TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 22

Innovative Plant Productivity and Quality

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24th Malaysian Society of Plant Physiology Conference (MSPPC 2013)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

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Organized by

Malaysian Society of Plant Physiology

Publisher

Malaysian Society of Plant Physiology
(Persatuan Fisiologi Tumbuhan Malaysia)
Beg Berkunci No. 282, Pejabat Pos UPM
43409 UPM, Serdang, Selangor
URL: http//mspp.org.my

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

ISBN 978-967-10840-3-8
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CHAPTER 1

PLANT GROWTH, DEVELOPMENT AND PRODUCTION

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Preliminary Study on the Effect of Organic Soil Amendment and Fertilizer on Growth and Phytochemical Concentration of *Cynodon dactylon* (L.) Pers.

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Introduction

Cynodon dactylon L. (Pers.) or Bermuda grass is one of the important grasses in the world. Its cultivars are among the best turfgrasses for warm and temperate regions. It is also known for its antioxidant and antibacterial properties as well as medicinal value to treat diabetes, diarrhea, ulcer and arrhythmia (Suresh et al., 2008; Rai et al., 2009; Badri and Renu, 2011; Rao et al., 2011). In preliminary study carried out by Syariel et al. (2012), local *C. dactylon* was found to contain bioactive compounds that inhibit some microorganism activities. The growth and phytochemical concentration in crop can be influenced by the modification of nutrient intake (Mohd Hafiz et al., 2011). However, to date, little is known about the phytochemistry and growth of organically cultivated Bermuda grass. Hence, the aim of this study was to investigate the effect of organic soil amendment and fertilizer on growth and phytochemical concentration of *C. dactylon.* The result can form the basis to plan the fertilization regime to produce *C. dactylon* for pharmaceutical purposes.

Materials and Methods

Field experiments

Wild ecotype of *C. dactylon* was collected at Batu 10, Sandakan Sabah. Healthy and disease free stolons of the grass were collected and replanted in trays measuring 33 cm x 25 cm x 8.5 cm. Three treatments were studied (NPK 15-15-15, bioorganic fertilizer 5-5-5 and Mushroom Medium Residue, MMR) at three nitrogen (N) concentrations (1.5, 2.5 and 3.5 gm^{-2}). Control treatment was standard turfgrass medium (1 soil: 1 sand). Shoot density, shoot length and leaf width was measured for 18 weeks, before the grass was harvested for phytochemical analysis**.**

Plant extraction

The grass from each treatment was cleaned under running tap-water, air-dried in drying chamber at room temperature for 24h, and ground into powder. Fifty (50) gram of the grass powder was soaked in 100 ml of 95% (v/v) ethanol and kept in platform shaker for 24h at 150 rpm. The extract was filtered using Whatmann filter paper No. 1. The residue was re-collected and re-extracted twice to obtain a complete extraction. The filtrate was concentrated under reduced pressure at 55 ºC using rotary evaporator.

Total phenolics and total flavonoid quantification

Phenolic content was determined using the modified method of Sunita and Dhanajay (2010). One ml of the extract was mixed with 5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 4 ml of 7.5% (w/v) sodium carbonate. The mixture was left for 30 minutes at room temperature and absorbance was measured at 760 nm. Total phenolic content was expressed as mg gallic acid equivalent (GAE) per g dry sample. Flavonoids content was determined using aluminum chloride method with quercetin as the standard (Chang et al., 2002). The mixture was left for 30 minutes at room temperature, and absorbance was measured at 415 nm. The reading was expressed as mg quercetin equivalent (QE) per g dry sample.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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Statistical analysis

The data were analyzed using One-Way Analysis of Variance (ANOVA; SPSS® software version 21). Significant difference of the test was evaluated at $P<0.05$.

Results and Discussion

Stolon density, leaf length and leaf width of the grass are shown in Table 1. MMR at all levels of N concentrations (1.5, 2.5 and 3.5 g N/m^2) was relatively better than the other treatments in improving new shoot formation (212-218 tillers per 825 cm sq^2) and stolon length (20-22 cm long). All treatments did not affect leaf width. This result was concurrent with previous study reported by Gobilik et al. (2011), that mixture of MMR and soil (1:1) improved density, leaf width and moisture content of *Zoysia matrella* turfgrass. The MMR addition also increased the growth and total yield of tomato and cucumber as reported by Polat et al. (2009) and Zhang et al. (2012). This beneficial characteristic of MMR might be supported by its high organic matter content, high nutrient capacity holding and neutral pH (Sues and Curtis, 2006).

Table 1. Effect of organic soil amendment and fertilizer on growth, total phenolic and total flavonoid contents of *C. dactylon*

Treatment	Total N (gm^{-2})	Stolon density (no. of tillers)	Stolon length (cm)	Leaf width (mm)	Total phenolic (mg GAE/g dry sample)	Total Flavonoid (mg QE/g dry sample)
Control	0	68 ± 12.19^{e}	14 ± 3.35^{d}	1.78 ± 0.20	0.26 ± 0.01^{f}	0.62 ± 0.01^{d}
NPK (15-15-15)	1.5	115 ± 14.36^{d}	18 ± 2.19^{bc}	1.84 ± 0.15	0.17 ± 0.01^{h}	0.28 ± 0.00^{i}
	2.5	136 ± 9.79^{c}	19 ± 1.12^{abc}	1.86 ± 0.21	0.37 ± 0.01^{b}	0.67 ± 0.00^{b}
	3.5	168 ± 16.98^{b}	19 ± 2.76^{abc}	1.90 ± 0.07	0.36 ± 0.01^{b}	0.47 ± 0.00^{f}
Bioorganic fertilizer (5-5-5)	1.5	82 ± 9.73^{e}	16 ± 1.88^{cd}	1.88 ± 0.11	0.51 ± 0.01^{a}	0.65 ± 0.00^{c}
	2.5	104 ± 5.59^{d}	17 ± 1.77^{bcd}	1.86 ± 0.17	0.33 ± 0.01^{c}	0.37 ± 0.00^{g}
	3.5	111 ± 15.01^{d}	17 ± 2.19^{bcd}	1.88 ± 0.08	0.30 ± 0.01^{d}	0.47 ± 0.02^{f}
Mushroom Medium Residue	1.5	217 ± 19.88^{a}	21 ± 3.67^{ab}	1.76 ± 0.09	0.34 ± 0.01^{c}	0.75 ± 0.01^{a}
	2.5	212 ± 17.99^{a}	20 ± 2.88^{ab}	1.72 ± 0.04	0.27 ± 0.01^{e}	0.50 ± 0.01^{e}
	3.5	218 ± 12.65^{a}	22 ± 1.85^{a}	1.76 ± 0.15	0.20 ± 0.00^{g}	0.29 ± 0.00^{h}
Sig.		0.000	0.001	0.432	0.000	0.000

Means followed by different letters in the same column are significantly different at p<0.05 (Duncan's multiple range test).

The total phenolic and flavonoid contents of the grass are shown in Table 1. Highest total phenolic content (0.51 ± 0.01 mg GAE/g dry sample) was found in bioorganic fertilized grass (at 1.5 g N/m^2). For total flavonoid content, it was in MMR fertilized grass (0.75 ± 0.01 mg QE/g dry sample at 1.5 g N/m^2 MMR). Production of the compound was poor in the medium with no nitrogen content. This result also suggested that low amount of nitrogen in bioorganic fertilizer and MMR (1.5 g N/m^2) increased the amount of phenolic and flavonoid content of *C. dactylon* compared to the same treatments with higher nitrogen concentration. This result was supported by earlier studies which indicated that amendment of low nitrogen fertilization increased the production of phenolic and flavonoid content in cassava tuber (NurFaezah et al., 2012), broccoli (Naguib et al., 2012) and Kacip Fatimah (Mohd Hafiz et al., 2011). The increased phenolic content under bioorganic fertilization can be associated with the ability of the fertilizer to induce the acetate shikimate pathway, a pathway that could lead to higher production of flavonoids and phenolics (Sousa et al., 2008).

Conclusions

The results suggested two important points. First, MMR allowed better growth of *C. dactylon* as compared to the standard turfgrass medium or medium with NPK and bioorganic application. Another point was, MMR and bioorganic fertilizer at low concentration (1.5 g N/m^2) induced higher phenolic and flavonoid production, meanwhile over fertilization suppressed the production of the compound. Future study suggested is to repeat this experiment under controlled environment.

Acknowledgements

This study received funding from the Ministry of Higher Education (MOHE), Malaysia (Grant code: FRG0288-STWN-2/2010). We also thank the staff of the field laboratory of the School of Sustainable Agriculture, Universiti Malaysia Sabah for helping us in various ways throughout the course of this study.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Viability of *Gigantochloa albociliata* (Buluh Madu) Plantation for Shoot Production in Peninsular Malaysia

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Introduction

Currently, most bamboo shoots in the local market are imported from China and Thailand. Bamboo shoots in Malaysia are produced in small quantities by the local people. They collect the bamboo shoots from the forests for the local market (Abd. Razak and Aminuddin, 1992).

The species *Gigantochloa albociliata* (buluh madu), a popular source of bamboo shoots, is not from Malaysia. It is native to Burma and Thailand. Most *G. albociliata* planted in Malaysia originated from Thailand. This bamboo was introduced and planted in Malaysia because of the high demand for bamboo shoots. Other bamboo species usually used by the local people for shoot production are *Gigantochloa levis* (buluh beting), *Dendrocalamus asper* (buluh betong), *Gigantochloa ligulata* (buluh tumpat), *Bambusa blumeana* (buluh duri), *Bambusa vulgaris* (buluh aur) and *Gigantochloa* sp. (buluh brang).

In terms of final crop, bamboo is different from other crops. It has short harvesting rotation over many years. Bamboo can produce shoots after one year of planting and production is continued until 20 years or more. Not all bamboo species produce edible shoots. This is due to the toxicity of cyanogens in the bamboo shoots that also give a bitter taste. The popular bamboo species for shoot production in Peninsular Malaysia are *D. asper* (buluh betong), *G. levis* (buluh beting), *G. ligulata* (buluh tumpat), *B. vulgaris* (buluh aur) and *G. albociliata* (buluh madu).

Cut rhizomes are suitable for propagation of *G. ligulata* (buluh tumpat) (Abd Razak et al., 1995). The same method has been used for *G. albociliata* (buluh madu). Some bamboo species are propagated using branch cuttings, e.g. *D. asper* (buluh betong), *B. vulgaris* (buluh minyak), *G. 'brang'* (buluh 'Brang') and *G. levis* (buluh beting) (Azmy et al., 2009). Bamboo can grow in various soil types. The suitable planting distance is 4 x 4 m, with a density of 625 clumps per hectare (Abd Razak and Jamaluddin, 1998). They studied the planting of *G. levis* (buluh beting) for shoot production in one hectare. Results of the study showed that the total cost was RM21,372 and net benefit was RM13,524 for shoot production only and RM10,436 net benefit for shoot and bamboo stick production. Ex-farm price of bamboo stick was RM1.00 $stick^{-1}$while it was RM1.60 kg^{-1} for bamboo shoot.

The viability of bamboo plantation could be determined by the benefit-cost analysis (BCA). BCA is a commonly used technique for financial and economic evaluations. The main components of BCA are net present value (NPV), internal rate of return (IRR) and benefit-cost ratio (B/C) (Gittinger, 1982). The objectives of this paper are to assess *G. albociliata* resources, shoot production and costs in Peninsular Malaysia; and to determine the viability of establishing a *G. albociliata* plantation

Materials and Methods

A ground survey and inventory of existing plantations were conducted to collect relevant information and data based on the distribution areas, acreage, current productivity, acceptance study and its economic value of shoot production. The standard questionnaire forms for both resource and economic survey were developed. Resource and economic survey on buluh madu was conducted in Perlis, Kedah, Pahang, Selangor, Negeri Sembilan, Johor and Kedah/Perlis respectively.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The economic valuation of bamboo shoot in the plantation areas focused on the potential (stocking) value that was available in the existing plantations. The information on the ex-farm price, amount of bamboo shoots harvested, cost of harvesting and others is determined through the market survey. In addition, for financial analysis, a cash flow was developed in order to determine the viability of the bamboo shoot harvesting from the existing plantations. Other financial analysis parameters incorporated in this study were B/C ratio, IRR and NPV.

Results and Discussion

Nutrient composition of the bamboo shoots (G. albociliata)

Bamboo shoots can be taken fresh, preserved or cooked. They contain many nutrients for our body needs. The nutrient composition of fresh bamboo shoots is presented in Table 1.

Table 1. Nutrient composition of fresh shoots

Item	Amount
Moisture (g/100 g)	92.8
Protein (g/100 g)	2.3
Fats (g/100 g)	0.1
Carbohydrates (g/100 g)	4.0
Crude fibre (g/100 g)	1.0
Total sugar (%w/w)	0.4
Minerals: Ca (mg/100g)	22.0
K(mg/100g)	297.1
Mg(mg/100g)	11.1
P (mg/100g)	57.3
Calorific value (Kcal)	26.0

Summarized information of bamboo plantation in Peninsular Malaysia

Sixteen (16) bamboo smallholders in Peninsular Malaysia were interviewed. Their main activities surveyed were planting, processing the shoots and selling the fresh and preserved bamboo shoots. The smallholders' average age was 50 years. The average area of the plantation was 0.89 ha. The respective smallholders started planting bamboo in 2002 to 2008. Bamboo seedlings were obtained from Thailand. The planting material, planting, fertilizer and other aspects are discussed in this paper. Based on the survey, bamboo was planted in different soil types by the respective smallholders such as foothills and ex-paddy fields. Some smallholders use organic fertiliser (foliar fertiliser) for their bamboo.

Two (2) smallholders sold fresh shoots to the local people, while fourteen (14) smallholders sold fresh and preserved shoots. Moreover, the smallholders also sold bamboo sticks and seedlings as an additional income. Most of their fresh and preserved shoots were sold in local community. The demand of bamboo shoots was high, hence they could not supply to other states. A total of 5,341 kg (RM 32,046.00) preserved shoots and fresh shoots were sold in a year. Fresh and preserved shoots were sold at around RM6.00 kg^{-1}. Fresh shoots were sold in two forms, i.e. as whole shoots (with or without sheaths) and as sliced fresh shoots soaked in water. The preserved shoots were shoots that had been sliced, boiled, mixed with some salt and kept in PVC storage jars without exposure to the air. The preserved shoots can be kept for a period of 6-12 months. Table 2 shows the average management costs and others of establishing a *G. albociliata* plantation.

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Trans. Malaysian Soc. Plant Physiol. 22
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Household preferences for bamboo shoot

A total of 37 households were interviewed to determine their preferences for taste, colour and smell of bamboo shoots (Table 3). Results showed that the households liked the bamboo shoots because of their taste (48.65%) and smell (70.27%), while colour was acceptable (51.35%).

Table 2. Management costs of establishing a G. albociliata plantation

Item	Description
Site clearing	RM 300.00 at year 1 only
Planting material-seedling	RM 5,646.00 at year 1 only
Planting	RM 474.00 at year 1 only
Fertilizer	RM 250.00 ha^{-1}, 3x per year
PVC pipe for watering	RM 360.00 at year 1 only
Harvesting	RM 240.00 $harvest^{-1}$
Maintenance – treatment	RM 167.00, 3x $year^{-1}$
Transportation	RM 56.00 $harvest^{-1}$
PVC container	RM 231.00 at year 1
Selling price	RM 6.00 kg^{-1}
Production (sale): Preserved and Fresh shoot	RM 2,670 $month^{-1}$ or 445 kg/ha
Production (sale): Bamboo stick	36 sticks $month^{-1}$; Price = RM 1.00/stick
Production (sale): Seedling	RM 349.00 $month^{-1}$; Price = RM 7.00/seedling

Table 3. Household preferences (no. of respondents) for taste, colour and smell

Item	Taste	Colour	Smell
Like very much	11 (29.73%)	2 (5.41%)	2 (5.41%)
Like	18 (48.65%)	16 (43.24%)	26 (70.27%)
Able to accept	8 (21.62%)	19 (51.35%)	9 (24.32%)
Total	37 (100%)	37 (100%)	37 (100%)

Financial analysis

The financial analysis showed that establishing bamboo plantation was profitable. The total cost including the contingency cost was RM 15,272.25. This gave the net benefit of the bamboo plantation for shoot production at only RM 30,117.75 and for shoot production, bamboo sticks and seedlings at RM 34,953.75. After discounted at a rate of 10%, the NPV, IRR and B/C are shown in Table 4. Option 2 is more profitable than Option 1 due to the additional income from selling seedlings and bamboo. The gross income for bamboo plantation (shoot only) was RM 2,670 $month^{-1}$. Sensitivity analysis on the prices and costs at 10% interest rates shows that plantation of *G. albociata* is still viable and profitable.

Table 4. Net present values (NPV), internal rates of return (IRR) and benefit-cost ratios (B/C) for bamboo shoot production

	Investment criteria	
	Option 1: Fresh bamboo shoots and preserved shoots only	Option 2: Fresh bamboo shoots, preserved shoots, sticks and seedlings
NPV	RM2,003.05	RM2,927.41
IRR	12.18%	12.99%
B/C	1.22	1.33

Note: interest rate was 10%

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Conclusions

Planting bamboo would be more attractive if all costs could be reduced and the plantation areas were managed on a large scale. The consumption of preserved shoots is very popular than fresh bamboo shoots. The households preferred bamboo shoots from *G. albocilliata* over others such as those from *D. asper* (buluh betong) and *G. ligulata* (buluh tumpat). The demand for bamboo shoots was high from July to December, especially during the school holidays.

Based on the criteria of the project evaluation (NPV, IRR and B/C), plantation of *G. albiciliata* is viable. The IRR of the project is about 12%. The smallholders will gain more profit if they sell the mature bamboo stems and seedlings at a higher price, produce other products from the bamboo shoots and export the bamboo shoots to other states in Peninsular Malaysia.

Based on the information gathered from the smallholders, the demand for bamboo shoots in the form of preserved and pickled in salt is high in the west Peninsular Malaysia, Singapore and the Middle-East countries such as Saudi Arabia and Egypt. The high demand for bamboo shoots from *G. albociliata* is a chance for local people to extend their plantations of bamboo to operate on a large scale. The ex-paddy areas and degraded areas could be developed for planting of selected bamboo species to produce bamboo shoots.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Harvesting Evaluation of *Dendrocalamus asper* (Buluh Betung) on Shoot Productivity

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Introduction

The two most popular bamboo species planted for bamboo shoots production are *Dendrocalamus asper* (Buluh betung) and *Gigantochloa albociliata* (Buluh madu). In Peninsular Malaysia, *D. asper* is a widely distributed indigenous large size species (Figure 1). The largest area planted with *D. asper* for shoot production is situated in Kulai, Johor, having acreage of 70 acres. The harvested shoots are sold locally and exported to Singapore. Locally, the only information on *D. asper* was based on research that focused on the establishment techniques and site suitability (Abd Razak, 1999).

Figure 1. *Dendrocalamus asper* clump

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

The bamboo shoots of *D. asper* is widely planted and consumed in the states of Negeri Sembilan and Johor. Most shoots are 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 Kg. Kundur Hulu, Rembau, Negeri Sembilan. The average annual rainfall in this area was about 2,800 mm with 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 height of bamboo shoots (30, 40, 50 and 70 cm) from the ground level were harvested and recorded to determine the shoot production (Figure 2). Ten shoots were harvested for

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

each harvestable height treatment. The parameters recorded were 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.

Figure 2. Harvestable height (left), raw shoot weight (centre) and edible shoot weight measurement (right)

Results and Discussion

From the observations made, the mean duration of harvesting, diameter, raw and edible weight and percentage of shoot recovery weight according to harvestable height of *D. asper* are given in Table 1. The results also show the relationship between harvesting height and percentage of shoot recovery weight. The recovery weight increased with the harvesting height and then started to reduce after the shoots reached 50 cm in height. The harvesting height of 30, 40, 50 and 70cm will produce 59%, 50.4%, 51.8% and 42.5 % of shoot recovery weight respectively.

From these results, shoot productivity can be determined by the height of bamboo shoot to be harvested. The highest mean of edible weight produced was found to be at 70 cm harvestable height. It took 15 days after sprouting from the ground to reach this height. It was followed by 50, 40 and 30 cm shoot height that produced 2,209.6, 1,570.2 and 1,162.2 g respectively.

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

Shoot height (cm)	Duration of harvesting (days)	Diameter (cm)	Raw weight (g)	Edible weight (g)	% shoot recovery weight
30	7	14.2	1970.3	1162.2	59.0
40	9	15.0	3115.6	1570.2	50.4
50	11	15.8	4263.7	2209.6	51.8
70	15	17.7	8106.0	3446.1	42.5

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 was also highly significant effect of shoot basal diameter on harvestable height of bamboo shoots.

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

Source of variation	F - values			
	df	Diameter	Raw weight	Edible weight
Treatment	3	0.001**	0.001**	0.001**

*** - highly significant at P<0.05*

Results in Table 3 show that the raw weight of bamboo shoots increased according to harvestable height. However, the best harvestable height to produce optimum edible weight was 70cm (produced 3,446.1 g of edible shoot weight). It is interesting to note that the basal diameter of shoots 17.7 cm gave significant effect on shoot production only at 70cm shoots harvestable height.

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

Harvesting height of shoot (cm)	Diameter (cm)	Raw weight (g)	Edible weight (g)
30	14.2 a	1970.3 a	1162.2 a
40	15.0 a	3115.6 b	1570.2 a
50	15.8 a	4263.7 c	2209.6 b
70	17.7 b	8106.0 d	3446.1 c

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

Conclusions

The studies conducted showed that the recommended harvesting height of *D. asper* (Buluh betung) shoots is 70cm and it takes 15 days after sprouting from the ground. It can produce 3446.1 g mean edible weight per shoot.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Shade and Morphological Characterization of Janggut Adam (*Tacca* sp.) as a Promising Native Ornamental and Medicinal Plant

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Introduction

The genus *Tacca*, from the family Discoraceae, consists of ten species of flowering plants (Zhang et al., 2005). *Tacca* typically inhabits moist and shaded understory environments in the lowland tropical forest and hilly areas, at an altitude of about 1,300 m above sea level (Chooi, 2004). *Tacca* is an interesting evergreen, perennial, and herbaceous plant with thick rhizomes or tubers and possess the most stunning inflorescence where it bears whisker-like filiform bracteoles and the colour of the two conspicuous inner involucral bracts range from white, green, purple, brown to near black colour.The true flowers of *Tacca* are dark purple, brown, or near black in colour and they are actinomorphic and hermaphroditic with six stamens (Zhang et al., 2005). A great diversity of *Tacca* species can be found in Malaysia where five of the species are distributed in this region: *Tacca integrifolia, Tacca chantrieri, Tacca nivea, Tacca leontopetaloides* and *Tacca palmata* (Henderson, 1954). Although Malaysia has a wide *Tacca* germplasm pool, the study of the morphological differences among *Tacca* species is lacking. Study on seed characteristic and development of *Tacca* species has never been carried out. The objectives of this study are to describe morphological characterization among selected *Tacca* species and effect of shading to produce good quality plants with suitable plant height as potted plants, vigorous growth and uniform flowering and also intense flower colour.

