGTTCAA
```

Figure 2: Sequencing result: Partial nucleotide sequences of CMV using primer CMV 168-483. Total RNA extracted from Chilli leaves.

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Sequences producing significant alignments:

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
FR820446.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	573	573	99%	2e-160	99%
FN552542.1	Cucumber mosaic virus CP gene for coat protein, genomic RNA, isolat	573	573	99%	2e-160	99%
FM999062.1	Cucumber mosaic virus CP gene for coat protein, isolate HC-56, genc	573	573	99%	2e-160	99%
EF608461.1	Cucumber mosaic virus isolate KPS 10 coat protein (CP) gene, compl	573	573	99%	2e-160	99%
FR820463.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	568	568	99%	1e-158	99%
FR820461.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	568	568	99%	1e-158	99%
FR820460.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	568	568	99%	1e-158	99%
FR820459.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	568	568	99%	1e-158	99%
FR820458.1	Cucumber mosaic virus partial CP gene for coat protein, isolate PaN4	568	568	99%	1e-158	99%
FR820457.1	Cucumber mosaic virus partial CP gene for coat protein, isolate PaN3	568	568	99%	1e-158	99%
FR820456.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	568	568	99%	1e-158	99%
FR820455.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	568	568	99%	1e-158	99%
FN552545.1	Cucumber mosaic virus CP gene for coat protein, genomic RNA, isolat	568	568	99%	1e-158	99%
FR820474.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RatS	562	562	99%	5e-157	98%
FR820473.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RatS	562	562	99%	5e-157	98%
FR820472.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RatS	562	562	99%	5e-157	98%
FR820469.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RaS8	562	562	99%	5e-157	98%
FR820468.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RaS7	562	562	99%	5e-157	98%
FR820466.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RaS4	562	562	99%	5e-157	98%
FR820465.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RaS3	562	562	99%	5e-157	98%
FR820464.1	Cucumber mosaic virus partial CP gene for coat protein, isolate RaS2	562	562	99%	5e-157	98%
FR820462.1	Cucumber mosaic virus partial CP gene for coat protein, isolate KhaP	562	562	99%	5e-157	98%

Figure 3: The BLAST results of the amplified product with CMV coat protein genes in GenBank database.

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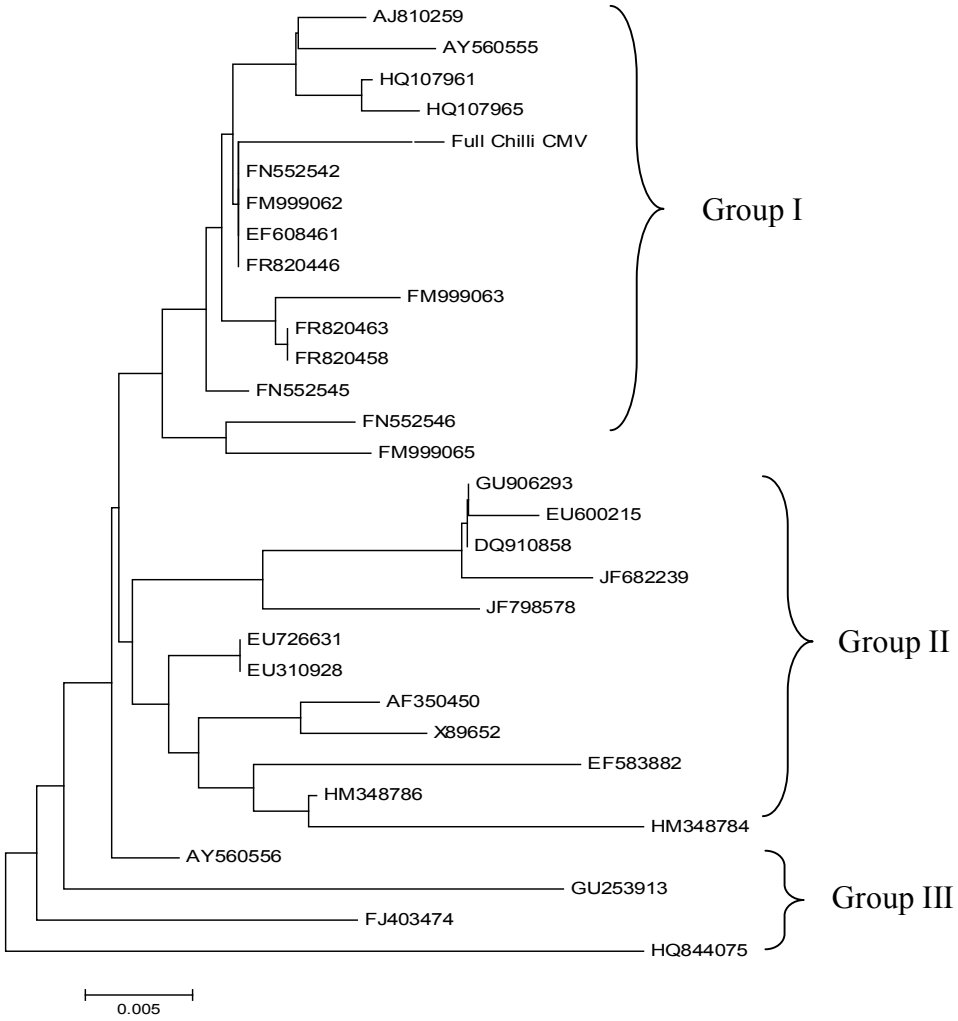

Figure 4. Phylogenetic tree of partial coat protein gene of cucumber mosaic virus from chilli leaves.

Conclusions