Materials and Methods

Study 1: Effect of shade on growth and flowering

In the first study, the effects of shade levels on the early growth and flowering response of bat lily, *Tacca integrifolia* were carried out. The plants were grown under four shading levels consisting of S1 (0% shade, without black net), S2 (30% shade), S3 (50% shade), S4 (70% shade). Black netting used as shading material was mounted on the bricked frame with measurement of 12.4 x 12.4 m and 2.26 m height. The average light intensity measured were 1,066.87 µmol/m²/s in S1, 695.35 µmol/m²/s in S2, 468.30 µmol/m²/s in S3 and 273.33 µmol/m²/s in S4. The light intensity was measured by using light meter (LI-COR Model LI- 250). Three readings of light intensity were taken and the average was recorded. The growth and physiological parameter (net photosynthesis, stomata conductance, chlorophyll content, number of leaves, leaf length, leaf width, petiole length, leaf growth rate, leaf area, specific leaf area and number of days to senescence of the plants) were determined.

Study 2: Morphological characterization among Tacca species

Vegetative and reproductive morphological characters for each species and accession were measured and recorded at selected location in Penang, Selangor and Sarawak. The plants were established at Ladang 2 UPM under rain shelter of 75% humidity and 70% shade. Qualitative and quantitative characteristics were categorized and given scores to represent each category. Multivariate analysis was done by transforming the scores from each species and analyzed using Gower's Similarity Index. Dendrogram and coefficient of similarities was produced using MVSP programme.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Results and Discussion

Shading did play a highly significant role in the growth and flower development of *T. integrifolia*. The growth and flowering of the plant was hastened with shading treatments. Increased shade levels gave a better growth and flowering environment for *T. integrifolia.* Shading also significantly influenced the net photosynthesis, stomata conductance, chlorophyll content, number of leaves, leaf length, leaf width, petiole length, leaf growth rate, leaf area, specific leaf area and number of days to senescence of the plants (Table 1). This showed the importance of shading treatment on the growth of the species.

Table 1. The effects of different shade levels on growth and physiological responses of *T. integrifolia*

Parameter	Shade Level			
	0% (S1)	30% (S2)	50% (S3)	70% (S4)
Photosynthesis Rate (µmol/m^2/s)	0.593 b	3.743 a	3.373 a	3.700 a
Stomata conductance (mmol/m^2/s)	0.003 b	0.050 a	0.067 a	0.042 a
Chlorophyll content (µg/cm²)	1.206 c	29.425 b	25.631 b	39.750 a
Number of Leaves	0.313 c	4.750 a	4.700 b	4.813 a
Leaf length (cm)	1.950 c	29.431 b	27.223 b	40.481 a
Leaf width (cm)	0.625 c	8.856 b	8.936 b	12.469 a
Petiole length (cm)	0.500 c	11.075 b	10.686 b	17.994 a
Leaf area (cm^2)	3.63 c	190.00 b	182.87 b	348.14 a
Specific leaf area (cm^2/g)	15.62 b	199.40 a	176.21 b	185.36 a
No. of days to senescence	0.0 d	6.0 a	3.2 c	5.2 b

Means within the same row having the same letter are not significantly different based on Duncan's multiple range test at (P<0.05) level

Shade level of 70% was the most suitable level to produce a good quality plants with intense colour and suitable flower stalk length (Figure 1). Open planting (control, 0% shade) was not suitable for *T. integrifolia*. This was because full sunlight will delay and slow down the growth of plants and cause death of plants.

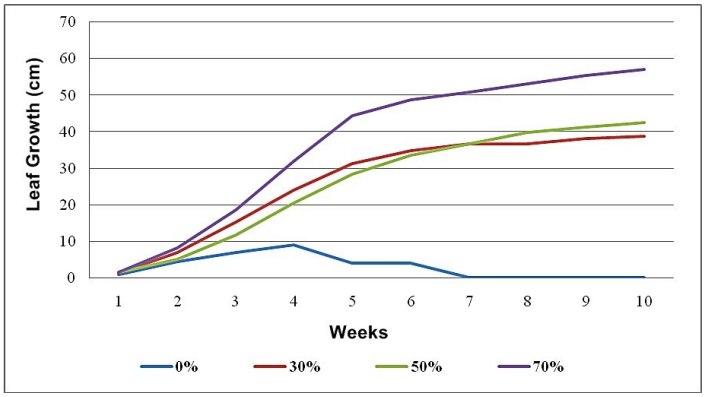

Figure 1. The effects of different shade levels on leaf growth rate of *T. integrifolia.*

The overall size of inflorescence was also a distinct characteristic; *T. nivea* had large inflorescence and foliage, *T. leontopetaloides* had longer inflorescence stalk with lobed leaves, the inflorescence of *T. integrifolia* and *T. chantrieri* were moderately sized. The vegetative characterizations of *Tacca* species were described. A total of thirty five qualitative and quantitative morphology characters with 140 descriptions were developed. Three different groups were determined from group cluster and dendrogram (Figure 2) based on 23 qualitative and 12 quantitative characteristics among *Tacca* species using MVSP programme.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

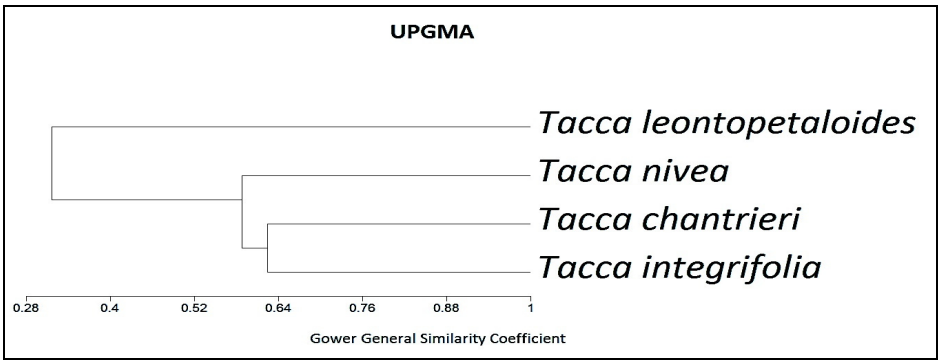

Figure 2. UPGMA dendrogram showing relationship of selected *Tacca* based on morphological characterization

The data obtained clearly shows the similarities and dissimilarities amongst selected *Tacca* species based on their morphological characterization (Table 3).

Table 3: Coefficient of similarity matrix showing pairwise comparisons of selected *Tacca* species

	T. intergrifolia	*T. chantrieri*	*T. nivea*	*T. leontopetaloides*
T. intergrifolia	1.000			
T. chantrieri	0.625	1.000		
T. nivea	0.625	0.550	1.000	
T. leontopetaloides	0.300	0.275	0.375	1.000

Evidently, the figure showed that the *T. integrifolia* and *T. chantrieri*, *T. chantrieri* and *T. nivea* shared similarities up to 62.5%, followed by *T. chantrieri* and *T. nivea* (55%), *T. nivea* and *T. leontopetaloides* (37.5%), *T. integrifolia* and *T. leontopetaloides* (33%) and *T. chantrieri* and *T. leontopetaloides* (27.5%).

Conclusions

Tacca integrifolia has a high potential to enhance the profitable floriculture industry in Malaysia because it has become a favourite in market as a potted plant as well as for exterior landscaping. For cut flower production of *T. integrifolia*, shade level of 30% was the most suitable to produce plants with longest flower stalk. Shade levels of 50% and 70% will be more appropriate treatments for production of potted *T. integrifolia* in Malaysia. Four morphological characteristics namely, seed shape, apices of innermost bracts, bracts and bracteoles color were greatly variable among the *Tacca* species.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Plant Beneficial Soil Bacteria and NPK Green Influenced the Yield of *Centella asiatica*

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Introduction

Pegaga or *Centella asiatica* is a herbal plant that is very popular among Malays, Chinese and Indians. It is believed that this plant has various advantage effects to health and has been recommended by the World Health Organization (WHO) as one of the most important medicinal plant species to be conserved and cultivated (Indu, 2000). Recently, due to the high demand by local herbal producers on this herb, most pegaga plantings are done through contract farming with local farmers. However, the supply of this material still could not meet the demand that is increasing every day. Due to this, some herbal producers imported this material from Indonesia and India. Plant beneficial soil bacteria (PBB) or well known as plant growth promoting rhizobacteris (PGPR) such as *Bacillus subtilisis* is one of the applications that can be made to improve soil condition (Hayat et al., 2010) and productivity. This bacteria also believed to specifically enhance the phosphorus status of plants when it is been applied to the soils (Brown, 1974). Gray and Smith (2005) reported that, soil bacteria is able to give a positive effect on the growth of the plants, nutrients supply and help to protect plant from diseases (Brierley, 1985; Ehrlich, 1990). Besides, it also helps to optimize the nutrient cycle in the event of stresses due to the unsuitable weather or soil conditions (Hayat et al*.,* 2010). Due to this condition it is hoped that by applying plant beneficial bacteria it will increase the production of pegaga and the fertility of the soil. This study was carried out to determine the effects of plant beneficial soil bacteria on the growth of *C. asiatica.*

Materials and Methods

This study was carried out at Unit Ladang Universiti Teknologi Mara, Perlis for three months. Thirty polybags sized 30 cm (length) x 30 cm (width) were prepared and filled with 7 kg of soil mixture with a ratio of 2:2:3 (top soil: organic matter: sand). Six number of treatments that consists of T1 (without plant beneficial bacteria, 0 PBB + NPK green fertilizer, 0 NPK-G), T2 (10 g/L PBB + 0 NPK-G), T3 (0 PBB + 1.56g NPK-G), T4 (8 g/L PBB + 1.56g NPK-G), T5 (10 g/L PBB + 1.56 g NPK-G) and T6 (12 g/L PBB + 1.56 g NPK-G) were used in this study. Each treatment was replicated five times and arranged in a randomized complete block design. For the treatment under T2, T4, T5 and T6, the plant beneficial bacteria were sprayed on the media a week before planting the planting material was conducted. While, NPK green fertilizer was applied on day 21 and 49 days after the first day of planting. Stem cutting of *Centella* spp. that was recognized as 'pegaga kampung' was used as planting material in this study. Four plants were planted in each polybag with a distance of 12 x 12 cm between each plant.

Data on root volume and fresh weight was measured by destructive sampling on 75 days after planting. The root volume was measured by immersing the root part into 100 ml of water and the difference in the volume was taken as root volume for that part. The concentration of nitrogen and phosphorus in plant part was determined by the method of drying the leaves, petiole and root part in the oven for 48 hours. Then, the sample was grinded and subjected to H_2O_2-H_2SO4 digestion. Nitrogen concentration was analysed by Elemental Analyzer. Whereas, phosphorus concentration was analysed using a spectrophotometer. Data were analyzed using the General Linear Model Procedure of SAS (Statistical Analysis System, version 6.12, 1996). Mean separation was perfomed using the Tukey's Honestly Significant Difference (HSD) method at $P<0.05$.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Results and Discussion

The root volume was significantly affected by PBB and NPK-G application at 75 days after planting (P≤0.05) (Figure 1). A greater root volume was recorded in the plant that was applied with 12 mg/L of PBB and 1.56 g of NPK-G (T6), compared to the plant without PBB which are T1 and T3 and lower amount of PBB which are T2, T4 and T5. This showed that the amounts of PBB at 12 mg/L was able to change the root morphology of the plant by increasing the root surface area thus made the plant absorbed nutrient more compared to other treatment (Table 1). This result is comparable with the finding by Hayat et a1. (2010) that bacteria *B. subtilis* synthesizes and produces the hormone like indole-3-butyric acid (IBA) and indole-3-ethanol (TOL) that stimulates the plant and root growth (El-Khawas and Adachi, 1999), thus increased the root length for better nutrient absorption. However, combination with (T2, T3, T4 and T5) or without (T1 and T2) NPK-G with PBB does not affect the root volume of *C. asiatica.*

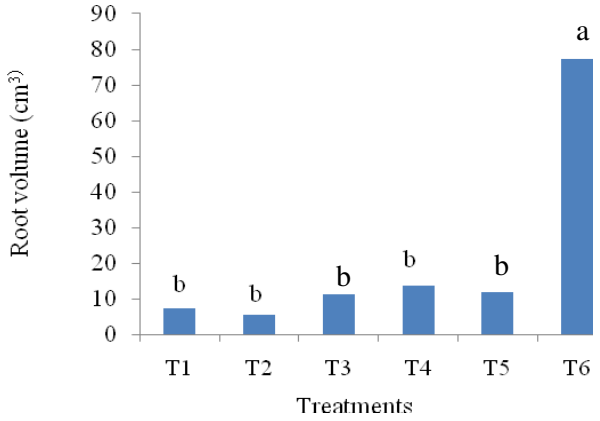

Figure 1. Effect of plant beneficial bacteria and NPK green fertilizer on a root volume of *C. asiatica* at 75 days after planting. Means followed by the same letter are not significantly different at 5% (Tukey's HSD).

Nitrogen and phosphorus concentration were statistically influenced by PBB and NPK-G application for all plant parts (P≤0.05) (Table 1). The concentration of nitrogen was higher in the leaf part compared other part of the plant. The concentration of nitrogen was significantly higher in the leaf and petiole but lower in root, as applied with 12 g/L of PBB and 1.56 g of NPK-G (T6). This describes that 12 mg/L of PBB able to facilitate the plant to absorb more nitrogen compare to plant with lower of PBB (T4 and T5). This condition will aid the plant to produce greater vegetative growth and contribute to higher yield in the future (Figure 2).

However, the concentration of phosphorus was highest in the petiole, followed by leaf and root. Similar to nitrogen concentration, phosphorus concentration was significantly higher in the plant that grown with 12 g/L of PBB and 1.56 g of NPK-G (T6) in petiole and root parts, but lower in the leaf part. This showed that *B. subtilis* was among the most effective bacteria that solubilize insoluble inorganic phosphate by creating an acidic condition in rhizhosphre that enhances the ability of the plant to uptake the phosphate in form of di- and- monobasic phosphate (Mahdi et al., 2010).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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Table 1. Percentage of Nitrogen (N) and Phosphorus (P) in the leaf, petiole and root as affected by plant beneficial bacteria and NPK green fertilizer**.**

Plant part	Treatment	N	P
Leaf	T1	2.54^{cd}	0.13^{d}
	T2	2.46^{d}	0.13^{c}
	T3	2.96^{cb}	0.17^{a}
	T4	2.94^{c}	0.11^{e}
	T5	3.41^{b}	0.12^{d}
	T6	3.94^{a}	0.15^{b}
Petiole	T1	0.81^{e}	0.12^{f}
	T2	0.98^{d}	0.15^{d}
	T3	1.74^{b}	0.16^{b}
	T4	1.67^{c}	0.13^{e}
	T5	1.88^{b}	0.16^{c}
	T6	1.75^{a}	0.18^{a}
Root	T1	1.08^{e}	0.09^{e}
	T2	1.43^{c}	0.10^{d}
	T3	0.77^{f}	0.12^{b}
	T4	1.19^{d}	0.11^{c}
	T5	2.03^{a}	0.12^{b}
	T6	1.60^{b}	0.14^{a}

Means within columns for each plant part having different letters are significantly different at 5% (Tukey's HSD).

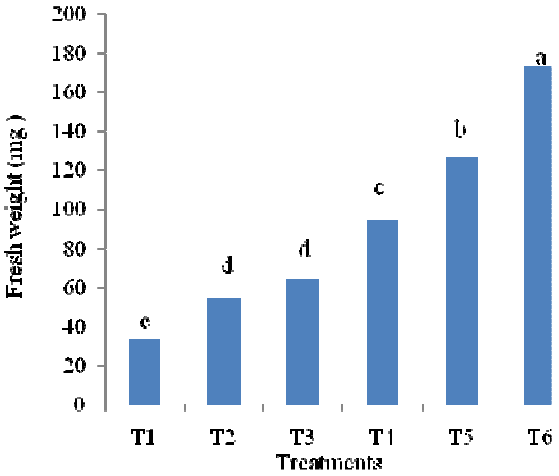

Figure 2. Effect of plant beneficial bacteria and NPK green fertilizer on a fresh weight of *C. asiatica*. Means followed by the same letter are not significantly different at 5% (Tukey's HSD).

Centella asiatica fresh weight significantly ($P \leq 0.05$) increased as weight of PBB increased from 8 to 12 mg/L (T4, T5and T6), however no significant changes between T2 and T3 were found at 75 days after planting (Figure 2). The plant that was applied with higher plant beneficial bacteria resulted greater fresh weight. This result indicates that the use of plant beneficial bacteria is efficient to improve the yield of *C. asiatica* by increasing the fresh weight of plant.

Conclusions

This result suggests that an application of NPK green with combination of plant beneficial bacteria is capable to increase the growth and yield produce by *C. asiatica* since the root volume and fresh weight was greater compared to other treatments.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Cultivation of Grey Oyster Mushroom (*Pleurotus sajor-caju*) on Different Agro-Waste Residues

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Introduction

Growing oyster mushroom is becoming more popular throughout the world because of their abilities to grow in a wide range of temperatures, utilizing various lignocelluloses (Khan and Garcha, 1984). Grey oyster mushroom (*Pleurotus sajor-caju)* is one of the most commonly grown speciality mushroom in Malaysia. The use of waste materials as the alternative for mushroom cultivation substrates, identified as a potential and viable way to generate new economic sources. Cultivation of edible mushroom with agricultural residues is a valued-added process to convert these materials, which are otherwise considered to be wasted, into human food (Zhang et al., 2002).

Malaysia has vast amounts (~4.2 million tonnes) of untapped agricultural waste or natural fiber materials available for the agricultural sector which is disposed into landfills annually. These fibers and biomass materials range from rice straws, rice husks, coconut trunk fibers, kenaf to oil palm biomass in the form of oil palm trunks (OPT), oil palm fronds (OPF) and empty fruit bunches (EFB), sugarcane and corn wastes and many more which can serve as alternative resources in the cultivation of mushroom and other agricultural industries as well as having the potential to be commercially manufactured into other value-added products (Agamuthu, 2009). Moreover, the uncontrolled wastes management led to environment pollution problems. Disposing of waste has huge environmental impacts and can cause serious problems. According to Philippousis et al. (2001) most agricultural residues are rich in lignocellilosic compounds whose handling and disposal are often problematic due to their chemical structure and properties. Burnt of lignocellulosic substances is hazardous which resulted in an oxygen deficient environment and respiratory diseases.

At the moment, mushroom producers rely mainly on sawdust. In addition, in many countries, including Malaysia the availability of sawdust supplies will not continue to meet the rising demand for very long. The limited supply and high demand of sawdust, lead to increase in its price as well. This phenomenon makes it imperative for other sources of substrates and additives to be utilized for mushroom cultivation. Therefore, the objective of this study was to determine the growth performances, yield and physico-chemical properties of oyster mushroom (*P. sajor-caju)* cultivated on five different agro-wastes, i.e sawdust (control), grasses, rice husk, dried leaves and coconut frond.

Materials and Methods

Agricultural wastes of rice husk, grasses, dried leaves and coconut frond were dried and chipped into small pieces. Each waste was mixed with rice bran, calcium carbonate in the ratio of 100:10:1 and water was later added to moisten the mixture. The mixed medium was then filled into polypropylene bags. The bags were compacted into a cylindrical shape and closed using necks and lids and sterilized at 121 °C for 30 minutes using an autoclave. After sterilization the bags were cooled overnight and ready to be inoculated with *P. sajor-caju* culture. The inoculated bags were incubated (spawn r unning) at 28-30 °C; 80-90% RH in the dark room (~30% light) until mycelia has completely covered the bags. The growth performances of mushroom from each different agro-waste residues were determined in terms of mycelium growth during spawning, the number of days for complete colonization of substrate (full bag). The days taken for the pin head emergence and fruiting body formation were also observed and recorded. The yield was measured in term of percentage biological efficiency.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Physico-chemical analyses: Colour measurements were done on the cap or pileus of mushroom using Minolta Chromameter. Presented in L* [lightness], a* [greenness (-) to redness (+)], and b* [blueness (-) to yellowness (+)] values. Texture of fresh mushrooms was determined by using Texture Analyzer using P/2 stainless steel probe. Moisture determination was done by heating an amount of mushroom in an oven at 105 °C for 4-5 hours. Ash was determined by heating sample in a Muffle furnace for 6 hours at 550 °C until a white residue of constant weight was obtained. Crude fiber of mushroom was determined by using Fibertec System 2021 FibreCap. Protein content in the mushroom was determined using the Kjeldahl method which involved digestion, distillation, and titration steps. The percentage of nitrogen was converted to protein by multiplying by the factor of 5.99.

This study used completely randomized design (CRD) with five replicates of each treatment. The data obtained were analyzed using one-way analysis of variance (ANOVA) with post-hoc Tukey multiple comparison test to determine the most efficient substrate which gave the highest yield and quality of the gray oyster mushroom. The statistical analyses were done using SPSS software.

Results and Discussion

The results of the mycelium growth on the different agro-waste residues are presented in Figure 1. Mushrooms were successfully produced from all treatments. Rice husk showed significantly the fastest ($p<0.05$) mycelium growth while coconut frond showed significantly the slowest among all treatments. Rice husk decreased the surface of mycelium density, but accelerated spawn running and therefore increased the mycelium growth. This could attribute to the physical nature, high porosity and how aerated the rice husks are that causing the mycelium to run through the substrate as also observed by Salmones et al. (1999). Moreover, these results are in line with Baysal et al. (2003) who reported that increasing amount of rice husk in the substrate mixture accelerated spawn running, pin head and fruit body formation. The slow rate of mycelium growth on coconut frond might be due to high cellulose or cellulose: lignin ratio since fungi that degrade lignin faced with several problems since the polymer is extremely large and highly branched (Hammel, 1997).

Rice husk showed the most rapid spawn running (25 days) among all treatments. The speed of complete mycelia runs to fill-up the bags showed positive correlation with the rate of mycelium growth. There were also significant differences ($p<0.05$) in the number of days for pinhead emergence. Coconut frond showed significantly ($p<0.05$) longer time (25 days) for pin head emergence. Pinheads emerged as small rounded lumps that were grouped at particular parts of substrate surfaces. Kimenju et al. (2009) indicated that the time taken by the mycelia to start pinning after ramification depends on the substrates used. These differences were attributed to nutritional variations among the substrates. Leafstalk of coconut had high quality lignin and cellulose content which reportedly takes longer time to start pinning compared to the substrates with low contents of lignin and cellulose. This is because high nutrition materials make the mycelia to remain vegetative for a longer period resulting in vigorous growth and late pinning. However, other factors such as high moisture content in a substrate could also cause delayed in pinning (Kimenju et al., 2009).

There was no significant difference in the number of days for fruiting body formation among all substrates. In the present study the pinheads grown to fruiting bodies in 1-2 days in different substrates. Different timings were reported for fruiting body formation of different mushroom species. For example, Shah et al. (2004) reported that the fruiting bodies appeared in 3-6 days after pin head emergence for *Pleurotus ostreatus.* Mondal et al. (2010) and Muhammad (1998) obtained the fruiting bodies after 3 and 4 days for *P. florida* and *P. sajor-caju,* respectively.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

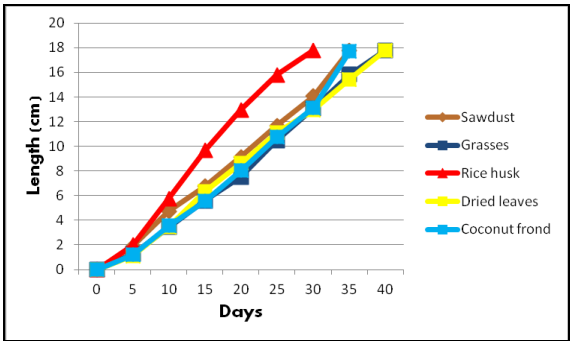

Figure 1. The mycelium growth of *P. sajor-caju* on different agro-waste substrates

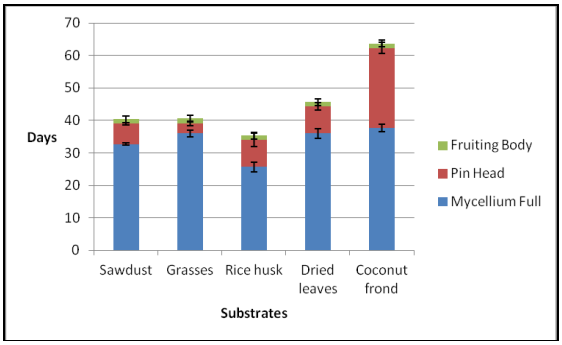

Figure 2. The growth performance of *P. sajor-caju* on different agro-waste substrates

The percentage conversion of substrate to fruiting bodies of the muhsroom has been indicated by the term 'biological efficiency' in the present work. Biological Efficiency (BE) was calculated to determine how the mushrooms utilized nutrients presence in the substrates efficiently. There were significant differences ($p<0.05$) in percentage of biological efficiency among all the treatments. Sawdust showed significantly the highest ($p<0.05$) percentage (55.7%) followed by grasses (30.8%), rice husk (28%), coconut frond (20.2%) where dried leaves showed the lowest percentage (16.89%) (Table 1). The variation in percentage biological efficiency of different substrates might be due to different lignolytic and cellulonotic activities of the substrates used (Pathak and Goel, 1988).

Substrates which are rich in usable nitrogen after spawn running may be a factor in enhancing the mushroom yield and quality, in addition to the mushroom species in bioconversion and bioaccumulation efficiency (Patil et al., 2008). A low yield of mushrooms showed on dried leaves. This might probably be due to low water retention of the substrates. The availability of water to be uptake by the mushroom has been suggested to be a limiting factor in yields (Kalberer, 1991).

Rice husk showed the best mycelium growth performance. However, in term of yield, it did not produce good yield. A good initial colonization is necessary but not sufficient to ensure a good mushroom yield, which probably could be influenced by other factors in the utilisation of nutrients by mushroom mycelium (Zhang et al., 2002). This results are in line with the rice husk for *P. ostreatus* (Obodai et al., 2003) and sawdust of *M. indica* for *P. citrinopileatus* (Liang et al., 2005), who reported that substrate that gave the fastest mycelium growth did not necessarily correspond well with yield. This also indicates that mycelium growth and yield of mushrooms have different requirements.

From Table 1, there was no significant difference ($p>0.05$) in colour L, a, b values and firmness of *P. sajor-caju* grown on different substrates. This is due to the colour of mushrooms is closely related to the intensity of light (Chang and Miles, 1999) and texture properties was independent on substrates and was more associated with the genetic factor.

Table 1. The yield and physical properties of *P. sajor-caju* on different agro-waste substrates

Substrates	Biological efficiency (%)	Color			Firmness (N)
		L*	a*	b*	
Sawdust	55.7 ± 7.9^{a}	75.6 ± 9.4^{a}	2.6 ± 1.4^{a}	14.6 ± 0.9^{a}	0.34 ± 0.02^{a}
Grasses	39.8 ± 9.6^{ab}	70.5 ± 10.4^{a}	4.2 ± 1.1^{a}	16.0 ± 0.9^{a}	0.30 ± 0.06^{a}
Rice husk	28.0 ± 12.4^{bc}	79.7 ± 3.3^{a}	2.6 ± 1.1^{a}	15.5 ± 3.5^{a}	0.29 ± 0.15^{a}
Dried leaves	16.9 ± 3.4^{c}	81.2 ± 1.8^{a}	2.1 ± 1.4^{a}	16.0 ± 3.4^{a}	0.44 ± 0.07^{a}
Coconut frond	23.5 ± 3.0^{bc}	73.3 ± 13.2^{a}	2.8 ± 2.1^{a}	14.7 ± 0.9^{a}	0.42 ± 0.11^{a}

Values are means ± standard deviation. The same superscript within the same column are not significantly different at 5% level ($p<0.05$).

Moisture content of 83.69 to 88.50% for fresh mushroom are considered normal, because mushrooms contain about 90% water (Stamets, 2000). There were significant differences ($p<0.05$) in the mushroom moisture content among all the treatments (Table 2). Mushroom cultivated on dried leaves showed significantly the lowest ($p<0.05$) moisture content which might due to the water retaining ability of the substrate. This is in agreement with the lowest percentage of BE because, the yield, size and quality of harvested mushrooms are related to the amount of moisture in the substrate or casing (Kalberer, 1987).

Table 2. The chemical properties of *P. sajor-caju* on different agro-waste substrates

Substrates	Moisture (%)	Ash (%)	Protein (%)	Crude fiber (%)
Sawdust	88.5 ± 2.6^{a}	0.7 ± 0.3^{a}	27.7 ± 4.3^{bc}	17.8 ± 2.5^{ab}
Grasses	83.7 ± 1.5^{a}	1.6 ± 0.8^{a}	35.0 ± 0.7^{ab}	10.7 ± 0.3^{ab}
Rice husk	86.3 ± 2.3^{a}	0.6 ± 0.2^{a}	34.8 ± 3.7^{ab}	19.7 ± 9.6^{ab}
Dried leaves	69.6 ± 2.4^{b}	1.5 ± 0.5^{a}	44.7 ± 3.2^{a}	5.7 ± 2.3^{b}
Coconut frond	83.8 ± 0.8^{a}	0.9 ± 0.3^{a}	19.0 ± 6.5^{c}	21.8 ± 6.4^{a}

Values are means ± standard deviation. The same superscript within the same column are not significantly different at 5% level ($p<0.05$).

There was no significant difference ($p>0.05$) in the ash content of mushrooms among different treatments which ranging from 6.88-15.73%. The results obtained were in agreement with Bisaria et al. (1987), Zhang et al. (2002) and Madan et al. (1987) who found variations from 6.3-6.9, 8.2-11.3% and from 6.4-6.8% of ash content respectively.

There were significant differences ($p<0.05$) in the protein content of the mushroom among different substrate used. The mushroom cultivated on dried leaves showed significantly the highest ($p<0.05$) protein content (44.73%) while coconut frond showed the lowest (18.02%) among all the treatments. This might probably be due to high composition of hemicelluloses, fiber and low nitrogen content in the coconut frond even though rice bran added in the substrate mix could provide some protein required. It has been reported that not only the protein content of the substrate but also the nature of protein in the substrate that influences the protein content of the fruiting bodies (Wang et al., 2001).

There were also significant differences ($p<0.05$) in the amount of crude fiber with different substrates. The variation in crude fiber content of the mushroom was 5.69-21.77%. Mushroom cultivated on coconut frond showed significantly ($p<0.05$) the highest crude fiber content (21.77%) whereas mushroom cultivated on dried leaves showed significantly the lowest (5.69%) amount. The results obtained might be due to the high amount of crude fiber present in the respective samples, as well as to the low protein contents present in those samples.

Conclusions

This study can be concluded that *P. sajor-caju* can be cultivated on range of lignocellulosic residues such as sawdust, rice husk, grasses, dried leaves and coconut frond. No significant difference was observed in the number of days of fruiting body formation, colour, firmness and ash content between all the treatments. Coconut frond took significantly longer time for flush formation but gave high crude fibre content. The rice husks showed the best mycelium growth performance, however, sawdust economically more profitable since it gave the highest yield. Dried leaves and coconut frond have less potential to be used in *P. sajor-caju* production since they had lower productivity and took longer time for fruiting body formation.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Asymbiotic Seed Germination and Early Seedling Development of Threatened Endemic Orchid *Phalaenopsis gigantea*

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Introduction

Phalaenopsis gigantea an epiphytic orchid is endemic to Borneo. It is found in Tenom and Tawau in Sabah and Kalimantan (Indonesia). Well known as the Elephant's ear orchid because of it large leaves up to 50-68.5 x 20-25.5 cm (Chan et al., 1994). Because of damage to its natural habitats by continuous destruction of forest for home sites, agriculture, habitat mismanagement and indiscriminate collection by orchid growers, this species has now become endangered. *Phalaenopsis* is propagated by seed and vegetatively *in vitro* by flower stalk buds (Tanaka and Sakanishi, 1978). Propagation via flower stalk buds is slow and not sufficient to surmount the threat of extinction. Seeds propagation increases genetic variability, which favors conservation. The number of orchid seeds produced per capsule varies from several hundred thousand to at least 4 million (Arditti, 1967). Unfortunately, less than 5% of the seeds germinate under natural conditions (Rao, 1977). The introduction of asymbiotic seeds germination *in vitro* culture methods for orchid propagation by Knudson (1946) greatly facilitates orchid seed germination (Kitsaki et al., 2004). Differences in the maturity of the seeds, characteristics of the species, composition of media, purity of chemicals and culture conditions also affect seeds germination (Oliva and Arditti, 1984). Germination rate and viability of the seeds differ to most of the orchid spesies (Rasmussen, 1995). There is no publish report on in vitro germination of *P. gigantea*. With this in mind the present investigation was undertaken with a view toward developing an efficient and inexpensive *in vitro* culture media for germinating of *P. gigantea*.

Materials and Methods

Flower of *P. gigantea*, grown in the Orchids World Nursery in Ranau, Sabah, were hand pollinated and were allowed to ripen by maintaining them in the Nursery. Ripen capsules were harvested 100, 130 and 170 days after pollinated and surface sterilization. The capsule will brush under the running water. The capsule then will surface sterilized by immersion into 75% (v/v) ethanol for 3-5 seconds before wash with distilled water for 3 times and later immersed with 30% (v/v) Clorox®, added with 2 drops of tween-20 for 20 minutes. The solution will decant and capsule washed thoroughly 4 times with sterile distilled water. The capsules were dissecting longitudinally with a surgical blade in the laminar flow. The seeds were scraped out and placed on the agar surface under sterile condition. Seeds were placed in Petri dishes containing 25 ml of respective medium supplemented with 2.0% (w/v) sucrose (Sigma) and darkened a with 1 g activated charcoal l^{-1} (BDH Chemicals, LTD Pool, England). The pH of the medium was adjusted to 5.6-5.8 before gelling with 10 g agar l^{-1} (Sigma). Media were autoclave at 104 kPa and 121^{o}C for 20 minutes. The seeds observed every 20 days interval. Parameters observed were percentage of germination. They were assessed through the use of stereomicroscope. Germination and protocorm development were scored on a scale of 1-6 (Arditti, 1967). Germination percentages were calculated by dividing the number of seeds in each individual germination and development stage by the total number of viable seeds in the sample. All developmental data were subjected to analysis of variance.

Results and Discussion

Germination and seedling development in orchids are different from other flowering plants (Niknejad et al., 2011) and considered difficult, as specific nutritional and environmental conditions are needed

(Rasmussen, 1995). Orchid capsules are dehiscent, and seeds for in vitro germinating maybe obtained after dehiscent or even before when the capsule is still unripe. Several studies have been shown that the seeds from immature capsules can germinate in vitro much earlier (Arditti et al., 1990).

Seeds of *P. gigantea* began swelling and greening within 8-10 days after being placed on medium and germinate within 30-50 days after inoculation (Figure 1). As higher as 90% of seeds from 170 day-old capsule germinated after 110 days of culture, whereas only 60% and none were observed from 130 and 100 day-old capsules, respectively. Protocorm derived from 130 day-old capsules look pale and died during cultivation compared to protocorm produced from 170 day-old capsule (Figure 2). Immature capsules are most suitable for *in vitro* germination as it is easy to surface disinfect than mature ones (Yam and Weatherhead, 1988) and embryo become viable and develop normally prior to the capsule ripening (Arditti, 1967). Usually orchid seeds lack endosperm and cotyledons and lipid droplets are the only storage materials localized within the embryo proper itself (Arditti and Ernst, 1984). These lipids are utilized during germination of orchid seeds (Manning and Staden, 1987).

Orchid seed induced to germinate symbiotically or asymbiotically due to an exogenous supply of simple nutrient for their further growth (Manning and van Staden, 1987). Result obtained showed that the up to 95% seed germinated well on XER and NDM whereas, KC and ½MS medium showed only 30.08% and 88.09% seed germination, respectively (Figure 3). This could be due to presence of sufficient endogenous growth regulator required for initial stages of germination. The KC and ½MS medium less not support the formation of seedling and most of the protocorm died after 150 days of culture (Figure 4). A possible reason for the inhibition of growth on the germination medium may be the depletion of nutrients and competition between seedlings.

Complex additives showed significant effect on seed germination. Both medium XER and NDM tested showed that addition of potato homogenate promote germination greatly (100%), followed by tomato juice (97-100%) and coconut water (79-100). Concentrations of potato homogenate, tomato juice and coconut water did not bring about significant effect on percentage of seed germination after starting the culture. However, germination reduces by increasing the banana homogenate concentration in both medium (Table 2 and Figure 5). A possible reason for the inhibition of germination may be the high osmotic pressure on the medium prevents water absorption by the seed. Protocorm development during the culture period is shown in Figure 6. Embryo becomes swollen and green after 8-10 days of culture. Protocorms formed after 30 days of culture. Pointed vegetative apex emerges after 70 days of culture. Discoid late protocorm showing leaflets, 1-2 mm after 90 days of culture. Seedling showing leaflets +2 mm after 150 days of culture. Protocorm in stage 6 continue further development and growing vigorously in sphagnum moss plus charcoal in ex vitro environment.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

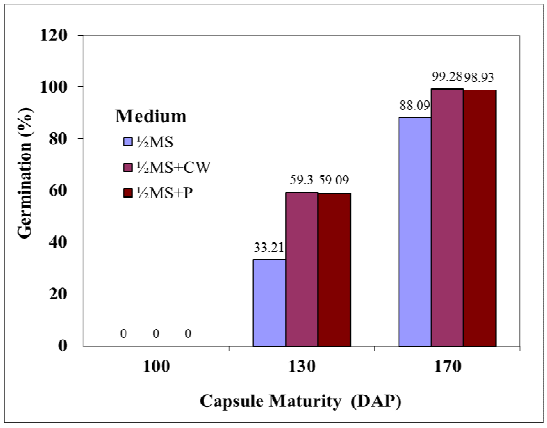

Figure 1. Effect of capsule maturity on germination of *P. gigantea* seeds on 110 day of culture. CW=Coconut Water, P= pepton. Bar=1mm.

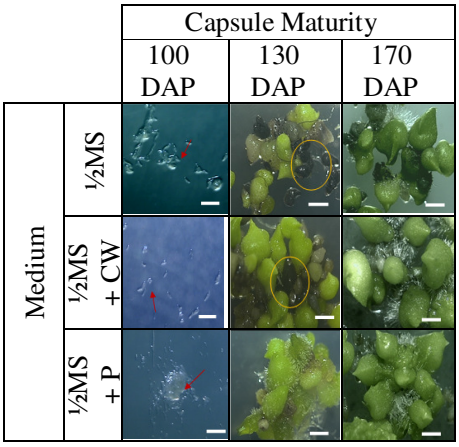

Figure 2. Effect of capsule maturity on germination of *P. gigantea* seeds on 110 day of culture. Arrow indicates ungerminated seeds. Circle showed died protocorms. DAP= Day After Polination, CW=Coconut Water, P= pepton. Bar=1mm.

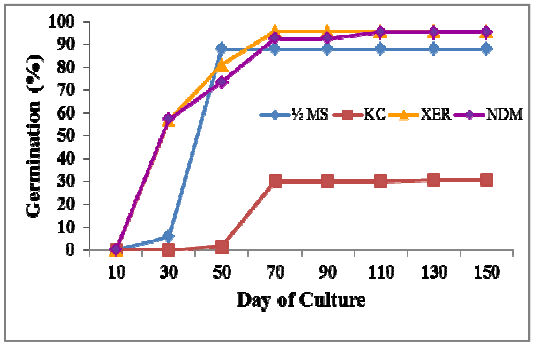

Figure 3. Effect of basal medium on germination of *P. gigantea* seeds during 150 day of culture.

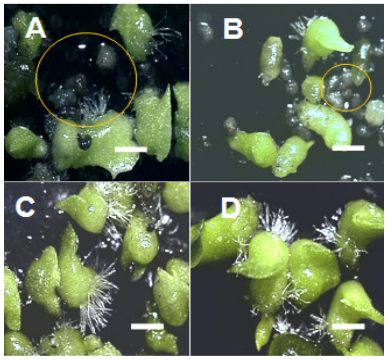

Figure 4. Effect of basal medium on germination of *P. gigantea* seeds 150 day of culture. Circle showed died protocorms. (A) ½MS, (B) KC, (C) XER and (D) NDM. Bar=1mm.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 2. Effects of four additives and their concentrations on *in vitro* germination of *P. gigantea* seeds after 90 days of culture

Additives		Basal Medium	
Type	Conc. (%)	XER	NDM
Control	-	95.66 ± 7.83	94.82 ± 4.29
CW	10	84.57 ± 16.19	100.00 ± 0.00
	15	86.76 ± 13.67	96.80 ± 7.00
	20	79.25 ± 16.90	93.00 ± 14.94
TJ	10	98.57 ± 4.52	100.00 ± 0.00
	15	97.31 ± 8.51	100.00 ± 0.00
	20	100.00 ± 0.00	100.00 ± 0.00
PH	10	100.00 ± 0.00	100.00 ± 0.0
	15	100.00 ± 0.00	100.00 ± 0.00
	20	100.00 ± 0.00	100.00 ± 0.00
BH*	10	28.28 ± 11.53	97.90 ± 4.43
	15	2.35 ± 2.63	71.95 ± 42.11
	20	0	11.36 ± 6.26

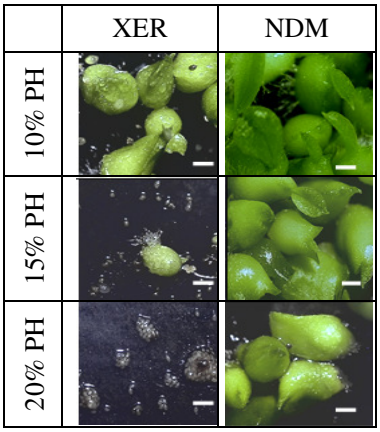

Figure 5. Seed germination of *P.gigantea* on NDM or XER basal media supplemented with potato homogenate at various concentrations after 150 days of culture.

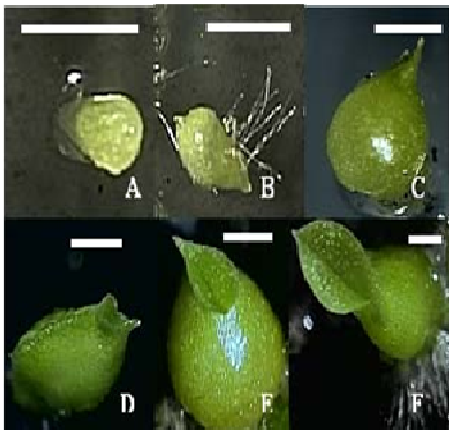

Figure 6. Protocorm development of *P. gigantea* during culture periods of different durations. (A) Swollen and green Embryo (B) Protocorms (C) Protocorm enlargement (D) vegetative apex. (E) leaflets (F) Seedling showing leaflets +2mm. Bars = 1mm.

Conclusions

These finding suggested that under standard culture conditions, seeds from 170 day-old capsule germinated on NDM or XER basal media with addition of potato homogenate at various concentrations can potentially be used to propagate *P. gigantea* in vitro for commercial and conservation purpose.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Different Rates of IBA Hormone Use on Rooting and Growth of Eksotika II Papaya (*Carica papaya* L.) Cuttings at Nursery Stages

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Introduction

Currently, most of the papaya plantings in Malaysia are established using seedlings. However, the seed segregation is rather high especially in the sex expression which is only noticed at flowering stage (Chong et al., 2008). Many cloning techniques have been developed to overcome the segregation problem. Allan reported in 2010, papaya can be propagated using grafting and tissue culture technique. However, the grafting technique has rather low survival rate and requires suitable size of stock and scion, while tissue culture plants are more expensive and not appropriate for small papaya farmers (Muhamad Hafiz et al., 2013). The microcutting technique, recently developed by MARDI showed some promise in the propagation of true to type hermaphrodite papaya.

Microcutting technique is the process whereby the pieces of the plants are cut and placed or sown in a suitable environment, so they can grow into a whole new plant (Awang et al., 2009). The rooting of cuttings is influenced by a few factors. Use of rooting hormones has been shown to influence the success rates of cuttings and has been extensively used in tea cuttings (Sirous et al., 2012). The application of rooting hormones plays an essential role in plant metabolism and can influence the cell cycle proteins (Sanchez et al., 2005). Furthermore, IBA hormone contains high proportion of auxin level, which crucially governs the rooting of cuttings. The level of rooting hormone can definitely influence the success rate of cuttings. A study on ornamental crops of *Ficus hawaii* has shown that the optimum level of rooting hormone should be used on various types of stem for good success (Ismail and Syed, 2007). It has been observed that certain concentration of IBA can enhance root induction, increase sprouting and ultimately increase the cuttings growth (Souidan et al., 1995). Similarly it is believed that the success of papaya cuttings can be improved by using the right concentration of IBA. Thus, a study was conducted to determine the optimum concentration of IBA for enhancement and improvement of rooting and growth in papaya cuttings.

Materials and Methods

This study was conducted in the nursery of MARDI Bukit Tangga, Kedah. The treatments consisted of different rates of rooting hormones (IBA) were arranged in a Completely Randomized Design (CRD) and replicated four times. The seeds of Eksotika II papaya were obtained from the Seed Production Unit located in Pontian, Johor. The seed was germinated in the germination tray. After 12 days, the seed was germinated and it was selected to be transferred into the polybags. The uniform seedlings in the polybags were selected at 3 weeks and each of the selected seedlings was cut at the main stem. The cuttings were dipped in the different solutions of IBA rooting hormone concentration for 1 min. All the cuttings were inserted in the rooting media and placed in the modified microcutting box. The cuttings were supplied with water twice a day using mist sprayer tool. The percentage of cuttings that has initiated rootings was recorded at day 7, 15, 22, 30 and 37. Meanwhile, the growth data for height, girth, canopy spread and leaves number of the rooted cuttings were taken at day 30, 35 and 40. The treatments were control (no rooting hormone added), 1000 ppm (T1), 2000 ppm (T2) and 3000 ppm (T3). The data was analysed using Statistical Analysis System (SAS).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Results and Discussions

Rooting percentage

The maximum rooting percentage was shown to be achieved by the cuttings treated with 3000 ppm (Treatment 3) of IBA at day 7 (16.66), 15 (41.67) and 22 (83.33) (Figure 1). The rooting percentages trend at 3000 ppm showed that by day 30, rooting reached 100%. In addition, the application of IBA at 3000 ppm was able to induce root initiation as early as 7 days after insertion. The use of IBA at 1000 ppm and 2000 ppm was not able to enhance the root initiation as reflected by the failure to root within 7 days. Furthermore, the control also showed the lowest rooting percentages at day 22 (41.67), 30 (83.33) and 37 (91.67), and failed to achieve 100% rooting after 37 days of insertion. Thus application of IBA at 3000 ppm enabled the cuttings to achieve 100% rooting earlier. IBA has been known to play important role in promoting rooting and enhance rooting through shortening the rooting time (Souidan et al., 1995; Ismail and Asghar, 2007).