The developed primer set CMV168-483 managed to amplify coat protein gene of CMV from chilli leaves. Nevertheless further experiments need to be done to test this primer with samples of related virus species to check for any cross reactivity. Field trial with samples of other plant species should be strongly required.

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A Survey on Contamination of *E.coli* O157:H7 on Butterhead Lettuce

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Introduction

Escherichia coli O157:H7 is a bacterial contaminant which may affect the product quality (Sudheer and Indira, 2007) including butterhead lettuce. Mukherjee et al. (2004) stated that lettuce posed a food safety risk because it is consumed raw and susceptible to be contaminated during growth, harvest, packaging, transportation, processing and handling. The symptoms of the disease caused by *E. coli* O157:H7 ranged from mild diarrhea and hemorrhagic colitis to complicated infections such as hemolytic uremic system (HUS) and thrombocytopaenicpurpura (Law 2000; Willshaw et al., 2001; Meng et al., 2007). In order to control the bacterial contamination on butterhead lettuce, the microbial recommended limit was suggested for safe consumer consumption. Study on *E. coli* O157:H7 contamination of lettuce seemed inevitable especially in Malaysia because until now, no specific data regarding this contamination have been reported. Furthermore, this is gaining ground since awareness among the Malaysians on the food safety aspects is on the increase. Therefore, the objective of this study was to determine the contamination of *E. coli* O157:H7 on butterhead lettuce.

Materials and Methods

Sample collection

The fresh butterhead lettuces (*Lactuca sativa* var. capitata) were purchased from four local producers which were assigned as producer 1, 2, 3 and 4. The samples from each producer were replicated four times. The samples were immediately transported to Postharvest Laboratory, Crop Science Department, Universiti Putra Malaysia, for microbial analysis. The outer damaged leaves and core were removed aseptically and discarded.

Enumeration of bacteria

The whole heads were shredded and only ten grams of shredded lettuce was weighed and transferred to 90 mL sterile distilled water in sterile conical flask. The samples were homogenized by shaker for two minutes at 230 rpm. Serial dilutions of 10-fold up to 10^{-5} for each homogenized sample was made and surface spread (100 µl $plate^{-1}$) in triplicate onto selective media (Hicrome *E. coli* O157:H7). The plates were incubated at 36 ºC for 18 to 24h (Zadik et al*.,* 1993). After incubation, the number of colonies which appeared as dark purple to magenta coloured moiety was estimated using standard plate count method.

Statistical analysis

The data was analyzed by ANOVA using SAS program. When F test was significant, the multiple mean comparisons of LSD were conducted at $p < 0.05$.