Height of rooted cuttings

By day 40, the height of rooted cuttings treated with 3000 ppm IBA was significantly higher than other treatments (Figure 2). At earlier stage, day 30 and 35, height of cuttings treated with 3000 ppm and 2000 ppm IBA were not significantly different but were significantly higher than those treated with 1000 ppm and the control. The higher plant height achieved was probably related to the earlier root development of the cuttings treated with 3000 ppm IBA which allowed good uptake of nutrients and hence promoted good growth. This is in line with the findings of Ismail and Saghar in 2007.

Girth of rooted cuttings

On the 30th and 35th day of the application of hormones at all rates (1000, 2000 and 3000 ppm) seemed to result in higher cutting girth compared to the control (0 ppm) (Figure 3). However by the 40th day the cutting girth was not influenced by the application of hormones. This was probably due to the very small value of the cutting girth. Perhaps a better trend would be observed at the later stage of the cuttings development.

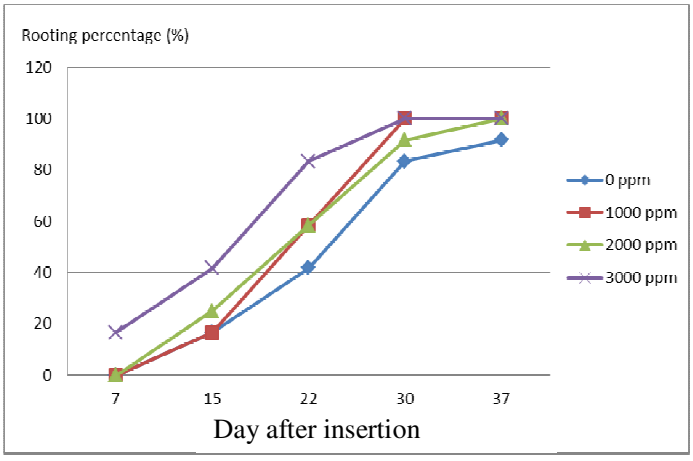

Figure 1. Effect of different rooting hormone use on rooting percentages (%) of cuttings at day 7, 15, 22, 30 and 37 after insertion.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

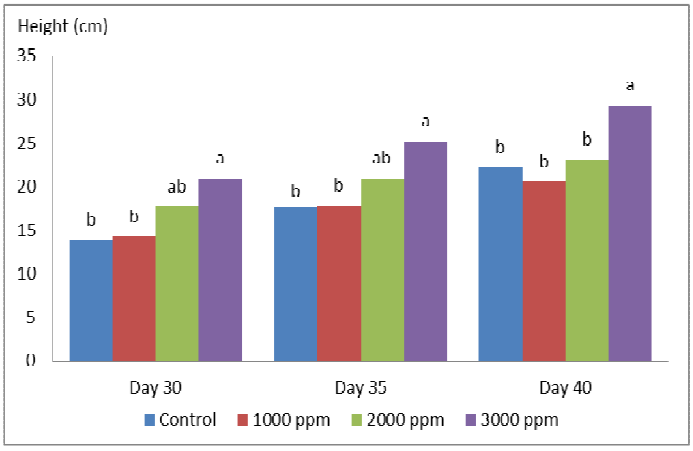

Figure 2. Effect of different rooting hormone use on height (cm) of rooted cuttings at day 30, 35 and 40 after insertion. Means followed by the same letter are not significantly different (P<0.05) according to the Duncan's multiple range test

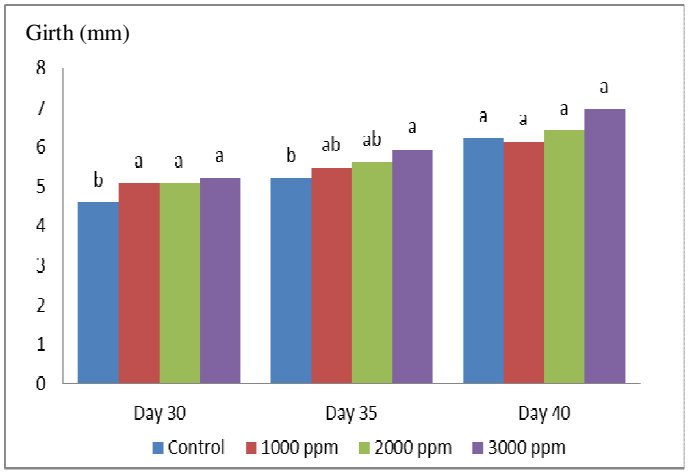

Figure 3. Effect of different rooting hormone use on girth (mm) of rooted cuttings at day 30, 35 and 40 after insertion. Means followed by the same letter are not significantly different (P<0.05) according to the Duncan's multiple range test

Canopy of rooted cuttings

The canopy spread of cuttings treated with 3000 ppm IBA was significantly higher than the other treatments (Table 1). This indicated that application of hormones at 3000 ppm induced early rooting and thus improved early plant vigour where the canopy spread increased concurrently with the root expansion (Muhamad Hafiz et al., 2013).

Leaves number of rooted cuttings

Cuttings treated with 3000 ppm IBA had significantly higher leaf number at day 30 and 40 compared to all other treatments (Table 1). By day 30, the cuttings treated with 3000 pppm IBA has six leaves which indicated that these cuttings came to rooting earlier and thus initiated leaf emergence earlier compared to other treatments.

Table 1. Effect of different rooting hormone use on leaves canopy spread (cm) and leaves number of rooted cuttings at day 30 and 40 after insertion.

Trt. No.	Treatments	Day 30		Day 40	
		Canopy Spread (cm)	Leaves number	Canopy Spread (cm)	Leaves number
Control	0 ppm	16.338 b	4.5 b	27.208 b	8.3333 b
T1	1000 ppm	12.05 b	3.4167 b	23.379 b	6.5833 b
T2	2000 ppm	15.033 b	4.25 b	25.888 b	7.5833 b
T3	3000 ppm	21.779 a	6.25 a	31.342 a	9.9167 a

Means followed by the different letter within the same column are significantly different at p<0.05 according to the Duncan's multiple range test.

Conclusions

The study revealed that rooting of papaya cuttings can be improved with the application of IBA. Rooting of cuttings could be initiated as early as 7 days with application of 3000 ppm IBA. These cuttings achieved 100% success by day 30. Although application of lower rates, 1000 and 2000 ppm IBA resulted in 100% rootings at day 37 these cuttings were less vigorous. Cuttings not treated with IBA could not achieve 100% rootings within 37 days and also lacked in vigour. The results indicated that application of 3000 ppm IBA resulted in good early rooting and more vigorous cuttings in term of height, leaf number and canopy spread. Thus application of 3000 ppm IBA could be recommended for propagation of papaya through cutting technique.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Auxin and Gibberellin in Developing Parthenocarpic Roselle Calyx

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Introduction

Parthenocarpic is referring to the unpollinated flower of fruits that will not produce seed. Roselle (*Hibiscus sabdariffa* L.) has been reported to be a self-pollinating crop species and pollination naturally occurs in the bud stage before the flower blooms (Mohamad et al., 2009). Roselle also known as stingy flower due to the above situation and has become a barrier to natural or artificial hybridization to produce genetic variation. In addition, not much research has been conducted in conventional manner. The brilliant red and fleshy cup-shaped flower calyces are the most important part of roselle plants and are consumed worldwide as hot and cold beverages, jellies, jam, sauces, preserves, and also for medicinal applications. With the increased demand from beverages, pharmaceutical and cosmetic industries, roselle cultivation in Malaysia has gained attention from the farmers to produce it in a large scale. However, farmers face a problem in handling and processing roselle calyx. This may be due to the short shelf life and rapid deterioration of roselle calyx. Roselle generally deteriorates very quickly soon after harvest and this in turn reduces its postharvest quality and at the same time reduces the income of local farmer. Remove velvety capsule containing seed from roselle calyx is time consuming and high labour cost. Therefore, the application of two synthetic hormones such as auxin and gibberellin in developing parthenocarpic roselle calyx were investigated.

Materials and Methods

Twenty-one roselle varieties Terengganu (UMKL-1) were planted at greenhouse, Department of Agrotechnology, Faculty Agrotechnology and Food Science, Universiti Malaysia Terengganu. Two-week-old roselle seedlings were transferred to the polybags containing 30 kg of soil mixture (3:1:1). The duration of experiment was five months started from November 2012. The harvesting date was at 70, 80, and 90 days after transplanting (DAT). Roselle flower bud appeared and bloomed after 20 and 40 - 60 days after transplanting respectively. The flowers born singly in the leaf axis that 13 cm wide, yellow and pink in colour depend on its variety (Muller et al., 1995). Roselle flowers are self-pollination that hardly to measure when the pollination and production of the seed take place. Based on the trial conducted, the self pollination of roselle plant occurs after male and female flower are completely grown in the capsule (Figure 1). In the present experiment, we assume that self-pollination of roselle flower occurs between 1 and 10 DAT (Figure 2). The experiment was arranged in a randomized complete block design (RCBD), with two synthetic hormones i.e. Indoleacetic Acid (IAA) and Gibberellic acid (GA_3) at three different concentrations. The treatments were, Control (without synthetic hormones), 200 ppm GA_3, 400 ppm GA_3, 600 ppm GA_3, 400 ppm IAA, 600 ppm IAA and 800pm IAA with three replications. Three trees represent one experimental unit. All treatments were sprayed three times i.e. 40, 47 and 54 DAT on flower buds excluding control. All experimental plants received same cultural practices including fertilization, pesticides, and fungicides sprays during the experiment. Manual watering was done twice a day. Parameter assessments were number of seed, calyx volume, capsule size and fresh weight, colour [Lightness (L*), chromaticity value a* and b*, chroma (C*) and hue angle (h°)], soluble solids concentration (SSC), titratable acidity (TA), firmness, ascorbic acid (AA) and total anthocyanins concentration.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

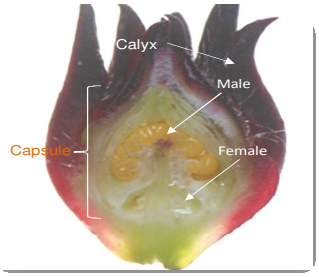

Figure 1. Roselle calyx with male and female organs in the capsule

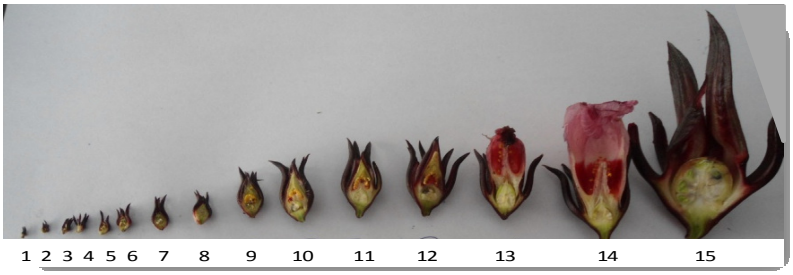

Figure 2. The development of roselle

Results and Discussion

The effect of synthetic hormones or plant growth regulators (PGRs) in developing parthenocarpic fruits have been discovered in various crops such as tomatoes, persimmons, avocado and peach (Supada and Chareonboonsit, 1985; Yamamura et al., 1989; Marianthi et al., 1995; Elina and John, 2006). Besides, PGRs also widely used to induce a variety of responses, including manipulation of fruit set, fruit size, fruit shape, and maturation (Ryugo, 1988). In the present study, various concentrations of PGRs significantly influence capsule diameter, number of seeds and calyx volume as in Figure 3, 4, 5 and 6. On day 70, both PGRs (IAA and GA_3) at higher concentrations tend to show the ability to develop parthenocarpic roselle calyx based on the smaller diameter and volume of capsule and lower number of seed. The lowest number of seeds (13 seeds) was recorded with the application of 800 ppm IAA. Similarly, Heuvelink and Korner (2001) and Aparna, (2011) reported that exogenous application of auxin on *Capsicum annuum* flowers before anthesis resulted in parthenocarpic fruit. Meanwhile, harvested roselle calyx on 80 and 90 DAT showed no apparent effect of different concentrations of IAA and GA_3 on diameter and volume of capsule and seeds number. Possibly, the response of PGRs on target cell (roselle calyx) reduced as its effectiveness also reduced. In addition, hormones are produce in tiny amount in plants. However, in order to maintain its effects, the hormones should be applied continuously. This may be the possible reason why the application of both PGRs was not noticeable in developing parthenocarpic roselle calyx. For future research, it is recommended that the spray application of IAA and GA_3 should be applied more frequent at higher concentrations and continuous supply of both PGRs were highly recommended.

As shown in Figure 7 and 8, IAA-treated plant significantly increased fresh weight and number of roselle calyx respectively. On day 70, 800 ppm IAA-treated plant significantly increased ($P \leq 0.05$) fresh weight (0.294 kg) and number of roselle calyx (32 calyces) compared to other treatments. This may be attributed to the higher IAA concentrations in plant induced the flowering and fruiting which resulted in higher yield (fresh weight and number of calyx) compared to other treatments. As mentioned earlier, IAA and GA_3 were sprayed thrice during bud stage which possibly increased flowering and fruiting of roselle, thereby increased its fresh weight and number of calyx. Moreover, PGRs such as auxin increased yield and quality of various crops such as cotton (Heuvelink and Korner, 2001), long beans (Resmi and Gopalakrishnan, 2004), date palm (Shaheen et al., 1988), capsicum (Oosterhuis and Zhao, 1993) and cherry (Francesco et al., 2012) are well documented.

The application of different PGRs improved red skin coloration of roselle calyx by lowering chromaticity value b*, L* and h° (data not included). Other calyx quality attributes such as firmness, TA, SSC, AA and anthocyanin concentration were also determined (data not included). IAA-treated plants had higher calyx firmness (3.381 N), TA (6.074%) and AA (256.08 mg/100g FW). Possibly, increased in firmness, TA and AA may be associated to stress and hormones uptake towards cells. In

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

addition, Cleland (1995) claimed that the auxin presumably causes increase in the extensibility of cell walls and induces uptake and retention of water and solutes. Many previous reports also conclude that the application of PGRs improved SSC, acidity and colour in corns and guavas (Farag and Kassem, 2000), bell pepper (Aparna, 2011), loquat (Takagi et al., 1994) and persimmons (Yamamura et al., 1989).

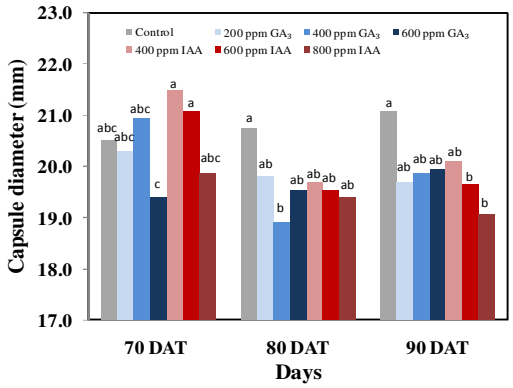

Figure 3. Effects of different concentrations of PGRs on diameter of roselle capsule. Means followed by the same letter are not significantly different (LSD, $P \leq 0.05$)

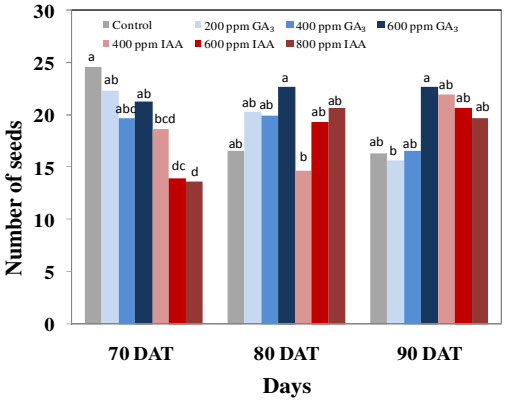

Figure 4. Effects of different concentrations of PGRs on number of roselle seed. Means followed by the same letter are not significantly different (LSD, $P \leq 0.05$)

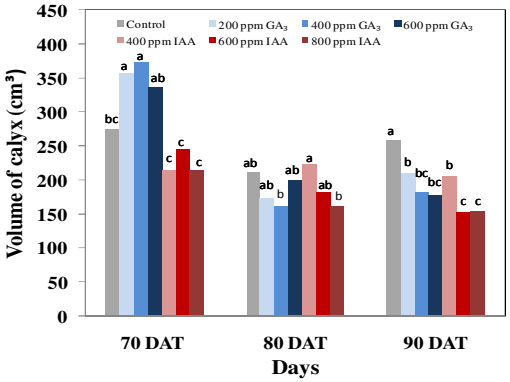

Figure 5. Effects of different concentrations of PGRs on calyx volume. Means followed by the same letter are not significantly different (LSD, $P \leq 0.05$)

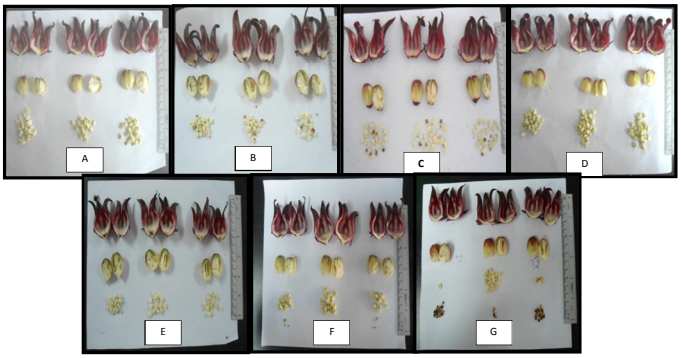

Figure 6. Diameter of capsule and number of seed on 70DAT. A:control, B:200 ppm GA_3, C: 400 ppm GA_3, D:600 ppm GA_3, E: 400 ppm IAA, F:600 ppm IAA and G:800 ppm IAA

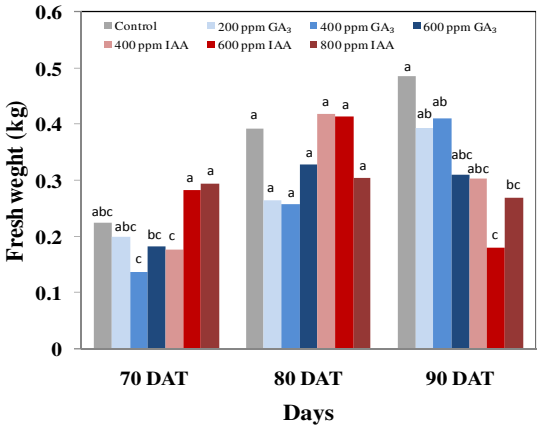

Figure 7. Effects of different concentrations of PGRs on fresh weight of roselle. Means followed by the same letter are not significantly different (LSD, $P \leq 0.05$)

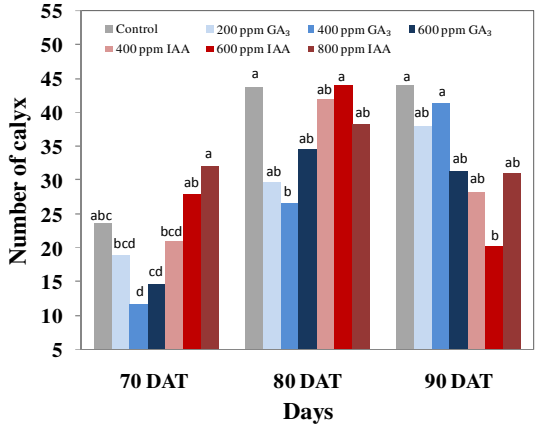

Figure 8. Effects of different concentrations of PGRs on calyx number of roselle. Means followed by the same letter are not significantly different (LSD, $P \leq 0.05$)

Conclusions

The synthetic hormone, IAA had a potential in developing parthenocarpic roselle calyx compared to GA_3. Three sprays of IAA at 800 ppm have the ability to develop parthenocarpic roselle calyx without a significant loss in yield and other quality attributes. In addition, a continuous supply of IAA was highly recommended.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of POMSC as Soil Ameliorant on the Growth and Yield of Roselle (*Hibiscus sabdariffa)* var. UKMR-2 and UKMR-3 Cultivated on Terengganu BRIS Soil

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Introduction

BRIS (Beach Ridges Interspersed with Swales) soil generally contained less water with less than 1% organic compound and pH below 5.0 (Aminuddin et al., 1982) and therefore low in cation holding capacity (CEC). Thus, it could not support the normal nutrient untake for plant growth and affect the quality and also yield (Wan Zaki, 2008). In addition, most of the BRIS soils in Terengganu were considered as non-fertile soil. BRIS soils contain 82-99% sand particles, mainly quartz, with the low cation exchange capacity (CEC) of 9.53 cmol kg-¹ and pH 4.3-4.4 (Yusoff et al., 2011). The soil has low water-holding capacity and poor in nutrients. Palm oil mill sludge cake (POMSC) has the ability to hold 0.5% N, 0.4% P, 0.5% K, 0.8% Ca and 0.3% Mg (Othman et al., 1990; Wan Zaki, 2008). Thus, it is envisaged that crops can still be planted on BRIS soil through improvement by enriching it with suitable soil treatments.

Previous study showed that EFB (empty fruit bunches) and POME (palm oil mill effluent) which is now known as POMSC had been used as soil treatments for fruit trees such as star fruit and sapodilla (Wan Zaki, 2008). The mixture of BRIS soils with EFB or POME in small underground hole or upper surface of the soil were proven successful in supporting the plant growth for the first 6 months. However, additional fertilizers as basal or foliar spray need to be applied to support subsequent plant nutrient needs. The potential benefits of POMSC are that it can increase the CEC and hence enhance the ability of cation exchange, availability of organic carbon and nutrient in soils and reduce the loss of nutrients.

Roselle (*Hibiscus sabdariffa* family Malvaceae) also commonly known as sorrel, mesta and karkade, is a popular plant in middle eastern countries (Morton, 1987; Abu Tarboush et al., 1997). In Malaysia roselle is known as ‘asam paya’, ‘asam susur’ and ‘asam kumbang’. Roselle can be found in almost all tropical countries in South East Asia such as Malaysia, Indonesia, Thailand, and Philipines (Chewonarin et al., 1999; Rao, 1996). It was first commercially planted in Terengganu in 1993 by the Department of Agriculture, Terengganu, Malaysia. In the beginning, 12.8 ha of roselle were cultivated on BRIS soil and the coverage increased to 500 ha by the year 2000.