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Results and Discussion

There was significant difference of *E. coli* O157:H7 counts between producers 2 and 4 (Figure 1). However, the number of *E. coli* O157:H7 from producer 1 was not significantly different from producer 2 as well as between producers 3 and 4. The highest mean of *E. coli* O157:H7 counts were 6.95 log $CFUg^{-1}$ from producer 2. It was still in the range indicated by several previous reports which the bacterial counts in unprocessed and minimally processed lettuce were 5 to 7 log CFU g^{-1} (Carlin et al., 1989; Khan et al., 1994; Nguyen-the and Carlin, 1994; Jacques and Morris, 1995; Ahvenainen, 1996; Babic et al,. 1996; Kaneko et al., 1999). The lowest mean of *E. coli* O157:H7 number was 4.59 log $CFUg^{-1}$ from producer 4.The bar chart showed that the lettuce contamination of *E. coli* O157:H7 from producers 1 and 2 were approaching the microbial recommended limit, 8 log $CFUg^{-1}$ or 10^8 $CFUg^{-1}$ as proposed by Debevere (1996). Overall, the contamination with *E. coli* O157:H7 of butterhead lettuce from all producers was still below the recommended limit.

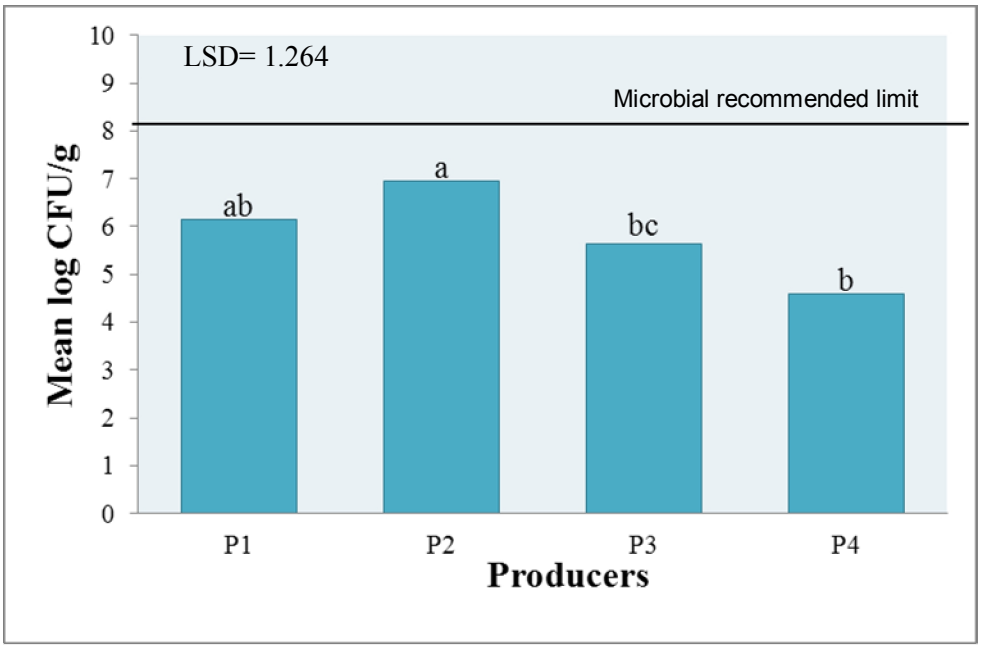

Figure 1. The mean log $CFUg^{-1}$ of *E. coli* O157:H7 contamination on butterhead lettuce from different producers

Conclusions

E. coli O157:H7 contamination of butterhead lettuce in Malaysia is still within the safe limit for the consumers. However, contamination of *E. coli* O157:H7 from farms of two producers, P1 and P2 were alarming since the counts obtained, 6.95 and 6.65 log $CFUg^{-1}$ were approaching the maximum recommended limit. If this is left uncheck, one day it may cause the outbreak of serious diseases from this bacteria. Hence, some interventions should be done to reduce this contamination besides hygienic practices which should be mandatorily applied at every point of production chain to ensure safe consumption by human beings.

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Overview of *Cucumber Mosaic Virus* Antibody Production in Rabbits

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Introduction

Cucumber mosaic virus (CMV) is the type species of the genus *Cucumovirus,* belongs to family Bromoviridae. It is distributed worldwide and has a very large host range, infecting more than 1000 plant species including monocots and dicots, herbaceous plants, shrubs, and trees. This common plant virus caused yellow mottling, distorted leaves and stunted growth in a wide range of garden plants, not just cucumbers. CMV is aphid transmissible in a non-persistent manner by more than 60 aphid species, and causes severe diseases in various crops all over the world (Roossinck, 2002). Damage to crop plants due to virus's infections is difficult to assess and actual figures for global crop loss are not available. According to Hsu (2002), losses due to plant diseases and infections are estimated to be $60 billion annually.

The development of effective control strategies is dependent mainly on the availability of reliable methods for detection and identification. Virus detection methods have improved greatly in recent years with the development of molecular diagnostic techniques such as real-time polymerase chain reaction (PCR) or microarrays (Vincelli and Tisserat, 2008). However, these techniques have their limitations. Therefore, it is suggested that serological based tests, such as lateral flow immunoassay (LFA) possibly be applied as a method for CMV detection in plants. LFA is a simple device used to detect the presence/absence of analytes including antigen and antibodies. This device is very sensitive, detecting viruses up to 90-97% accuracy and has been the method of choice for the detection and assay of various plant viruses because it is sufficiently sensitive for most applications (Salomone et al., 2004).

In an antibody LFA set up, plant-pathogen specific antibody are mainly based on injection of whole pathogens, surface fragments or soluble surface components into a suitable animal-host for polyclonal antibody (PAb) (Werres and Steffens, 1994). Obtaining pathogen-specific antibodies is a challenging task and antibodies should preferably be directed against a single target molecule that is surface exposed and constitutively expressed in the target species only (Leonard et al., 2003). Thus, the aim of this work was to produce a specific and sensitive CMV PAb in rabbits that will be utilized in the development of a CMV LFA.

Materials and Methods

CMV isolate

The same isolate of CMV was used throughout the experiments. The virus was cultured in tobacco plants, grown in a greenhouse at 20-25 ^{o}C. CMV was purified as previously described by Mossop et al. (1976). Virus concentration was estimated by measuring the absorbance with a spectrophotometer at 260nm.

Production of PAb

Male rabbit (3 months old) was immunized subcutaneously with purified CMV antigen as immunogen for 4 times and after a two week rest, blood samples were taken for antibody purification including pre-

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immune serum (blood taken before any immunization). Booster injections were given fortnightly. A total of 5 bleeds were conducted.

Purification of PAb

Immunized rabbit sera were collected and purified by precipitation with ammonium sulphate and passaged through a column protein A. Antiserum was applied to a column filled with sorbent and then allowed to flow through the chromatographic column with immobilized protein A. The column was washed with washing buffer, eluted with eluting buffer and neutralized with glycine-HCl buffer. The purified PAb was then subjected to three times dialysis buffer change before being stored at 4 ^{o}C until further use.

Results and Discussion

In this experiment, rabbit was immunized with purified CMV. After multiple immunizations, blood was collected and column chromatography was used for antibody purification. The purification of antibodies presents several practical complications, especially for polyclonal antibody production (Verdoliva et al., 2000). Separation and recovery of proteins from column chromatography are affected by factors such as buffer type and pH, length of gradient, flow rate of the mobile phase and characteristic of the proteins. The selection of ideal conditions for protein purification involves changing some or all of these parameters (Tishchenko et al., 1998). Furthermore, column chromatography is considered as an economical alternative to affinity and immunoaffinity chromatography.

Purification of polyclonal antibody involves two steps of procedures, ammonium sulphate precipitation and column chromatography using protein A. Figure 1 exhibits the peaks obtained during purification process. The first two peaks obtained were during the binding process with phosphate buffer whereas the third peak was obtained after the elution buffer (glycine-HCl) was run through the column.