However, several years later the cultivated areas under roselle in Terengganu decreased to a few hectares. Currently, there is a big market demand for roselle related products both domestically and internationally and therefore it can be considered as one of potential crops that can be grown on BRIS soil. This study was carried out to determine the effect of POMSC as ameliorant on plant growth and calyx yield of two new roselle varieties, UKMR-2 and UMKR-3 planted on BRIS soil (spodosoil, Series Rhu Tapai).

Materials and Methods

Plant materials and soil treatment

Two roselle varieties, UKMR-2 (red) and UKMR-3 (green) were planted on BRIS soil at the Commodity Development Centre, Rhu Tapai, Merang, Terengganu. These were the new varieties released by Universiti Kebangsaan Malaysia in 2009. It was planted in Completely Randomized

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Design with nine replications. POMSC were used at the rate of 0, 10, 20, 30 and 40 ton/hectare. The commercial fertilizers used were NPK with the ratio of 15:15:15 and 12:12:17 + TE added at predetermined intervals. Pesticides were applied when necessary. Irrigation was supplied using drip irrigation.

Determination of plant growth

Observations were made on overall plant growth and data on selected parameters were taken during the primary and secondary growth. These parameters were plant height (cm), canopy diameter (cm) and calyces yield in terms of weight of calyces and number of calyces per plant. The data were collected beginning at field transplanting until day 84 at 2 weeks intervals. Plant height was measured from the soil level to the shoot tip of plant using measuring tape. The plant canopy was also measured using measuring tape. The number of leaves was counted manually. The calyces per plant were harvested at maturity stage 3, weighed using digital balance and the numbers were counted manually.

Results

Vegetative growth parameters

Plant height: The data in Table 1 shows that the plant height was higher when Palm Oil Mill Sludge Cake (POMSC) was added to the BRIS soil compared to control in both varieties, UKMR-2 and UKMR-3. The plant height were the highest with treatment of 20 t/ha POMSC at 4, 6, 8, 10 and 12 weeks after planting (WAP) when compared to the other rates and control plant in both varieties.

Canopy diameter: Canopy diameter (Table 2) was significantly affected by POMSC except on 8 WAP in both varieties. At 4 WAP there were significant response to applied POMSC on canopy diameter for the treatments with 20 and 30 t/ha POMSC when compared with the control. At 6 and 12 WAP, the response to POMSC at 10, 30 and 40 t/ha POMSC were not statistically different from the control but for treatment with 20 t/ha POMSC the response was higher than that obtained from the control for both varieties. At 10 WAP, there was significant response to POMSC at 20 t/ha POMSC compared to control for both varieties, but the responses among 10, 30 and 40 t/ha POMSC were not statistically different (Table 2).

Table 1. Plant height of UKMR-2 and UKMR-3 roselle as affected by different rates of POMSC ameliorant

Treatment	Week After Planting						
	0	2	4	6	8	10	12
UKMR-2							
CONTROL	10.00±0.00^{Fa}	13.00±3.12^{Fb}	22.38±4.41^{Ec}	41.25±8.221^{Dc}	60.00±10.39^{Cc}	75.63±12.75^{Bc}	87.63±11.07^{Ac}
10 t/ha POMSC	9.81±0.37^{Ga}	14.63±3.34^{Fa}	27.50±6.69^{Ea}	46.75±6.98^{Db}	65.00±9.94^{Cbc}	80.50±12.39^{Bb}	87.75±8.71^{Ac}
20 t/ha POMSC	10.10±0.46^{Fa}	15.13±1.36^{Fa}	27.50±3.63^{Ea}	52.38±9.81^{Da}	71.13±12.47^{Ca}	93.50±16.97^{Ba}	97.38±17.46^{Aa}
30 t/ha POMSC	9.98±0.07^{Fa}	13.75±2.87^{Eab}	25.63±5.01^{Eab}	45.63±5.93^{Dbc}	68.50±15.12^{Cab}	82.38±12.21^{Bb}	91.00±13.62^{Abc}
40 t/ha POMSC	9.85±0.35^{Fa}	14.88±2.53^{Fa}	24.25±5.55^{Ebc}	50.00±5.50^{Dab}	66.25±8.45^{Cab}	80.5±11.69^{Bb}	92.63±17.95^{Ab}
UKMR-3							
CONTROL	6.88±1.37^{Fa}	15.13±3.82^{Fb}	25.13±6.27^{Ec}	46.00±6.79^{Dc}	71.38±8.37^{Cd}	95.00±12.53^{Bc}	106.25±11.32^{Ac}
10 t/ha POMSC	6.09±1.27^{Ga}	15.88±2.00^{Fb}	28.38±4.13^{Eb}	53.5±7.55^{Db}	84.00±10.24^{Cbc}	103.38±10.47^{Bb}	114.69±10.05^{Ab}
20 t/ha POMSC	7.24±1.68^{Ga}	19.00±1.88^{Fa}	36.63±4.67^{Ea}	63.75±8.17^{Da}	90.50±8.56^{Ca}	112.88±10.64^{Ba}	125.25±11.79^{Aa}
30 t/ha POMSC	6.00±2.23^{Ga}	18.00±3.46^{Fa}	35.13±6.25^{Ea}	62.63±6.02^{Da}	85.63±8.73^{Cb}	105.6±10.32^{Bb}	116.13±10.17^{Ab}
40 t/ha POMSC	7.44±1.6^{Ga}	15.75±4.03^{Fb}	27.75±5.59^{Ebc}	55.35±9.14^{Db}	81.13±10.55^{Cc}	105.75±17.98^{Bb}	115.75±18.47^{Ab}

Note: Values in table 1 are mean of 9 replicates. Mean (n=9) ± standard deviation
A-G – means bearing the same superscript within the same row are not significantly different at 5% level ($p<0.05$)
a-c – Means bearing the same superscript within the same column are not significantly different at 5% level $p<0.05$)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 2. Plant canopy of UKMR-2 and UKMR-3 roselle as affected by different rates of POMSC ameliorant

Treatment	Week After Planting						
	0	2	4	6	8	10	12
UKMR-2							
CONTROL	4.00±0.00^{Ga}	14.13±5.46^{Fb}	14.50±5.24^{Ec}	29.40±10.54^{Dc}	37.38±7.58^{Cc}	41.13±6.17^{Bb}	48.88±4.911^{Ab}
10 t/ha POMSC	4.00±0.00^{Ga}	16.63±5.29^{Fa}	16.63±3.66^{Eb}	29.63±7.11^{Dc}	41.13±8.53A^{Cb}	42.13±6.75^{Bb}	48.13±14.66^{Ab}
20 t/ha POMSC	4.00±0.00^{Ga}	17.06±3.89^{Fa}	20.50±4.31^{Ea}	35.50±3.82^{Da}	45.38±4.78^{Ca}	45.38±5.09^{Ba}	52.88±5.25^{Aa}
30 t/ha POMSC	4.00±0.00^{Fa}	13.00±6.63^{Eb}	16.00±5.61^{Dbc}	32.75±8.12^{Cb}	40.50±7.84^{Bb}	43.00±6.32^{Bb}	50.50±12.68^{Aab}
40 t/ha POMSC	4.00±0.00^{Fa}	16.50±4.00^{Ea}	15.00±4.57^{Dbc}	32.75±7.69^{Cb}	41.50±5.15^{Bb}	41.13±5.59^{Bb}	51.50±3.85^{Aa}
UKMR-3							
CONTROL	5.75±2.57^{Ea}	13.00±4.41^{Db}	14.50±4.68^{Dc}	26.50±5.18^{Cc}	36.00±3.20^{Bc}	39.50±7.43^{Bc}	43.63±8.65^{Ac}
10 t/ha POMSC	6.50±1.72^{Ea}	13.75±2.24^{Db}	14.88±3.22^{Dc}	28.50±7.14^{Cbc}	37.25±5.24^{Bbc}	33.88±6.86^{Bd}	45.00±10.26^{Ac}
20 t/ha POMSC	6.88±1.58^{Ga}	16.00±3.64^{Fa}	24.25±4.82^{Ea}	37.00±8.15^{Da}	47.25±8.90^{Ca}	52.50±12.61^{Ba}	58.25±9.87^{Aa}
30 t/ha POMSC	6.88±1.87^{Ga}	15.63±3.23^{Fa}	22.51±4.08^{Ea}	35.63±6.35^{Da}	45.25±7.07^{Ca}	48.88±8.43^{Bb}	53.00±7.77^{Ab}
40 t/ha POMSC	6.38±2.35^{Fa}	15.25±4.25^{Ea}	17.25±4.17^{Eb}	30.50±7.19^{Db}	38.88±4.93^{Cb}	34.63±5.58^{Bd}	45.75±9.07^{Ac}

Note: Values in table 1 are mean of 9 replicates. Mean (n=9) ± standard deviation
A-G – means bearing the same superscript within the same row are not significantly different at 5% level (p<0.05)
a-d – Means bearing the same superscript within the same column are not significantly different at 5% level (p<0.05)

Number of leaves: The data in Table 3 shows that the number of leaves per plant of roselle for both variety UKMR-3 were significantly (p<0.05) influenced by the application of POMSC at 2 and 4 WAP. However increased application of POMSC did not produce significant differences (P>0.05) in the leaves number of roselle var. UKMR-2 at 4 WAP. Generally, the highest number of leaves per plant were obtained from 20 t/ha followed by 30 t/ha, 40 t/ha and 10 t/ha POMSC and the lowest was obtained from non-treated BRIS soil (control) for both varieties.

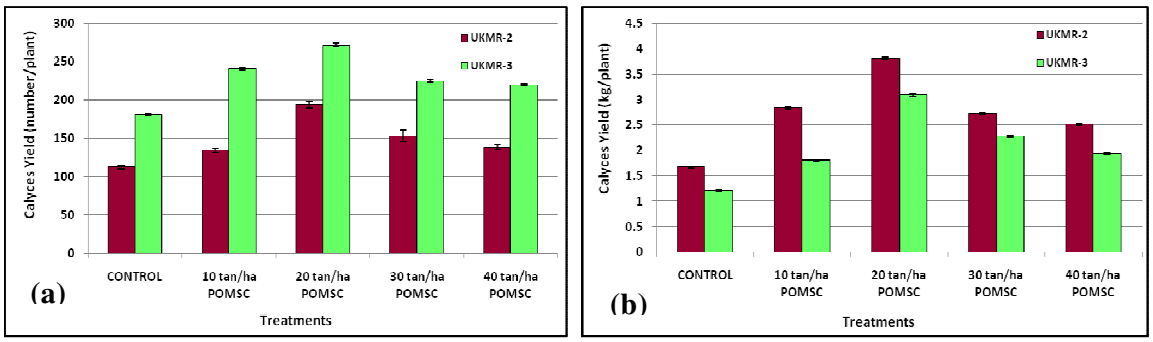
Table 3. Leaf numbers of UKMR-2 and UKMR-3 roselles as affected by different rates of POMSC ameliorant

Treatment	Week After Planting		
	0	2	4
UKMR-2			
CONTROL	4.00±0.00^{Ba}	8.75±0.46^{Bd}	26.38±1.69^{Ab}
10 t/ha POMSC	4.00±0.00^{Ba}	11.62±1.06^{Bc}	30.00±0.93^{Aa}
20 t/ha POMSC	4.00±0.00^{Ca}	19.12±1.13^{Ba}	32.50±2.67^{Aa}
30 t/ha POMSC	4.00±0.00^{Ba}	9.50±0.53^{Bd}	32.00±2.00^{Aa}
40 t/ha POMSC	4.00±0.00^{Ca}	14.50±1.51^{Bb}	30.75±0.71^{Aa}
UKMR-3			
CONTROL	4.00±0.00^{Ca}	8.38±0.74^{Bc}	23.12±1.81^{Ae}
10 t/ha POMSC	4.00±0.00^{Ca}	8.62±0.52^{Bc}	28.12±1.64^{Ad}
20 t/ha POMSC	4.00±0.00^{Ca}	13.38±1.30^{Ba}	45.25±4.17^{Aa}
30 t/ha POMSC	4.00±0.00^{Ca}	12.12±2.03^{Bab}	38.75±1.91^{Ab}
40 t/ha POMSC	4.00±0.00^{Ca}	11.50±0.93^{Bb}	33.75±0.71^{Ac}

Note: Values in table 1 are mean of 9 replicates. Mean (n=9)±standard deviation
A-C – means bearing the same superscript within the same row are not significantly different at 5% level (p<0.05)
a-e– Means bearing the same superscript within the same column are not significantly different at 5% level (p<0.05)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Yield parameters: Fresh calyces yield (weight and number per plant) of roselle varieties were significantly ($p<0.05$) influenced by the applications of POMSC as soil ameliorant. Varieties variation showed that fresh calyces yield of UKMR-2 recorded the highest at 20 t/ha POMSC (3.09kg and 194/plant) while the lowest was obtained from control (1.21 kg and 113/plant). While for UKMR-3, the highest fresh calyces yield was 3.81 kg and 302/plant (20 t/ha POMSC) and the lowest mean was 1.67 kg and 181/plant (control). The highest yielding variety was UKMR-3 at 20 t/ha POMSC (Figure 1a and Figure 1b).



Figures 1a and 1b. Calyx number and weight (kg) of calyces of two roselle varieties (UKMR-2 and UKMR-3) as affected by different rates of POMSC ameliorant. Values are expressed as mean ± S.E.M of triplicate measurements and analysed statistically using one way ANOVA.

Discussion

The results showed that POMSC as soil ameliorant promoted the growth parameters such as plant height, plant canopy diameter and increased the calyx yield parameters (calyx weight and number of calyces/plant) compared to untreated BRIS soil. This result indicated that the amelioration of POMSC on BRIS soil had improved the BRIS soil health. A large amount of sand (>90%) and very poor physical and chemical characteristics of BRIS soil affect the overall growth development and resulted in inferior calyx yield and quality parameters. Therefore, the yield was significantly decreased under BRIS soil condition (Figure 1a and 1b).

Deficiency of nutrients affects roselle yield, e.g., potassium deficiency decreased grains production (International Potash Institute, 2008). An application of POMSC as soil ameliorant improved the soil structure and thus, reduced nitrogen leaching from soil; it can also reduce the amount of commercial nitrogen fertilizer to be applied, and decreased the possibility of nitrate groundwater contamination (Shiralipour et al., 1992). The positive response of calyx yield to POMSC and fertilizer application confirmed the findings of Tindall (1983) who reported that economic yield of roselle was only obtained in soils which were well supplied with organic materials and essential nutrients.

Conclusions

Generally, the morpho-agronomic traits of two roselle varieties (UKMR-2 and UKMR-3) were improved by the addition of POMSC as soil ameliorant. Irrespective of POMSC rate, analyzed data showed that higher plant height was obtained with the application of POMSC. The application of POMSC also resulted in better plant canopy diameter growth. Similarly the number of leaves and calyces yield of roselle plants (calyx weight and number of leaves) was higher in POMSC treatments as compared to control for both varieties. The above advantages imply that POMSC can be used effectively as potential soil ameliorant for commercial roselle planting on BRIS soils.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Growth and Flowering of *Chrysanthemum* in Subtsrate Culture under Root Restricted Volume

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Introduction

Chrysanthemum (*Dendranthema grandiflorum or Chyrsanthemum moliforium* Ramat) is a flower in the family Asteracae. It can be used as cut flower, pot plant and garden plant (John and Harold, 2004). For cut chrysanthemum production, growers mainly cultivate in soil culture. Production of chrysanthemum in hydroponics system or soilless culture has been tried more than two decades ago such as nutrient film technique (van Os, 1980), ebb-flow system (Bulwada et al., 1986), and aeroponics (Molitor and Fischer, 1997). There are few studies on the effect of substrate and volume on growth of chrysanthemum. The objective of this study was to compare the growth and flowering of chrysanthemum with different substrate type and volume to find suitable substrate and optimum substrates volume for cut chrysanthemum production.

Materials and Methods

Chrysanthemum cv 'Reagan white' were grown in soil as control recommended by Mohammad et al., (2003) compared with nine treatments in substrate at density 64 plants/m^2 included coconut peat 34 ml (T1), burn rice husk 34 ml (T2), coconut peat mixed with burn rice husk 34 ml (T3), coconut peat 73 ml (T4), burn rice husk 73 ml (T5), coconut peat mixed with burn rice husk 73 ml (T6), coconut peat 140 ml (T7), burn rice husk 140 ml (T8), coconut peat mixed with burn rice husk 140 ml (T9). Plants in soil were fertigated 4 times/day by drip tape while substrate culture were fertigated by micro-sprinkler with nutrient solution contained N,P,K, Ca, Mg,Fe,Mn,Zn,B,Cu, Mo at 200, 30, 200, 150, 50, 1.05, 0.58, 0.35, 1, 0.05, 0.05 mg/l (MARDI, 2003). Relative water content, proline content (Bate et al, 1973) and photosystem II efficiency was measured. Plant growth was observed as plant height, total leaf area (Li-COR model 310, USA), root surface area (WinRhizo, Canada) and total dry weight. Flower quality was measured as number of flower, flower diameter, number of petals, day to harvest and vase life. Analysis of variance of all parameters was implemented by SAS statistical package, and means were compared by LSD at $p<0.05$.

Results and Discussion

Plant height of chrysanthemum grown in substrate tends to increase when substrate volume increased regardless of substrate type (Table 1). This result was in agreement with Latimer (1991) who reported a reduction of plant height of marigold. Coconut peat 73 ml (T4) produced the tallest plant 75.38 cm. of substrates, however, it was still lower than control 130.39 cm. Chrysanthemum grown in substrate produced lower leaf area and total dry weight than control, but had greater root shoot ratio than control significantly. According to (Wageningen UR Greenhouse Horticulture, 2013) reduced substrate volumes also reduce growth of chrysanthemum in soilless system. Relative water content were not significantly different between control and other treatments (Figure 1). However, proline, an amino acid, which accumulated when plant were water stressed was detected in all treatments. Chrysanthemum grown in soil had lower proline content than in substrate (Figure 2). Proline content varied among treatments, but tends to decrease with increasing substrate volume. This was in correspondence with previous result by Drüge (1997) who reported that chrysanthemum grown in hydroponic system had higher proline than conventional cultivation. Photosystem II efficiency of plant grown in all treatments had Fv/Fm values lower than 0.70 (Table 2). When Fv/Fm is lower than

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

0.84 this indicated that plants had low photosynthetic efficiency due to stress (Maxwell and Johnson, 2000).

Table 1. Effect of substrate types and volumes on plant height, leaf area, root surface area, total dry weight and root shoot ratio

Treatments	Plant height (cm)	Leaf area (cm^3)	Root surface area (cm^2)	Total dry weight (g)	Root shoot ratio
control	130.39 a	1556.98 a	73.41 bcd	26.01 a	0.03 c
T1	56.03 d	178.04 d	52.82 de	5.40 de	0.10 ab
T2	54.05 d	139.51 d	37.52 e	4.01 e	0.09 b
T3	51.60 d	170.84 d	54.25 cde	4.58 e	0.10 ab
T4	75.38 b	392.43 bc	91.15 b	11.86 bc	0.10 ab
T5	66.05 bc	244.03 cd	80.48 bcd	6.27 de	0.09 b
T6	60.79 cd	275.95 cd	89.19 bc	8.10 cde	0.09 b
T7	71.09 b	360.16 c	137.71 a	11.05 c	0.11 ab
T8	72.10 b	374.80 bc	158.47 a	9.02 cd	0.13 a
T9	74.88 b	521.85 b	165.23 a	15.95 b	0.10 b
Sig	0.01	0.01	0.01	0.01	0.01

Mean were not significantly different when followed by the same character P<0.05

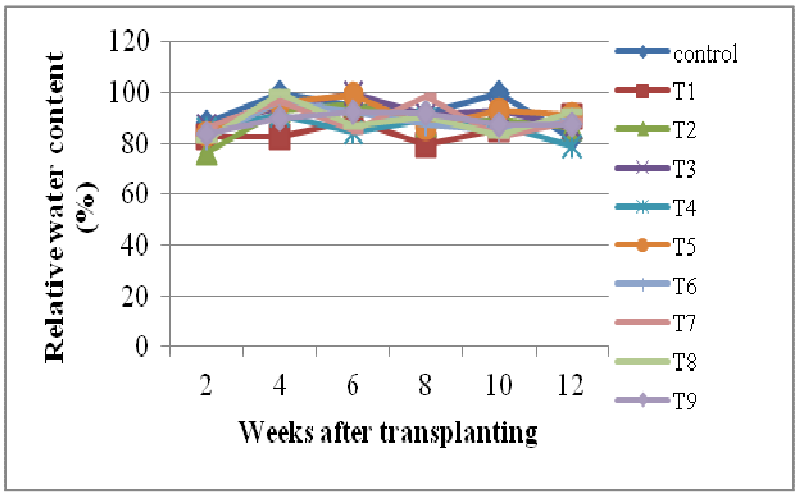

Figure 1. Effect of substrate type and volume on relative water content of chrysanthemum.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

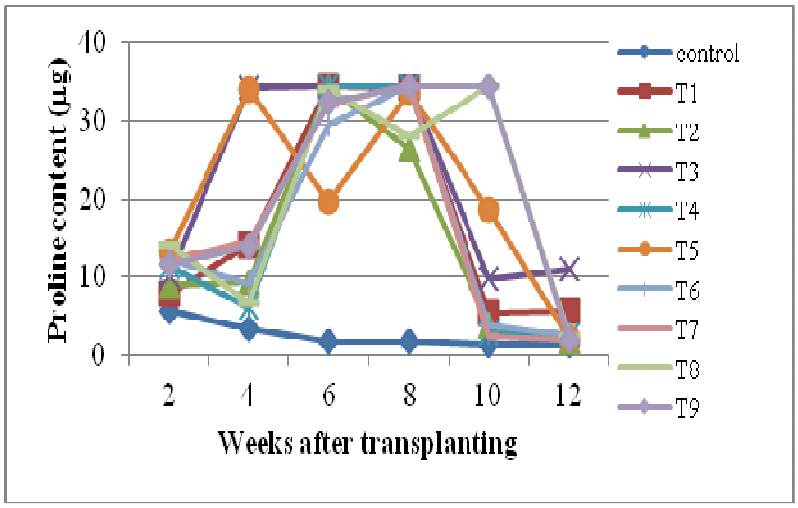

Figure 2. Effect of substrate type and volume on proline content of chrysanthemum.

Table 2. Effect of substrate type and volume on photosystem II efficiency (Fv/Fm)

Treatments	Weeks				
	4	6	8	10	12
control	0.81	0.40	0.77	0.32	0.49
T1	0.67	0.44	0.71	0.51	0.52
T2	0.58	0.64	0.72	0.39	0.57
T3	0.53	0.63	0.70	0.28	0.42
T4	0.76	0.68	0.53	0.72	0.57
T5	0.31	0.69	0.48	0.75	0.38
T6	0.63	0.27	0.53	0.36	0.47
T7	0.68	0.67	0.65	0.47	0.58
T8	0.64	0.54	0.41	0.33	0.35
T9	0.73	0.59	0.33	0.58	0.55
Sig	ns	ns	ns	ns	ns

ns=nonsignificant

The results obtained from plant analysis in Table 3 showed that there were no significant difference between macro element concentration of control and treatments while the same nutrient solution were use in all treatments in this study. Thus, nutrient uptake may be not limited factor of growth under restricted container volume.

Table 3. Effect of substrate types and volumes on macro nutrient concentration at six weeks after transplanting

Treatments	Nutrient Concentration (%)				
	N	P	K	Ca	Mg
control	2.62	0.22	4.83	0.44	0.18
T1	3.16	0.33	5.75	0.66	0.52
T2	3.32	0.41	6.13	0.67	0.46
T3	2.02	0.16	3.83	1.39	0.95
T4	2.02	0.21	4.43	0.56	0.52
T5	2.37	0.27	4.58	0.68	0.57
T6	2.51	0.27	5.22	0.60	0.58
T7	2.75	0.32	5.19	0.50	0.45
T8	1.62	0.16	3.59	1.37	1.14
T9	1.92	0.23	4.78	0.47	0.52
Sig	ns	ns	ns	ns	ns

ns=non significant

Chrysanthemum grown in soil had more number of flowers than substrate significantly. Between substrate cultures, chrysanthemum grown in coconut peat mixed with burn rice husk 140 ml (T9) produced highest number of flower (Table 4). Soil grown chrysanthemum had biggest flower diameter 16.94 cm. but it was insignificantly with chrysanthemum grown in coconut peat mixed with burn rice husk 140 ml (T9). Number of petal did not differ significantly among all treatments and control. There were no significant difference in days to harvest and vase life of chrysanthemum grown in all treatments.

Table 4. Effect of substrate type and volume on number of flowers, flower diameter, number of petal, day to harvest and vase life

Treatments	Number of flower	Flower Diameter (cm)	Number of petal	Day to harvest (day)	Vase life (day)
control	31.75 a	16.94 a	23.33	97.00 a	7.00 d
T1	7.38 ef	8.23 def	23.96	93.25 cd	14.00 ab
T2	7.13 f	6.52 f	15.46	94.75 b	14.25 a
T3	8.25 def	7.13 ef	15.04	94.00 bc	11.13 abcd
T4	15.00 cd	11.19 bcd	24.25	92.75 de	9.13 cd
T5	11.75 cdef	9.96 cde	24.96	92.25 de	11.38 abcd
T6	15.00 cd	9.18 cdef	14.83	92.25 de	9.38 bcd
T7	14.88 cde	10.83 bcd	23.5	92.00 e	12.13 abc
T8	15.88 bc	11.73 bc	24.46	92.25 de	10.00 abcd
T9	22.63 b	14.01 ab	23.08	92.00 e	10.63 abcd
Sig	0.01	0.01	ns	ns	ns

Mean were not significantly different when followed by the same character P>0.05, ns=not significant

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Conclusions

The results from this experiment showed that the growth and flowering of chrysanthemum was influenced by substrate volume and not substrate type. Smaller substrate volumes reduced growth and quality of chrysanthemum**.** However, it was still possible to produce cut chrysanthemum with limited root volume.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

CHAPTER 2

POSTHARVEST TECHNOLOGY AND QUALITY CONTROL

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Physico-Chemical Properties and Sensory Acceptance of Juices Made from Different Roselle (*Hibiscus sabdariffa* L.) Varieties

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Introduction

Roselle (*Hibiscus sabdariffa* L.) belongs to the family of Malvacea which also known as roselle, sorrel, mesta and karkade. Roselle is a tropical plant of considerable economic potential that can be found in almost all tropical countries of South East Asia such as Malaysia, Indonesia, Thailand and Philipines (Chewonarin et al., 1999). It is a new commercial crop of Malaysia, where it is reported to have been brought in from India. The calyces have been found to be rich in vitamic C and other antioxidants such as flavonoids and also mineral (Fasoyiro et al., 2005) and are widely used to prepare herbal drink, cold and warm beverages, and for making jams and jellies (Abu-Tarboush et al., 1997; Tsai et al., 2002). Roselle can also be used as natural food colourants (Duangmal et al., 2004).

Roselle calyces are largely used in the production of beverages. Roselle juice which is conventionally made from water extraction of fresh or dried roselle calyces has been reported as being a popular soft drink with daily consumption in many countries including Egypt, Sudan, Mexico, Nigeria and Thailand (Aurelio et al., 2007). Roselle juice is claimed to be a new pro-health drink due to its high contents of ascorbic acid, anthocyanins and other antioxidants. Earlier investigation on roselle indicated that it could prevent cancer, reduce high blood pressure, eased food digestion and other benefits that help to maintain health (Muhammad and Shakib, 1995). Duh and Yen (1997) who studied the antioxidative activity of three herbal water extract, including the calyx of roselle, found that roselle contained high levels of total phenolic compounds, showed reducing power and scavenging effects of radicals. This study focused on determining the physico-chemical properties and sensory acceptability of roselle juices made from five different roselle varieties namely UKMR-1, UKMR-2, Arab, Terengganu and UMTHS-01.

Materials and Methods

All of the roselle varieties were planted at Plant Research Lab, Department of Agrotechnology, Universiti Malaysia Terengganu (UMT). All the roselle samples were harvested at maturity stage 3 and brought to Postharvest Lab for juice processing and for analyses. Roselle juices were made from the roselle calyces by extracting it in boiling water and adding some sugar into the juice. After that, the physico-chemical characteristics of roselle juices were analysed as well as their sensory evaluation.

Physico-chemical analyses of roselle juices were measured in terms of their color by using chromameter according to the following color coordinate: lightness (L*), redness (a*, red-green) and yellowness (b*, yellow-blue). Total Soluble Solids (TSS) by using refractometer, pH value by using pH meter, ascorbic acid content was determined based on the colorimetric method byJagota and Dani (1982) and anthocyanins content was measured by pH differential method and calculated based on delphinidin-3-glucoside (Giusti and Wrolstad, 2001).

Sensory evaluation involved 30 panelists who evaluated roselle juice made from different varieties for the attributes of color, aroma, taste, after-taste and overall acceptability. The acceptability scores were ranging from 1 to 7 with 1 for very much unacceptable until 7 for very much acceptable.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Results and Discussion

There were significant differences ($p<0.05$) among the color L*, a*, b* values of roselle juice made from five different varieties. For L* value, juice of UKMR-1 variety showed significantly ($p<0.05$) the lightest color among other varieties which were 57.77 while Arab variety showed the darkest color with L* value of 40.28. Mean while for color a* value, juice from Terengganu variety showed the highest color a* value compared to other varieties which was 42.07 and juice of UKMR-1 showed the lowest color a* value which was 25.57.

Juice of UKMR-1 showed significantly ($p<0.05$) the highest b* value compared to other roselle varieties. Anthocyanins are water-soluble flavonoid pigments, which are responsible for the most spectacular red, blue and purple colours in many fruits and vegetables. The brilliant red pigments contained in the red roselle calyxes are anthocyanins (Du and Francis, 1973). Roselle calyces also contained natural constituents of organic acid such as malic, citric and 3-indolyl acetic acids (Al-Khatani and Hassan, 1990) which played an important role in giving brilliant red colour of the sample extract.

Table 1. The color (L* a* and b* values) of roselle juice from five different varieties

Roselle varieties	Color		
	L* value	a* value	b* value
UKMR-1	57.77 ± 8.73^{a}	25.57 ± 5.59^{d}	25.57 ± 4.13^{c}
UKMR-2	43.76 ± 4.44^{bc}	36.87 ± 4.58^{b}	36.87 ± 4.52^{ab}
Arab	40.28 ± 4.58^{c}	35.09 ± 4.75^{c}	35.09 ± 5.41^{b}
Terengganu	45.50 ± 2.02^{b}	42.07 ± 0.95^{a}	42.07 ± 1.17^{a}
UMTHS-01	46.73 ± 6.05^{bc}	41.96 ± 1.64^{a}	41.96 ± 3.04^{ab}

Note : Values are means of 3 replicates (3 readings/replicate). Mean (n=9) ± standard deviation.
a-d : Values bearing the same superscript within the same column are not significantly different at 5% level ($p<0.05$)

Juice of UMTHS-01 variety showed the highest ($p<0.05$) TSS value among juice of different roselle varieties which was 8.96°Brix. The TSS values in the juice was mostly due to the addition of the sugar during roselle juice preparation even though in fresh state roselle of Arab variety has the highest TSS value. According to Kirk and Sawyer (1997) the total sugar contents for roselle was around 3.28% which made up of glucose, fructose and sucrose. Glucose was found to be the major sugar for roselle followed by sucrose and fructose. Generally, the total sugar value in roselle was lower compared to blackcurrants, raspberries and strawberries which were 6.3%, 4.5% and 5.6% respectively.

The pH values of juice made of five different roselle varieties were significantly difference where UMTHS-01 variety showed the highest pH value which was 3.10 whereas UKMR-1 variety showed the lowest value (2.54). Wills et al. (1998) reported that the pH value of Arab variety which was 2.62. The pH depends on the concentration of free H ions or mirrored the changes in total organic acids. The free state of H ions is due to dissociation of H ions from the carboxylic group (-COOH) of organic acid. This increase in pH throughout maturation was due to a metabolic process in the fruit that resulted in the decrease of organic acids. This is because organic acids are an important source of respiratory energy in plant cell. The low pH value of the juice was due to the acidic nature of the roselle calyces and the fruits. Roselle is characterised as a highly acidic fruit rich in organic acids: oxalic, tartaric, malic and succinic (Wong et al., 2002).

Ascorbic acid is lost due to the activities of phenoloxidase and ascorbic acid oxidase (Salunkhe et al., 1991). The ascorbic acid content in juices made of five different roselle varieties showed that UMTHS-01 variety had significantly the highest ascorbic acid (60.17 mg/100g) as compared to other varieties. However, UKMR-1 variety showed significantly the lowest amount of ascorbic acid which was 21.40 mg/100g. Kirk and Sawyer (1997) reported that roselle contains higher amount of ascorbic

acid than oranges and mango. Ascorbic acid content of roselle calyces is related to the state of freshness. Lower value of ascorbic acid content in roselle juice reported in the present study could be associated with nutritional losses during juice preparation. Ascorbic acid is water soluble that can easily lost during boiling, heating and cooking.

The highest anthocyanin content was obtained from juice of Arab variety which was 458.25 mg/100g. Meanwhile juice of UKMR-1 variety showed the lowest amount of anthocyanin which was only 58.45 mg/100g. It is known that roselle calyces are very rich in vitamin C, anthocyanins, polyphenols and other water soluble antioxidants (Duke and Atchley, 1984).

Table 2. The chemical properties of roselle juice from five different varieties

Roselle varieties	TSS (°Brix)	pH	Ascorbic acid (mg/100ml)	Anthocyanin (mg/100g)
UKMR-1	8.66 ± 0.47^{ab}	2.54 ± 0.03^{b}	21.40 ± 0.95^{c}	58.45 ± 1.64^{d}
UKMR-2	8.56 ± 0.16^{ab}	2.67 ± 0.02^{a}	37.82 ± 2.3^{bc}	188.64 ± 3.9^{b}
Arab	8.38 ± 0.36^{b}	2.70 ± 0.01^{a}	44.92 ± 4.76^{b}	458.25 ± 33.33^{a}
Terengganu	8.00 ± 0.00^{b}	2.59 ± 0.01^{b}	59.17 ± 1.78^{ab}	171.27 ± 3.11^{c}
UMTHS-01	8.96 ± 0.05^{a}	2.66 ± 0.01^{a}	60.17 ± 0.73^{a}	187.44 ± 2.04^{b}

Note : Values are means of 5 replicates. Mean (n=5) ± standard deviation.
a-d : Values bearing the same superscript within the same column are not significantly different at 5% level (p<0.05)

From the present study, the acceptability for the attribute of aroma showed no significant different ($p<0.05$) among five different varieties. Meanwhile For the attributes of color, taste, after-taste and overall acceptability, Terengganu variety showed significantly the highest scores of acceptability which were 6.93, 6.33, 6.10 and 5.90 respectively. However, UKMR-1 showed the lowest score of acceptability for color, taste and overall acceptability which were 4.73, 4.90 and 4.03 respectively. This might probably be due to the lighter juice color and more acidic taste.

Table 3. The sensory acceptability of roselle juice from five different varieties

Roselle varieties	Sensory Acceptability				
	Color	Aroma	Taste	After-taste	Overall acceptability
UKMR-1	4.73^{b}	5.80^{a}	4.90^{b}	4.10^{b}	4.03^{b}
UKMR-2	6.40^{a}	5.97^{a}	5.13^{b}	4.23^{b}	4.77^{ab}
Arab	6.80^{a}	5.70^{a}	5.23^{ab}	4.03^{b}	4.23^{ab}
Terengganu	6.93^{a}	5.53^{a}	6.33^{a}	6.10^{a}	5.90^{a}
UMTHS-01	6.87^{a}	5.83^{a}	5.20^{ab}	4.03^{b}	4.43^{ab}

Note : Values are means of 30 replicates.
a-b : Values bearing the same superscript within the same column are not significantly different at 5% level (p<0.05)

Conclusions

In conclusion, roselle juice from UKMR-1 variety showed the highest in colour L* value but the lowest in color a*, b*, ascorbic acid and anthocyanin contents. Meanwhile juice from Arab variety had lower colour L* and TSS values but the highest in anthocyanin content. Higher color a* and b* values were observed in roselle juice from Terengganu variety with lower TSS and pH values. Juice made from roselle UMTHS-01 variety was found to be the highest in TSS and ascorbic acid content. The sensory evaluation done on the roselle juice made from different roselle varieties revealed that juice from Terengganu variety was the most accepted among the panellists in almost all the attributes where juice from UKMR-1 variety was the least accepted.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Different Soilless Growing Media and Biochar on Growth, Yield and Postharvest Quality of Lowland Cherry Tomato (*Solanum lycopersicum* var. Cerasiforme)

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Introduction

Cherry tomato (*Solanum lycopersicum* var. Cerasiforme) is classified under family Solanaceae and thought to be originated in Peru and Northern Chile (Smith, 1994). It is an important horticultural crop grown commercially worldwide and available all-year-round. Currently global production is dominated by China (48 million metric tonnes, mt), India (16 million mt) and United States of America (12 million mt) (FAO, 2013). In Malaysia, among local vegetables, tomatoes are one of the few vegetables that are able to penetrate domestic retail sector as well as export market. In 2011, the production of tomatoes in Malaysia was 134,338 mt from an area of 1,354 hectares (FAO, 2013). The major commercial tomato productions in Malaysia are concentrated in Cameron Highlands, Pahang and Kundasan, Sabah. To date, the demand for healthy and safety tomatoes have been increased in both at the export as well as domestic markets in Malaysia. However, insufficient supply of tomato due to intensive demand from local and export markets entail more tomato cultivation in Malaysia. In recent years, fertigation is the most widely used soilless culture system in growing tomato. Soilless culture technique is a growing system that eliminates the usage of soil in cultivation of crops (Nikki, 2012). The purpose of using soilless growing media is more likely to avoid soil-borne disease and pest. The common soilless growing medium used is coco peat which is mainly from coconut husk strand. Coco peat (CP) has the appearance and texture of soil but contains no mineral content. However, CP alone may not sufficient to enhance yield, growth and postharvest quality of tomato. Thus, combination of CP and other organic growing media such oil palm fruit bunch (OPFB) and biochar could be the effective tools to be applied. Nowadays, the use of newly developed organic medium, OPFB has increased attention due to its nutrient content and ability to hold soil moisture. OPFB is a by-product of oil palm plantation while biochar is charcoal-like material produce by thermo chemical pyrolysis of biomass materials (Laird, 2008). Biochar is used to stimulate the ability to hold carbon, improve food safety and discourage deforestation (Anon, 2010), lessen the ability of microbes to mineralise the carbon (Baldock and Smernik, 2002) and increase growth (Siti et al., 2012).Therefore, this study aimed to evaluate the effects of CP, OPFB, biochar media and its combination on yield, growth and postharvest quality of lowland cherry tomato.

Materials and Methods

Thirty six of cherry tomato plants were grown in this experiment. The experiment was conducted at Greenhouse, Department of Agrotechnology, Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu. After four weeks, the tomato seedlings were transferred into polybag containing various treatments of soilless growing media. Fertilizers used in this study were type A and B. Irrigation was equipped with droplet system and scheduled for 2 min/day (1000 mL). Irrigation water filled with fertilizers then applied at every 0800 h to 1000 h/day. The experiment was arranged in a randomized complete block design (RCBD) with three replicates. Two plants represented as one experimental unit. The treatments were (i) coco peat alone, (CP, 3 kg), (ii) coco peat + 150 g Biochar (CP+BC), (iii) coco peat + Oil Palm Fruit Bunch (OPFB) (1:1) (CP+OP), (iv) OPFB alone (3 kg), (v) OPFB + 150 g Biochar (OP+BC), and (vi) coco peat + OPFB + 150 g Biochar (CP+OP+BC). All plants experienced similar cultural practices such as irrigation and pesticide spray. The experiment

duration was 114 days (November 20, 2012 – March 14, 2013). Stem diameter was recorded at weekly basis (0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 and 77 days after transplanting, (DAT). Meanwhile, postharvest parameters assessed at harvest were fruit skin colour (lightness (L*), chromaticity value a* and b*, hue angle and chroma), fruit firmness, soluble solids concentration (SSC) and titratable acidity (TA). The data were subjected to the analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

Yield of various vegetables such as cucumber, tomato, and lettuce (Zabiholah et al., 2013) tend to be higher when grown in mixing soilless media compared to those grown in the soil as reported by Olle et al. (2012). A variety of combination growth media has been studied which include soil, peat, perlite and sand alone with promising outcomes mainly in improving fruit quality attributes. In the present study, stem diameter, fresh weight, number of fruits, fruit colour (except for chromaticity value a*) exhibited a significant influence of different growing media applied but firmness, SSC also TA were not (data not included) (Figures 1, 2, 3, 4, 5, 6, 7 and 8). It was noticeable that the production of cherry tomato grown in CP, CP+BC and CP+OP was the same based on number of fruits (104 fruits). However, tomatoes grown in CP resulted in slightly higher fresh weight (562 g) as compared to CP+BC and CP+OP (529 g and 521 g, respectively). A comparable fresh weight of CP+OP and CP+BC to CP could be the possible reason to replace the used of CP alone. Besides, the combination of CP+OP and CP+BC may reduce the waste from oil palm and rice industry also helps to preserve the nature. The current price of CP for 1 tonne was ranged from RM 500 to RM 1000 meanwhile the price for OPFB was only RM 100 per tonne (Sabran, 2013; Mohd. Asri, Pers commn., 2013). The combinations of CP+OP or CP+BC are more economical based on the price of CP and also the promising results of the growth and yield of cherry tomato. Increased in stem diameter, fruit number and fresh weight of cherry tomato treated with CP+OP and CP+BC maybe associated to the changes in soil structure, soil organic content also aeration capacity (Wira et al., 2011). In addition, Wira et al. (2011) claimed that the fruit quality and plant growth were improved in combination of OP+CP as CP triggered OPFB properties to increase plant nutrients availability, pH and cation exchange capacity (CEC). This combination also resulting better growth and higher yield of cauliflower and Pak Choy compared to CP alone as reported by Ismail et al. (2004). Ghehsareh et al. (2011) reported that combination of CP+OP was the best growth medium in enhancing the fruit diameter, fresh weight and SSC of rock melon var Waka Natsu 1. Meanwhile, Zabiholah et al. (2013) claimed that combination of CP and other growing media had better results for tomato transplant production than other combinations of growing media and soil cultivation. Hochmuth et al. (1998) reported that perlite and CP had a positive correlation in increasing the yield components of strawberry.

In the present study, the application of CP and OPFB alone resulted in a small stem diameter, low number of fruits and fresh weight of cherry tomato also fruit colour development. Possibly, the growth of fungus on OPFB may cause the reduction of above mentioned parameters. Asiah et al., (2004) claimed that the growth of these fungi caused the OPFB to harden hence reduce nutrients and water uptake of the plant. In addition, Zabiholah et al. (2013) reported that palm media is not suitable for tomato transplant as it resulted in lowest root fresh weight and yield of tomato. Thus, CP and OPFB application will be more effective if it combines with other growth media. Mami et al. (2008) claimed that BC can replace peat to increase the production of tomato. Fruit colour development in cherry tomato was enhanced with the application of different soilless growing media. All treatments exhibited red colour pigmentation of cherry tomato (Figures 4, 5, 6, 7 and 8). Skin colour of cherry tomato plants treated with CP, CP+OP and CP+BC showed lower chromaticity b*, h° and L* which indicates redder skin colour. Similarly, Luoto (1984) claimed that tomatoes grown in peat based had redder skin colour, softer and tastier. Possibly, the increased of red skin colour in tomato may be attributed to the increased of lycopene concentrations. In addition, the degradation of chlorophyll will induce chloroplasts change to chromaplasts which indicates red skin colour of tomato (Frazer et al.,

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

1994). In the present study, combination of CP+OP and CP+BC resulted in the highest C*, lower h° and bigger stem diameter compared to CP. Although no apparent effect was recorded in SSC, TA and fruit firmness, both combinations tend to have the best postharvest quality of lowland cherry tomato. Among all treatments, combination of CP+OP and CP+BC were promising to replace current soilless growing medium in term of stem diameter, number and fresh weight as well as skin colour.