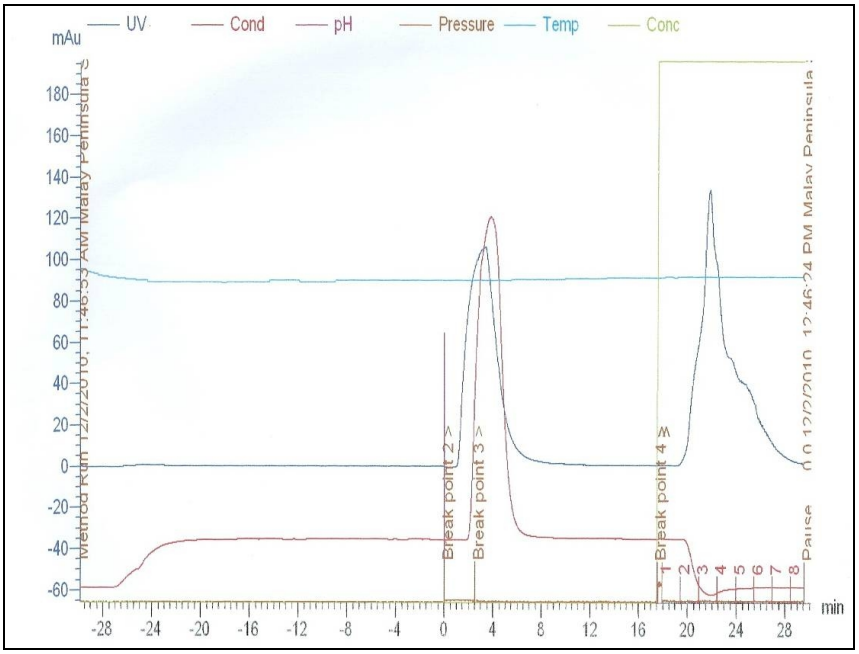

Figure 1: Peaks obtained during the antibody purification process using column chromatography Protein A.

The column chromatography analysis showed that purification of antiserum resulted in a purified product. Purified PAb was collected during the elution process. The production of purified PAb is an efficient means of long-term maintenance of antibodies. This is especially important since Protein A has a significant role in immunomodulation for example in treatments for various types of inflammatory diseases in humans and plant disease detection (Low et al., 2006).

Conclusion

It was observed that the antibody purification was successful and the antibody was purified through the process.

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Floristic Composition of Weed Community in Selected Organic Vegetable Fields in Malaysia

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Introduction

There is a high demand for organic vegetables as they are believed to be clean and safe. Thus, the Malaysian government is promoting organic farming. Organic farms occupy about 11,000 hectares of agriculture land (Ramli, 2004). Weeds are known to cause up to 45% of economic and yield losses in crop growing areas (Kumar and Jagannathan, 2003). Therefore, a weed survey could be useful for determining the occurrence and importance of weed species in any production system and area (Frick and Thomas, 1992; McCloskey et al., 1998). Data from surveys can be useful for research and extension programs by identifying troublesome weeds in a given area over time (Coble, 1994; Elmore, 1984) and by evaluating the rate of weed development (Loux and Berry, 1991; Webster and Coble, 1997). The latter is particularly beneficial when considering the threat of potentially invasive species from becoming established in greater abundance or the development of herbicide resistant weed populations. The objective of the study was to determine the composition of weed community in selected organic vegetable fields.

Materials and Methods

A survey was conducted, to identify weeds, in five organic leafy vegetable fields. The leafy vegetables were mustard, sweet potato, salad, kangkung and bayam grown during August-October, 2010. The survey was done according to the quantitative survey method described by Thomas (1985). An inverted "W" pattern was used to collect systematic samples in each field. Five locations were sampled along each arm of the "W" pattern, resulting in 20 locations. In a uniform field, the first-encountered corner of the field was the starting point. One hundred paces along the field edge and 100 paces into the field marked the first weed counting site. The size of quadrat used was 0.5 m x 0.5 m. The distance between each quadrat depended upon the size and shape of the field and obstructions present in the field. All weeds, in each quadrat, were identified, counted and recorded. Species that were not identified in the field were tagged and transported to the laboratory for later identification (Chancellor and Williams, 1982; Chancellor and Williams, 1984). Care was taken to ensure that anomalies such as shoulder and foot slopes, potholes, ditches, bluffs, power lines and paths were not sampled. The data were summarized using four quantitative measures as outlined by Thomas (1985); frequency (F), field uniformity (FU) over all fields, field density (FD), and relative abundance (RA). F was calculated as the percentage of the total number of fields surveyed in which a species occurred in at least one quadrat.

$$F_k = \frac{\sum_{1}^{n} Y_i}{n} \times 100$$

Where F_k= frequency value for species k
Y_i= presence (1) or absence (0) of species k in field sized quadrat used
n = number of fields surveyed.

FU was calculated as the percentage of the total number of quadrats sampled in which a species occurred.

$$FU_k = \frac{\sum_{1}^{n}\sum_{1}^{20} X_{ij}}{20n} \times 100$$

Where FU_k = field uniformity value for species k
X_{ij}= presence (1) or absence (0) of species k in quadrat j in the field$i$
n = number of fields surveyed.

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The density of each species in a field was calculated by summing the number of plants in all quadrats and dividing by area of 20 quadrats.

$$D_{ki} = \frac{\sum_{1}^{20} Z_i}{A_i}$$

Where D_{ki} = density (in numbers m-2) value of species *k* in the field*i*
Z_i= number of plants of a species in quadrat *j* (a quadrat is 0.252 m)
A_i= area in m2 of 20 quadrats in the field*i*.

MFD is the mean number of plants m^{-2} for each species averaged over all fields sampled.

$$MFD_k = \frac{\sum_{1}^{n} D_{ki}}{n}$$

Where MFD_k = mean field density of species *k*
D_{ki} = density (in numbers m-2) of species *k* in the field*i*
n = number of fields surveyed.

RA was used to rank the weed species in the survey. It was assumed that the F, FU, and MFD were equally relevant in describing a weed species.Such values have no unit, but the value of one species in comparison to another, indicates the relative abundance of the species (Thomas and Wise, 1987). The relative frequency (RF), relative field uniformity (RFU) and relative mean field density (RMFD) were calculated by dividing the parameter by the sum of the values for that parameter for all species and multiplying by 100. The relative abundance of species k (*RA*k) was calculated as the sum of RF, RFU and RMFD for that species;

RA*k*= RF*k*+ RFU*k*+ RMFD*k*

RA value is an index that was calculated using a combination of F, RFU and RMFD for each species, as described by Thomas (1985). The sum of the RA values, for all species in a community was 300, and it allowed for comparison of the overall abundance of one weed species vs. another.

Results and Discussion

A total of 27 different weed species belonging to 12 families were identified in the different organic vegetable fields, of which 18 were annuals and 9 were perennials; 3 grassy weeds, 7 sedges and 17 broadleaf weeds (Table 1). In terms of frequencies among the grasses, the most common and frequent grasses were *Axonopus compressus* (Sw.) P. Beauv., *Eleusineindica* (L.) and *Eleuthranthera ruderalis.*Among the sedges, the most wide spread weed species in terms of frequencies were *Cyperus kyllingia* Endl. *Cyperus iria* L. and *Cyperus aromaticus* L. occurred in frequencies of > 40%. In broadleaves, the most frequent weed species was *Ageratum conyzoides* L. along withother weeds of frequencies ≥ 40%, *Amaranthus spinosus* L., *Borrerias setidenns, Cleome rutidiosperma* D.C., *Lindernia crustacean* (L.), *Hedyoti scorymbosa* (L.) Lamk, *Ipomoea triloba* L., *Mimosa pudica* L., *Phyllanthus niruri* L., *Phyllanthus urinaria* and *Scoparia dulcis*. Frequencies of the remaining grasses, sedges, and broadleaves were 20 to 40% (Table 1). FU is a quantitative measure of the spread of a weed species within a given field. Grasses (*Paspalum scrobiculatum* Linn. and *Cynodon dactylon* L. Pers.), sedges (*Cyperus rotundus* L., *Cyperus kyllingia* Endl. and *Cyperus iria* L.), and broadleaf *(Ageratum conyzoides* L.) weeds were uniformly distributed throughout the fields (Table 1). *Ageratum conyzoides* L. was the most abundant weed, with a density of 42.5 plants m^{-2}, while *Amaranthus spinosus* L. was second in abundance, with a density of 31.9 plants m^{-2} (Table1).When the field weed's density was examined. It was found that the density of most species increased compared to densities obtained from all fields. For brevity, only species that appeared in ten or more fields were ranked according to RA value (Table 2). RA

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provides an indication of the overall weed problem posed by a species. In descending order, the top 10 species that had high RA values included *Amaranthus spinosus* L. Thomas (1985) observed from weed survey that the RA value clearly indicated a remarkably few dominated weed species. Similarly, Moody and Drost (1983) observed that the dominant weedflora in any crop field is usually about 10 species of which the dominant one rarely were more than 3 to 4.