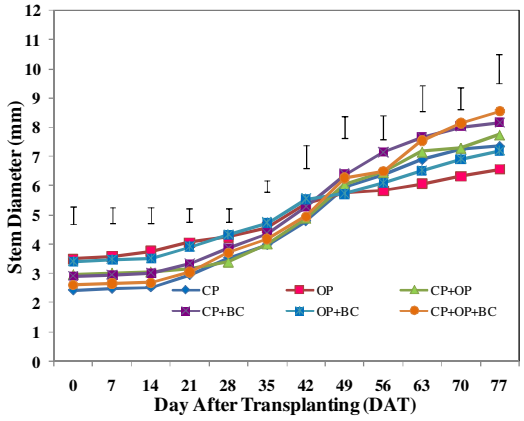

Figure 1. Effect of different soilless growing media on stem diameter of cherry tomato. Vertical bars represent LSD (P≤0.05)

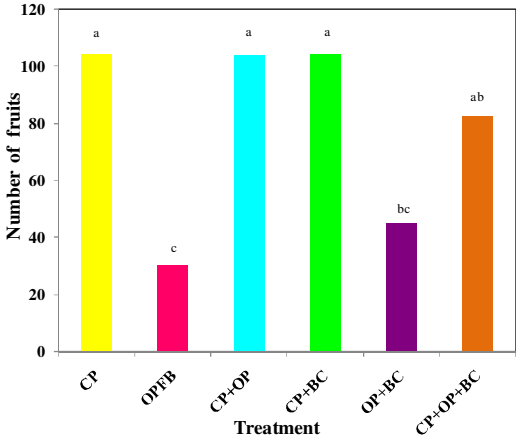

Figure 2. Effect of different soilless growing media on numbers of cherry tomato. Means with the same letter are not significantly different (LSD, P≤0.05)

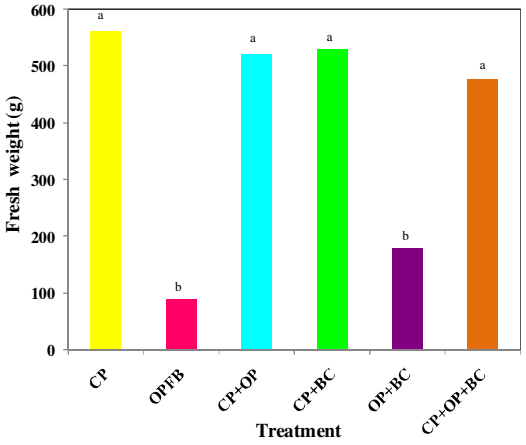

Figure 3. Effect of different soilless growing media on fresh weight of cherry tomato. Means with the same letter are not significantly different (LSD, P≤0.05)

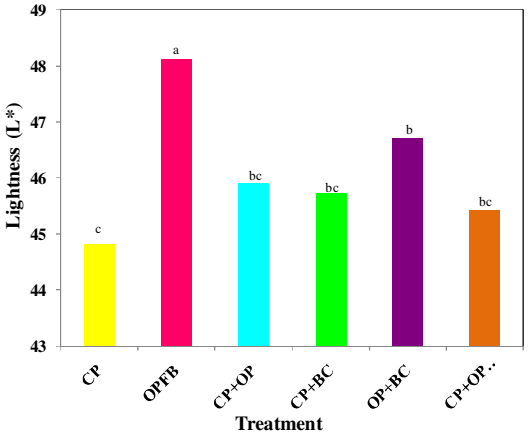

Figure 4. Effect of different soilless growing media on lightness (L*) of cherry tomato. Means with the same letter are not significantly different (LSD, P≤0.05)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

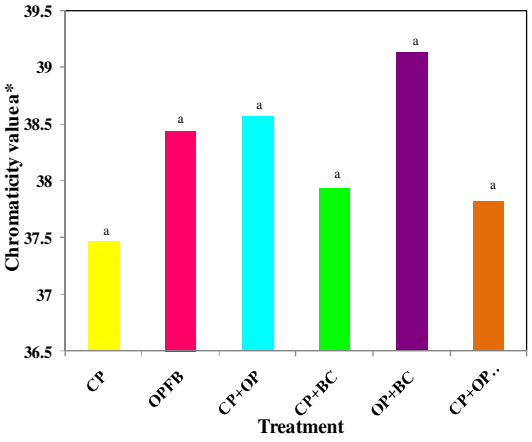

Figure 5. Effect of different soilless growing media on a* value of cherry tomato. Means with the same letter are not significantly different (LSD, P ≤0.05)

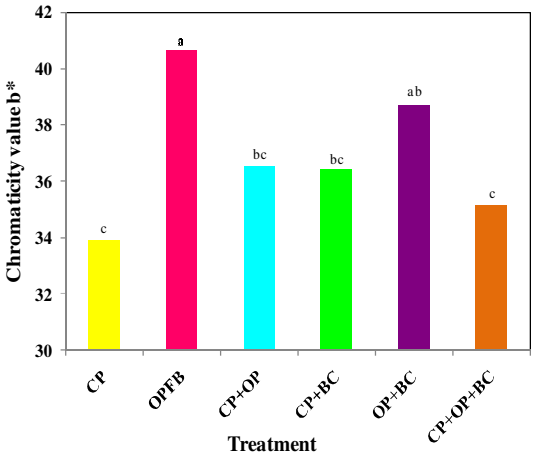

Figure 6. Effect of different soilless growing media on b* value of cherry tomato. Means with the same letter are not significantly different (LSD, P ≤0.05)

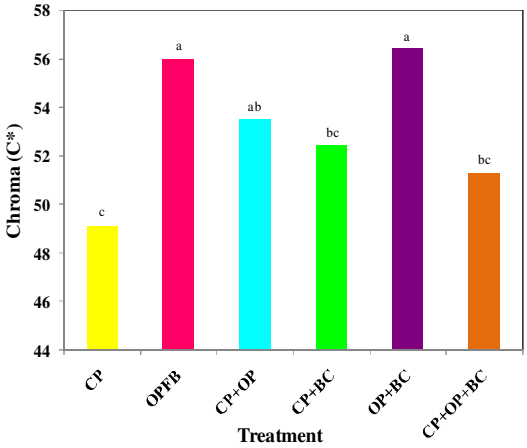

Figure 7. Effect of different soilless growing media on chroma (C*) of cherry tomato. Means with the same letter are not significantly different (LSD, P ≤0.05)

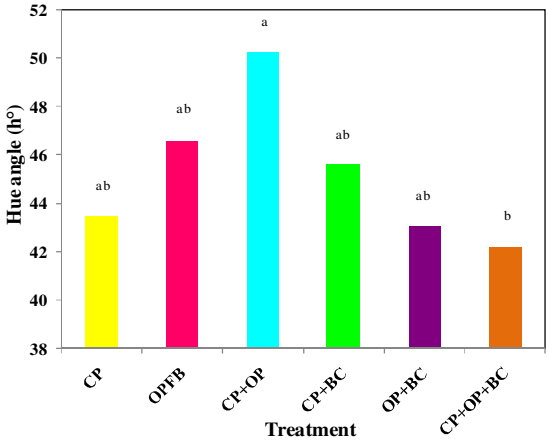

Figure 8. Effect of different soilless growing media on hue angle (h°) of cherry tomato. Means with the same letter are not significantly different (LSD, P ≤0.05)

Conclusions

Newly-developed growth media such as CP+OP and CP+BC increased growth and yield without adversely affecting postharvest quality of lowland cherry tomato. Both combinations have the potential to replace current soilless growing media based on the promising outcomes also low cost of substrate.

Acknowledgements

We thank Department of Agriculture, Setiu and Kilang Beras Bernas, Tumpat for providing Oil Palm Fruit Bunch (OPFB) and Biochar, respectively as a gift sample for the experiment.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Postharvest Performance and Physico-Chemical Changes of Two Cashew Apple (*Anacardium occidentale* L.) Cultivars at Different Maturation Stages

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Introduction

Cashew apple (*Anarcadium occidentale* L.) is a pseudo-fruit that thought to be native to the Northen part of South Africa, including Brazil and the West Indies. The cashew apple is one of the tropical fruits most highly consumed and an extremely important agricultural trade product for Brazil. The cashew tree is well established in many tropical regions, including Malaysia and India (Maia et al., 2000). In 1972, the cashew cultivated area in Malaysia particularly in Terengganu, corresponds to approximately 142,000 hectares (ha) (Noor, 1972). However, the planted area of cashew gradually decreased due to the lack of technology and knowledge in commercializing it into food products. In 1982, a total of more than 3,600 ha were under cashew; 2,400 ha at Besut and the other 1,200 ha at Kuantan (Zambri et al., 1982). However, in 2010, the planted area has decreased sharply to 5 ha (Pers. comm., 2010) and the crops have been abandoned and neglected by the farmers. Cashew can grow successfully in Terengganu and that the trees are particularly adaptable to the infertile sandy Beach Ridges Interspersed with Swales (BRIS) soil. Botanically, cashew apples planted in Terengganu are not considered established cultivars, but it can be classified according to form, rounded or elongated and colour, red or yellow. Two cultivars of cashew apples were recorded in Terengganu based on their skin colour namely red and yellow cultivars.

In Brazil, cashew apple has been consumed as fresh fruits and being developed, and exploited into commercial products such as juice, beverages and others (Paiva et al., 2000). Cashew apple juice has been reported to have antitumor (Cavalcante et al., 2005), urease inhibitory (Kubo et al., 1999) and lipoxygenase activity (Ha and Kubo, 2005). Moreover, the cashew also contributes to human health and nutrition by supplying vitamin C, four and ten times higher compared to orange juice (Menezes and Alves (1995) and pineapple (Ohler, 1988), respectively. In Malaysia, the cashew apple is still underutilized even it is known to contain high polyphenolic compounds such as tannin which acts as anti-mutagenic (Cavalcante et al., 2005), anti-microbial and anti-carcinogenic (Kozubek et al., 2001). No work, however, has been reported on the two cultivars of cashew apple from Malaysia particularly in Terengganu. Therefore, identification and quantification of important bioactive compounds in both cashew apple cultivars warrant further investigation.

Cashew apple like any other climacteric fruits undergoes a variety of physical and chemical changes before and after harvest. The stage at which the fruit gets ripe at harvest period is an indication of its final quality (Wills et al., 1989). Filgueiras et al. (1999) reported that there are several indices that can be used to determine the optimal harvest stage of cashew apple, notably colour, firmness, composition and specific gravity. However, cashew can be harvested when apple is fully developed, firm, without any shade of green colour and easily detachable from the plant where flavour, aroma and sugar concentrations are maximum and acidity and astringency are minimum (De Figueiredo et al., 2002). With respect to the highly potential to be commercialized and its beneficial effects to human, it is crucial to revitalize the cashew apple as a commercial crop. Therefore, the objectives of this present study were (i) to explicate the changes of physico-chemical characteristics of two cashew apple cultivars at seven maturation stages, and (ii) to provide information for future research.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Materials and Methods

Two cashew apple cultivars, red and yellow were harvested at two different locations i.e. Department of Agriculture and Kg. Saujana, Setiu, Terengganu. The flower bloom of the cashew was observed from February 2012 to May 2012. Seven different maturation stages of cashew apple were recorded based on the external colour. For red cultivar, the maturity stages were determined as according to de Filgueiredo et al., (2002). Meanwhile, for yellow cultivar, the maturity indices were based on; stage (1) green with green nut; stage (2) green with ripe dry nut; stage (3) light green; stage (4) yellow colour started; stage (5) pale yellow; stage (6) light yellow and stage (7) deep yellow. Red and yellow cashew apples which free from decay were chosen for the experiment. The experiment was laid out by following a complete randomized design with three replications. Fifteen fruit per replicate were used for postharvest performance following harvest such as fruit colour (lightness (L*), hue angle (h°), chromaticity value a* and b*, and chroma), fruit firmness, titratable acidity (TA) and soluble solids concentration (SSC) (Wan Zaliha, 2009). Meanwhile for physical characteristics include length, diameter and fresh weight (whole cashew and apple alone). The data were subjected to two-way analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

The growth and physico-chemical properties of cashew apple which includes whole weight (apple+kernel), whole length (apple+kernel) and firmness were significantly affected by the interaction between cultivars and stages, but cashew apple weight without kernel, diameter and length (without kernel) were not (Table 1). Both cashew cultivars showed an increased trend in fruit weight and length as growth and development progressed. This trend is in line with other cashew cultivars such as early dwarf cashew clone CCP-76 in Brazil. As expected, whole weight and length of cashew increased with advancing maturity in both cultivars and nearly showed a slight sigmoid growth pattern. For red cultivar, whole weight and length increased from 23.5 to 86.2 g and 68.2 to 79.6 mm respectively, while for yellow cultivar, increased from 17.3 to 66.0 g and 62.3 to 75.7 mm, respectively (Table 1). Red cultivar had a bigger size than yellow. Both cashew cultivars had the same trend in fruit firmness. The fruit firmness decreased as the ripening stage advanced. Red and yellow pulp of cashew experienced a tremendous decline in fruit firmness at stage 4 (59 and 30% decreased from stage 3 respectively). Possibly, the fruit softening in both cultivars may be attributed to the starch degradation and action of pectinolytic enzyme (De Filgueiredo, 2002).

Fruit colour indices such as chromaticity value a*, b*, chroma and h° significantly affected by the interaction between factors except for L* (Table 1). In general, both cultivars had an increasing value of chromaticity a* and b* as fruit ripening progressed. The a* values of red cultivar were higher than yellow which indicate redder skin colour. The greatest change in a* in red cultivar was recorded at stage 5 (19.1). In contrast, h° of both cultivars showed a decreasing trend with advancing maturation, as the fruit changed from green to red colour. Decreased h° in red cultivar may be due to the accumulation of anthocyanins. However, the decreasing of h° in yellow cultivar may be attributed to the degradation of chlorophylls to carotenoids (yellow-orange). This was in agreement with Filgueiras et al. (1999). He reported that the changes in colour of cashew apple during maturation were due to the chlorophyll loss as well as synthesis of other pigments. No specific trend can be deduced for C* in both cultivars as the values fluctuated throughout the fruit development observed. The red cultivar had darker colour as the lower values of L* (ranged from 47.6 to 63.1 L*) were observed which coincide with the increased of a*. On the other hand, yellow cultivar resulted in higher values of L* (ranged between 56.2 and 75.3 L*) which is lighter and indicates yellow-orange skin colour.

The SSC, pH and TA in both cashew cultivars were significantly affected by the interaction between cultivar and stage (Table 2). In general, both cultivars had a decreasing value of SSC and TA as the

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

maturity advanced. In contrast, a decreasing trend was observed for pH. The increase in pH was paralled by a decrease in TA due to the lost of malic acid. Yahaya et al. (2010) explained that the acidity level decreased as a result of ripening process. The yellow cultivar was more acid and less sweet than the red. For red cultivar, the SSC and TA ranged from 14.1 to 11.4 % and 1.04 to 0.21 % malic acid, respectively. However for yellow cultivar, the SSC and % malic acid ranged from 13.5 to 9.9 and 0.58 to 0.14 %, lower than red. Contrarily, the pH yellow cultivar resulted in higher values than red cultivar. Moura, (1998) reported that the SSC in clone CCP-76 increased as the ripening progressed. This was contradicting with our finding. Probably, the decreased in SSC due to the changes of some chemical in the fruit as the ripening progressed. This variation also may be ascribed to several factors such as seasons of harvesting (Moura, 1998) and environmental differences (soil, temperature and solar intensity) and that can affect nutrient values (Assunção and Mercadante, 2003).

Table 1. Physical change of two cashew apple cultivars during development and maturation.

Cultivar	Stage	Whole Weight (g)	Apple Weight (g)	Diameter (mm)	Length (mm)	Apple Length (mm)	Firmness (N)	Lightness (L*)	a*	b*	Hue (h°)	Chroma
Red	1	23.5 f A	14.4 g A	27.6 f A	68.2 f A	37.4 f A	23.2 a B	47.6 e B	-15.1 e A	29.6 d B	116.9 a A	62.0 ab A
	2	30.0 g A	22.4 f A	33.5 e A	68.9 ef A	41.8 e A	13.5 b B	56.2 d B	-15.0 e A	38.0 c B	111.6 b A	71.1 a A
	3	41.3 e A	33.8 e A	39.6 d A	71.0 de A	45.9 d A	10.4 c B	60.7 c B	-6.9 d A	43.5 b B	98.7 c B	66.2 ab A
	4	51.1 d A	43.4 d A	44.2 c A	73.7 bc A	49.5 c A	4.2 d B	62.3 c B	9.9 c A	44.1 b B	76.8 d B	60.2 ab A
	5	60.6 c A	53.2 c A	48.3 b A	72.9 cd A	49.9 bc A	4.9 d A	66.5 a A	19.1 c A	49.4 a B	68.6 e B	54.6 b B
	6	71.1 b A	63.5 b A	50.9 ab A	75.5 b A	52.0 b A	5.4 d A	65.5 ab B	24.7 a A	48.8 a B	63.3 f B	55.4 ab B
	7	86.2 a A	78.7 a	53.2 a A	79.6 a A	55.4 a A	5.2 d A	63.1 B	27.2 a A	45.7 b B	59.2 g B	53.3 b A
	Mean	52.0	44.2	42.5	72.8	47.4	9.5	60.3	6.3	42.7	85.0	64.0
Yellow	1	17.3 f B	10.1 c B	24.7 e B	62.3 d B	34.5 e B	27.8 a A	56.2 c A	-16.4 f B	36.7 e A	114.2 a B	72.2 a A
	2	23.1 e B	17.4 bc B	30.6 d B	64.7 d B	40.5 d A	16.6 b A	66.0 b A	-16.8 f A	45.3 d A	110.5 b A	48.4 f B
	3	31.6 d B	41.2 a A	35.5 c B	68.2 c A	45.2 c A	12.6 c A	73.6 a A	-13.5 e B	51.8 c A	104.7 c A	53.6 e B
	4	44.3 c A	38.9 ab A	41.2 b A	72.2 b A	50.5 b A	8.7 d A	77.9 a A	-7.8 d B	57.2 b A	97.7 d A	57.8 cd B
	5	51.1 b B	42.9 a A	42.9 b B	73.1 ab A	51.3 b A	7.6 de B	76.6 a A	-5.3 c B	58.6 ab A	95.2 e A	58.9 bc A
	6	63.5 a A	59.3 a A	48.1 a A	74.4 ab A	52.5 ab A	6.2 e A	75.5 a A	-2.5 b B	59.6 a A	92.4 f A	59.7 b A
	7	66.0 a B		47.9 a B	75.7 a B	54.3 a A	4.0 f A	75.3a A	0.6 a B	56.7 b A	89.4 g A	56.8 d A
	Mean	42.4	35.0	38.7	70.1	47.0	11.9	71.8	-8.8	52.3	100.6	58.2
LSD ($P \leq 0.05$)												
Cultivar (C)		2.00	5.71	0.91	0.91	0.83	0.62	1.63	0.83	0.81	1.08	4.11
Stage (S)		3.74	14.5	1.70	1.70	1.54	1.16	3.04	1.55	1.52	2.01	7.69
C x S		5.28	NS(5.00)	NS (0.83)	2.40	NS(0.75)	1.63	NS (1.49)	2.20	2.15	2.85	10.87

Means followed by the same letter within the column in capital letter are not significantly different between the cultivar and in small letter within the column are not significantly different between stages (LSD, P≤0.05). Values within the brackets represent standard error of means. NS = not significant.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 2. Chemical changes of two cashew apple cultivars at seven maturity stages.

Cultivar	Stage	SSC	pH	TA (%)
Red	1	14.1 a A	3.9 d B	1.04 a A
	2	13.2 b A	3.9 d B	0.81 b B
	3	11.5 c A	3.9 d B	0.70 b A
	4	11.5 c A	4.0 d B	0.55 c A
	5	11.3 c B	4.3 c B	0.47 c A
	6	11.0 c B	4.8 b B	0.33 d A
	7	11.4 c A	5.0 a B	0.21 d A
	Mean	12.0	4.2	0.6
Yellow	1	13.5 a A	4.0 g A	0.58 b B
	2	10.9 b B	4.1 f A	1.01 a A
	3	9.9 e B	4.1 e A	0.33 c B
	4	10.0 d B	4.4 d A	0.26 d B
	5	10.3 c B	4.5 c A	0.21 de B
	6	9.9 e B	5.0 b A	0.17 ef B
	7	9.9 e B	5.6 aA	0.14 f B
	Mean	10.6	4.5	0.4
LSD (P ≤0.05)				
Cultivar (C)		0.21	0.05	0.04
Stage (S)		0.40	0.09	0.07
C x S		0.57	0.12	0.10

Means followed by the same letter within the column in capital letter are not significantly different between the cultivar and in small letter within the column are not significantly different between stages (LSD, P≤0.05). Values within the brackets represent standard error of means. NS = not significant (P≥0.05).

Conclusions

The variation in fruit morphology and physicochemical properties of the two cashew cultivars was noticeable. The red cultivar had better physiological and biochemical attributes than the yellow. Information on maturity indices such as fruit colour, firmness, SSC and TA contribute to better understanding of physiological maturity status of both cultivars, and hence provide the valuable information to postharvest handling and future research as well as for commercialize it into industrial products.

Acknowledgements

The authors wish to thank Universiti Malaysia Terengganu and Ministry of Higher Education for the grant provided under Fundamental Research Grant Scheme (FRGS) (59227).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The Effects of Partial Rootzone Drying on the Yield, Growth and Postharvest Performance of Roselle (*Hibiscus sabdariffa* L.) Grown on BRIS Soil in Lysimeter

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Introduction

Roselle (*Hibiscus sabdariffa* L.) is a vegetable that belongs to the family of Malvaceae. It is an annual shrub crop which is relatively a new plant in Malaysia. Roselle is cultivated for its succulent calyces, leaves and young shoots which are eaten either raw or as cooked vegetables. Roselle once had a wide and vigorous activity of plantation in Malaysia around 1990's. Due to low market demand, roselle plantation was slowed down and most farmers gave up planting roselle and switched to other crops. Terengganu was once the top roselle producer in Malaysia which the area of plantation covered around 12.8 ha in 1993 and increased drastically to 506 ha in 2000. However, in 2003, the cultivated area was less than 150 ha. Department of Agriculture (2009) claimed that within years of 2005 until 2010, the cultivated area covered was decreasing year by year by 40 ha with declining roselle production from 714 metric tons (mt) in year 2006 to 300 mt in 2010. Even though, in the last three years (2006-2010), it showed that both acreage and production of roselle were gradually increasing indicating roselle demand was rising in the state of Terengganu. In fact, the demand was higher than the supply even until at these days. Roselle can be planted in most type of soils as well as in Beach Ridges Interspersed with Swales (BRIS) soil which has high porosity and low water holding capacity. Hence, to maximize roselle production in Terengganu which consists of BRIS soil largely, amount of water irrigated must be determined precisely so that no water wastage will occur without affecting growth, yield and postharvest performance of roselle.

Partial rootzone drying (PRD) is one of the deficit irrigation (DI) methods together with water withholding and regulated deficit irrigation (RDI). PRD requires the frequent irrigation of approximately half of the root system while the other half is left to dry. After a certain period of time the 'wet' and 'dry' zones are alternated, allowing the former 'wet' zone to dry while the 'dry' zone is irrigated (Dry et al., 2000). By letting the crop to water stress, this will trigger the root-to-shoot chemical signal by secreting abscisic acid (ABA) which plays an important role in adapting crops to stresses. Secreting of ABA will then trigger the closing of stomata to reduce transpiration, stimulate root growth and apical dominance. Hence, it is believed that PRD is able to increase fruit quality. Besides, PRD is also proven its ability to saving water by optimizing water use efficiency. Water supply is limited worldwide and there is an urgent need to identify and adopt effective irrigation management series. More than 83% of the world water is used for agricultural irrigation. Therefore, adoption of PRD could make substantial contribution to saving water. The levels implied is the different amount of water that irrigated to see what and which level does best for postharvest quality and the growth of the plant. Hence, amount of water that a plant needs can be estimated and saving water can be implemented by supplying only the amount of water needed to the plant.

Materials and Methods

Twenty-four roselle plants variety Terengganu (UMKL-1) were grown in controlled environment of Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu. The BRIS soil was taken from Department of Commodities Centre located at Rhu Tapai, Setiu, Terengganu and roselle seeds were purchased from Department of Agriculture, Kuala Berang, Terengganu. Twenty-four lysimeters were constructed using polyvinyl chloride (PVC) cylinder (71 cm x 21 cm). A special drainage hollow was made below each lysimeter. The lysimeters then set up on a wooden rack with

distance between each lysimeter was 30 cm. All lysimeters were then divided into two parts using plastic cardboards placed vertically inside them before 30 kg BRIS soil filled inside each lysimeter. Roselle seeds were sowed at the greenhouse on 25th October 2012. Within 14 days, the seedlings were transferred into lysimeter. All experimental trees received similar cultural practices including fertilization, pesticides and fungicides application excluding irrigation water.