Table 1. Scientific and common names, frequency (F), field uniformity (FU), Mean field density (MFD) of weeds in selected organic vegetable fields of Malaysia. A= annual, P=perennial.

Scientific name	Common name	Life cycle	F	FU	MFD
Amaranthus spinosus L.	Spiny pig-weed; Needle burr	A	71.4	40.7	749.7
*Ageratum conyzoides*L.	Goat weed	A	100.0	65.0	848.6
Borreria setidenns	Buttonweed	A	57.1	15.0	202.3
Axonopus compressus (Sw.) P.Beauv.	Carpet grass; Common lawn grass	A	57.1	30.7	364.0
Cleome rutidiosperma DC.	Yellow cleome	A	71.4	24.3	204.6
Cyperu srotundus	Purple Nut sedge	P	28.6	13.6	572.0
Cyperu skyllingia Endl.	White kyllingia	P	100.0	22.9	246.9
Cyperus iria L.	Grasshoppers cyperus	A	14.3	4.3	36.6
Cyperus aromaticus L.	Greater kyllingia	P	28.6	1.4	43.4
Cyperus esculentus L.	Yellow nutsedge	P	57.1	19.3	294.3
Cyperus imbricatu Retz.	C. RadiatusVahl	P	14.3	0.7	1.7
Eleusine indica (L.) Gaertn	Goosegrass	A	14.3	10.7	161.7
Euphorbia hirta L.	Hairy sprurge	A	71.4	17.9	59.4
Edipta prostata	American false daisy	A	28.6	8.6	57.7
Echinochloa colonum(L.) Link.	Jungle rice	A	28.6	2.9	57.1
Eleuthranthera ruderalis	E. ovataPoit. ; E. Prostrata Sch. Bip.	A	85.7	39.3	595.4
Emilia sonchifolia (L.) DC.	Purple sowthistle	A	14.3	1.4	3.4
Fimbristylis miliacea (L.) Vahl.	Lesser fimbristylis	A	14.3	0.7	2.9
Hedyotis corymbosa (L.) Lamk.	Two flowered oldenlandia	A	14.3	1.4	5.1
Hedyotis verticillata L.	Woody borreria	P	28.6	5.7	22.9
Ipomoea triloba L.	Little bell	A	57.1	25.7	160.6
Lindernia crustacea (L.)	Malaysian false pimpernel	P	14.3	4.3	22.9
Mimosa pudica L.	Sensitive plant	P	42.9	30.0	18.3
Portulaca oleracae L.	Pig-weed	I	14.3	4.3	36.0
Phyllanthus niruri L.	Purple Nut sedge	A	71.4	11.4	33.7
Phyllanthus urinaria	Chamber bitter	A	28.6	1.4	2.3
Scoparia duscis	Sweet broom weed	A	14.3	1.4	2.3

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Table 2. Scientific name, relative abundance (RA) and weed types in selected organic vegetable fields in Malaysia.

Scientific name	RA	Weed type
Ageratum conyzoides L.	42.5	Broadleaf weed
Amaranthus spinosus L.	31.9	Broadleaf weed
Eleuthranthera ruderalis	29.6	Broadleaf weed
Axonopus compressus (Sw.) P.Beauv.	20.2	Sedges
Cyperus kyllingia Endl.	19.5	Sedges
Cyperus rotundus	17.8	Sedges
Cleome rutidiosperma DC.	16.5	Broadleaf weed
Euphorbia hirta L.	15.9	Broadleaf weed
Ipomoea triloba L.	14.7	Broadleaf weed
Borreria setidenns	12.9	Broadleaf weed

Conclusions

A useful feature of the survey system was the method of ranking species based on RA values. This survey provides the first quantitative comparison of the common species. Among the 10 abundant species, *Ageratum conyzoides* L. and *Amaranthus spinosus* L., were the most abundant weeds in vegetable fields followed by *Eleuthranthera ruderalis*, *Axonopus compressus* (Sw.), P. Beauvand and *Cyperus kyllingia* Endl. More survey work is needed to identify problematic weeds and weed population shifts and direct research towards new or improved control measures.

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