The experiment was arranged in a randomized complete block design (RCBD) with treatment comprising of four different regimes of irrigation, i) control (100% full irrigation), ii) 20% PRD (80% irrigation), iii) 40% PRD (60% irrigation) and iv) 60% PRD (40% irrigation) with three replications. Two plants represented one experimental unit. Drippers with flow rate of 8 L·ha were used for irrigation. Irrigation was applied twice a day at 0830-0900 hours and 1630-1700 hours. All irrigation treatments were applied alternately (wet and dry) for every 7-day intervals. The irrigation was imposed for 84 days after transplanting. Full irrigation or control was 2.15 L per day, applied based on evapotranspiration calculation: $Et_c=ET_o$ x K_c; where ET_c=crop evapotranspiration, ET_o=reference crop evapotranspiration and K_c= crop coefficient. Average Kc was taken as 1.2 from a generalized Kc chart by Doorenbos and Pruit (1977). Average ET_o was 1.99mm/day (Niazuddin, 2007). The ET_o was calculated from Penman-Monteith equation as according to Allen et al. (1998). The preharvest parameters evaluated were plant height, stem diameter and number of branches. Meanwhile, postharvest parameters were calyx fresh weight, colour (lightness (L*), chromaticity value a*, chromaticity value b* chroma (C*) and hue angle (h°)) and firmness, number of calyx, titratable acidity (TA), and soluble solids concentration (SSC). The data were subjected to the analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

As expected, the application of different irrigation regimes on roselle plants did not significantly influence the growth performance and also the post harvest quality of roselle calyx (Figure 1, 2 and 3). However, 20% PRD treated plants tend to have higher and bigger size of plants compared to other treatments. Similarly, Nur Razlin et al. (2013) reported that no apparent effect was recorded on the growth of roselle grown on BRIS soil. In addition, Babatunde and Mofoke (2006) also reported that the plant height and number of branches per roselle plant was not significantly affected by irrigation schedules. Possibly, a moderate stress of irrigation water may have masked the effects on hydraulic status of soil and leaf of roselle trees which resulted in similar to control trees.

Fresh weight and calyx number of roselle were significantly affected by water deficit imposed (Figure 4, 5 and Figure 6). This was in agreement with the report by Babatunde and Mofoke (2006) and El-Boraie et al. (2009). However, in the present study, 20% PRD showed comparable results to control when it resulted in only 18.54% and 9.09% reduction in fresh weight and number of calyx in roselle which was not adversely affecting the production. Low fresh weight value in 60% PRD was an indicator to the reduced fruit size and so the weight as a result from water stresses in action with increase peroxidase activity which in turn restricting fruit growth.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

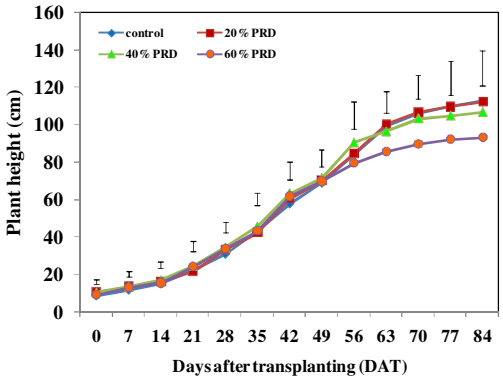

Figure 1. Effects of PRD on the plant height of roselle grown on BRIS soil. The vertical bars=LSD at P≤0.05

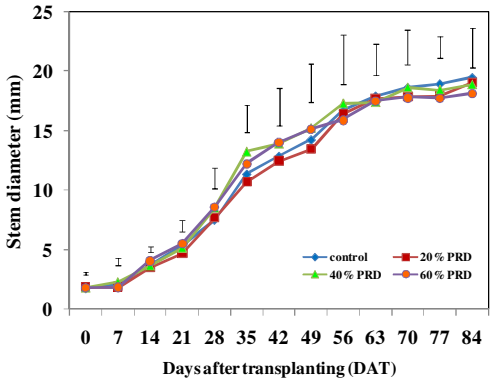

Figure 2. Effects of PRD on the stem diameter of roselle grown on BRIS soil. The vertical bars=LSD at P≤0.05

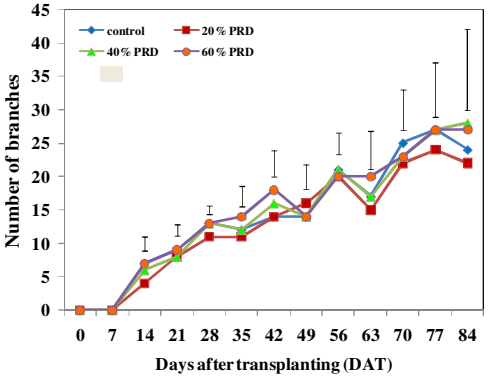

Figure 3. Effects of PRD on the number of branches of roselle grown on BRIS soil. The vertical bars=LSD at P≤0.05

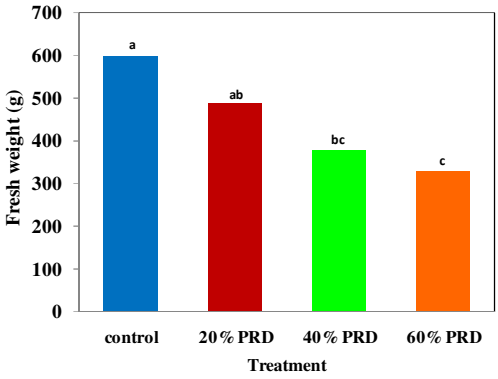

Figure 4. Effects of PRD on the fresh weight of roselle grown on BRIS soil. Means followed by the same letter are not significantly different (LSD, P≤0.05)

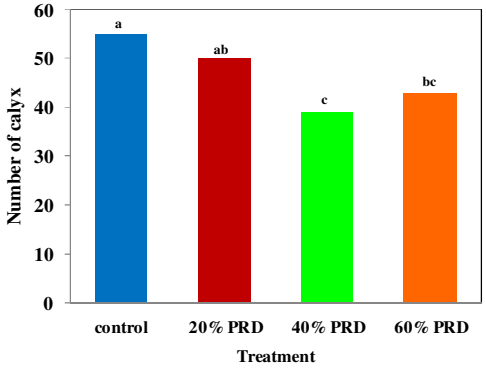

Figure 5. Effects of PRD on the number of calyx of roselle grown on BRIS soil. Means followed by the same letter are not significantly different (LSD, P≤0.05)

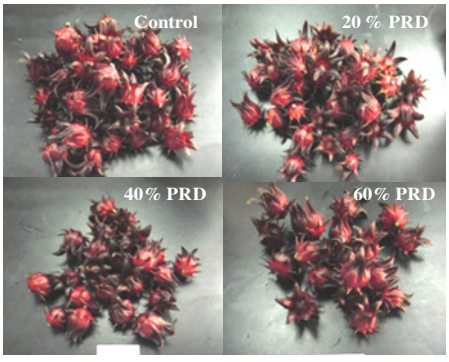

Figure 6. Yield of roselle

For postharvest quality of roselle under PRD treatments, SSC and calyx firmness, both were significant whereas calyx colour exclude a* value and TA were not (Figure 7, 8 and 9, some data not included). All PRD plants resulted in higher SSC value ranged from 6.67 to 7.55% which was more than 50% higher than normal SSC reported by Wong et al. (2002). Similar findings were also reported by Mandour et al. (1979) and El-Boraie et al. (2009). Increased SSC in roselle calyx may be due to higher starch to sugar conversion under water deficit condition (Zegbe-Dominguez et al., 2003). Meanwhile, in calyx firmness, water potential related to cell turgidity causing it to significantly affect by the water stress applied. Control plants had the highest value of firmness (4.18 N) because of high water potential that lead to turgidity of cells which needed high force to break into the cells. The results showed that lower water irrigated lower calyx firmness (Figure 8). Contrarily, Mpelasoka et al. (1997) reported that fruit firmness is influenced by fruit size which smaller fruits generally being firmer than bigger fruits due to higher cellular activity. In the present study, PRD treated plants tend to have smaller calyx size than control. Wan Zaliha and Singh (2010) also concluded that firmer fruits in RDI might be associated with the reduction in cellular hydration and increased flesh compactness that would cause source limitation of photosynthesis due to lower stomatal conductance in water deficit plants.

Meanwhile, all PRD plants had higher TA (ranged between 4.9 and 5.2) than control plants (Figure 9). Similar trend was also reported by Mills et al. (1994). Possibly, the higher TA recorded in water deficit plants may be ascribed to the higher concentration of acid contents such as tartaric, fumaric and succinic acid which an indicator to the contribution of organic acids to the fruit osmotic adjustments (Wan Zaliha and Singh, 2010). Colour of roselle showed no significant difference in PRD treated roselle except for a* value (data not included). Similarly, Nur Razlin et al., (2013) claimed that application of PRD did not affect calyx colour in terms of L*, chromaticity values a* and b*. Possibly, excess or lack of water supply during the vegetative growth and developmental stage of some plants decreased the chlorophyll and carotenoids while medium supply of irrigation water increased the pigmentation in the leaf and other organs (Mandour et al., 1979).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

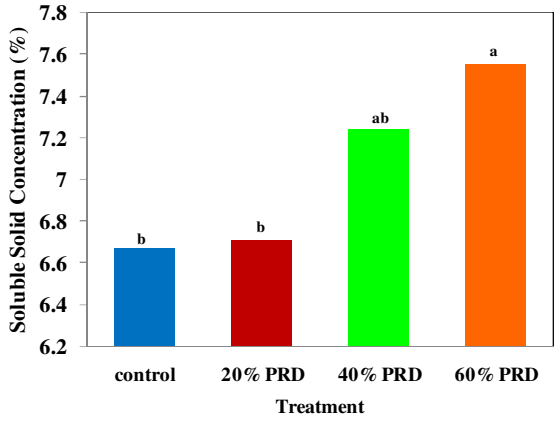

Figure 7. Effects of PRD on the SSC of roselle grown on BRIS soil. Means followed by the same letter are not significantly different (LSD, P≤0.05)

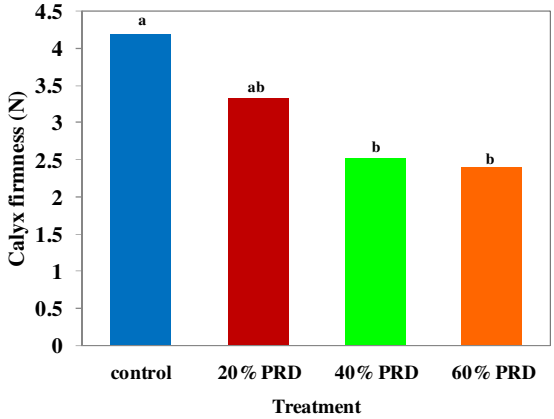

Figure 8. Effects of PRD on the calyx firmness of roselle grown on BRIS soil. Means followed by the same letter are not significantly different (LSD, P≤0.05)

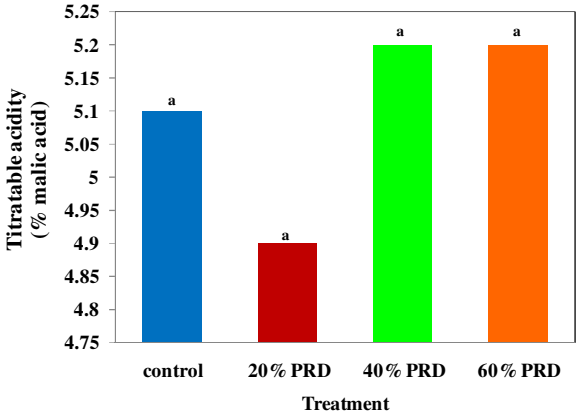

Figure 9. Effects of PRD on the TA of roselle grown on BRIS soil. Means followed by the same letter are not significantly different (LSD, P≤0.05).

Conclusions

The application of mild water stress, 20% PRD had higher SSC and calyx firmness of roselle grown on BRIS soil without giving significant reduction in yield (fresh weight and number of calyx). In addition, irrigation water reduced up to 20% (80% irrigation), increased water use efficiency and maintained other quality attributes such as growth (plant height, stem diameter and number of branches), titratable acidity, and colour. The 20% PRD could be the best irrigation scheduling for roselle plantation on BRIS soil as it increased water use efficiency and profit for local farmers. The exact amount of water to be applied is 19 m^3/ha or 1.72 L/tree/day.

Acknowledgements

The authors wish to thank Universiti Malaysia Terengganu and Ministry of Education Malaysia for the grant provided under Exploratory Research Grant Scheme (ERGS) (55054).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effects of Different Ethylene Removals on Berangan Banana (*Musa* sp. AAA Berangan)

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Introduction

Banana, a climacteric fruit belongs to the family of Musaceae is the most widely consumed fruits in subtropical and tropical regions due to its taste and aroma. The ripening of climacteric fruit is divided into four important phases i.e. a pre-climacteric minimum, a climacteric rise, a climacteric peak and a post-climacteric. A sudden increase in both ethylene synthesis and respiration rate occurs during the first stage of ripening. Being a climacteric fruit, banana produces enough ethylene bringing about speedy changes in physico-chemical characteristics, including colour, texture, aroma, chemical composition, respiration rate and senescence. Ethylene (C_2H_4), a colourless gas, is the main regulator of ripening in climacteric fruits which leads to the short marketable life and increase postharvest losses. Banana has been reported to have a short market life, an average of 1 to 10 days which subjected to serious postharvest losses. These losses can be reduced by adopting various postharvest management practices that are currently in practice all over the world. One of the practices in reducing post production losses in banana industry is the application of ethylene removal. One of the most effective ethylene removals is potassium permanganate ($KMnO_4$) which is widely and commercially used in reducing ethylene production in various climacteric fruits, including banana. However, due to the chemical properties of $KMnO_4$, precautions must be taken to prevent contamination of food products and also its waste disposal. Thus, other materials with ethylene removal capability such as zeolite and activated charcoal should be explored to replace current ethylene removal. Zeolite (Zeo) is crystalline aluminoslicate mineral with ion-exchange and gas-adsorption properties (Bernal and Lopez, 1992). While, activated charcoal (AC) is a non-graphitic form of black carbonaceous material with microporous structure, high adsorption capacity, and high degree of surface reactivity (Smisek and Cerney, 1970; Williams and Reed, 2006). Based on the properties above, Zeo and AC which are both unique and versatile adsorbents have been widely applied in food and chemical industries, wastewater treatment, solvent recovery and air pollution control. Therefore, this study aims to evaluate the effects of different types of ethylene adsorbents/removals in reducing ethylene production and delaying ripening of Berangan banana.

Materials and Methods

Berangan bananas (*Musa* sp. AAA Berangan) at maturity stage 1 were purchased from local farmers at Kuala Terengganu. The uniform fruits in weight and size were sorted from defects and decay. The fruits then immediately transferred to Postharvest Technology laboratory, Faculty of Agrotechnology and Food Science, University Malaysia Terengganu. The experiment was arranged in Complete Randomized Design (CRD) with three replications. The treatments were; i) control, without ethylene removals, ii) 75 g of zeolite (Zeo), iii) 75 g of potassium permanganate, $KMnO_4$ and iv) 75 g of activated charcoal (AC). Each hand of banana contains 13 fingers. The hands then washed with sodium hypochlorite. After that, the hands were air dried at room temperature (25 ± 2 °C) before being placed in sanitized polyvinylchloride, PVC air tight containers (20 cm x 29 cm x 19 cm). The PVC containers were sanitized with 70% of alcohol to avoid fungus infection on fruit. Each hand of banana was placed in the sealed PVC container containing treatments as mentioned above. Each container also contains 4 g calcium carbide, CaC_2, to promote ripening and silica gel to absorb water. Sixty sealed PVC containers then were stored at ambient temperature of 20 ± 2 °C for twelve days.

The parameters evaluated were ethylene production (Wan Zaliha, 2009), peel colour and firmness, soluble solids concentration (SSC), titratable acidity (TA) and starch pattern index (SPI). SPI was determined as according to Dadzie and Orchard (1997). All postharvest parameters were assessed on every four day intervals i.e. 0, 4, 8 and 12 except for ethylene concentration. Ethylene concentration was measured at daily basis. The data were subjected to the analysis of variance (ANOVA) by using GLM (General Linear Models) procedures and further separated by LSD for least significance at $P \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

As shown in Figure 1, climacteric peaks of ethylene production of Berangan banana treated with different ethylene removals occurred on day 1 and 3. Berangan banana treated with Zeo, $KMnO_4$ and without ethylene removal showed the earliest occurrence of climacteric peak (day 1) followed by AC (day 3). The response in delaying the occurrence of climacteric peak was more pronounced in AC treated fruit as compared to other ethylene removals. The highest climacteric peak was recorded in control fruit (457.87 µg/mL), followed by Zeo (457.87 µg/mL), $KMnO_4$ (387.60 µg/mL) and AC (374.37 µg/mL). Although AC delayed climacteric peak of ethylene production in Berangan banana, the concentration of ethylene was the highest (ranged from 460.0 µg/mL to 388.0 µg/mL) among all treatments after day 3 till day 12. Meanwhile, the lowest ethylene concentration was recorded in $KMnO_4$ treated fruit. On day 12, the production of ethylene in banana treated with $KMnO_4$ showed the lowest amount at 55.50 µg/mL. The possible reason of AC treated fruit delayed climacteric peak of ethylene production and at the same time increased its production, might be associated to its mode of action. While, low level of ethylene production in $KMnO_4$ treated fruit following climacteric peak in ethylene production (day 2) may be attributed to the fruit entering the next stage of fruit growth, senescence or may reflect to the reduced 1-amino-cyclopropane-1-carboxylic acid synthase (ACS) and/or 1-amino-cyclopropane-1-carboxylic acid oxidase (ACO) enzymes activities. Hoffman and Yang (1980) claimed that low level of ACC in climacteric fruit is a limiting factor that regulates ethylene production. ACC starts to accumulate due to increased activity of ACS which is responsible in the conversion of ACC to ethylene.

As fruit ripen, various physical and chemical changes occurs such as changes of peel colour from green to yellow, the accumulation of starch, fruit softening and increase of soluble solids content. Even though no significant effect was recorded among ethylene treatments, at the fully ripened stage (day 12), the L*, chromaticity b*, h^o and C* values were lower in all banana fruits treated with ethylene removals (data not included). The response of Berangan banana on ethylene removals showed the ability to retard peel colour development. Possibly, retardation in skin colour development in Berangan was associated to the activities of ACS and ACO enzymes that are closely related to the production of ethylene. $KMnO_4$ showed the ability in retarding fruit colour development. Previously, Santosa et al. (2010) reported that the application of $KMnO_4$ in banana was not desirable as it tints fruit peel colour into blue. Similarly, the application of AC on banana resulted in the same conditions on the peel colour. It showed that the absorption of ethylene by AC causes changes on fruit peel colour. Watada (1986) reported that there existed a potential problem in removing ethylene in commercial storage room such as; it induces loss of green colour (chlorosis), abscission and softening. Furthermore, the application of $KMnO_4$ on Berangan banana may be due to incomplete and uneven ripening, as reported by Golding et al. (1998) and Jiang et al. (1999). Other reasons behind this, it also may be attributed to the uneven ripening of Cavendish banana due to the variation in fruit maturity which was claimed by Harris et al. (2000).

In general, postharvest quality of Berangan banana such as SSC and TA was slightly affected with the application of different ethylene removals (Figures 2 and 3), but pulp firmness was not (Figure 4). A significant different was recorded in SSC of Berangan banana treated with different ethylene removals on day 0 and 12. On day 0, AC treated fruit resulted in a higher SSC (4.8%) as compared to zeolite (3.5%). However, on day 12, fruit treated with $KMnO_4$ showed the highest SSC (24.9%) as compared to control (20.8%). The application of different ethylene removals significantly affects TA, on day 4

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

only. On day 4, Zeo treated fruit resulted in the highest TA (0.99% malic acid) as compared to AC (0.66% malic acid). The TA showed a gradual increase from day 0 to day 4 (ranged from 0.18% to 0.90% malic acid) and followed by a sharp decrease on day 12 (ranged between 0.69% to 0.36% malic acid). The decreasing pattern of TA may be attributed to the fruit ripening in progress. While, pulp firmness in all treatments showed a decreasing trend as storage period prolonged. The loss of pulp firmness was closely associated with the breakdown of starch to sugar, and cell walls or reduction in the middle lamella cohesion which causes it to become softer. For SPI, control fruit had a score of 7 where almost 95% of starch has been degraded into sugars (Figure 5). Meanwhile, $KMnO_4$, AC and Zeo treated fruits had SPI scores of 5, 6 and 6, respectively. Among all treatments, Zeo was the less effective in delaying the production of ethylene in Berangan banana. Peiser and Suslow (1998) claimed that the heated zeolite at 150 ^{o}C for 15h had the ability to absorb 94% of the ethylene added. This could be the possible reason why Zeo in the present study was not effective in ethylene absorption.

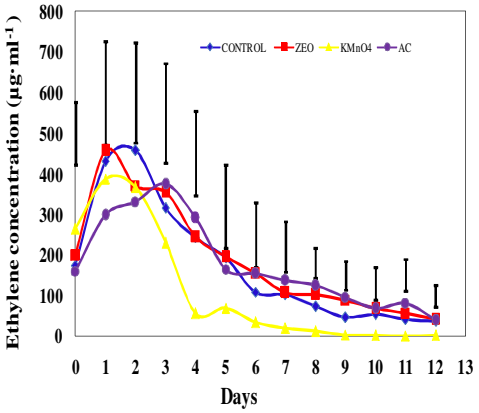

Figure 1. Effects of different ethylene removals on ethylene production of Berangan banana. Vertical bars represented LSD at ($P \leq 0.05$).

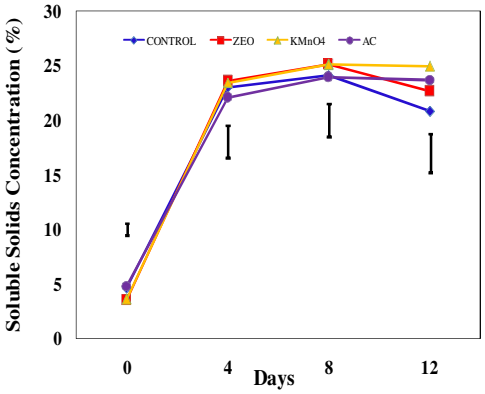

Figure 2. Effects of different ethylene removals on SSC of Berangan banana. Vertical bars represented LSD at ($P \leq 0.05$).

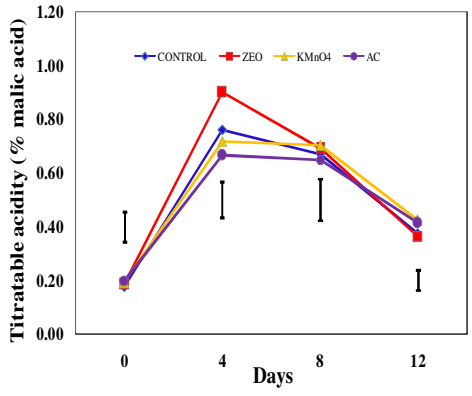

Figure 3. Effects of different ethylene removals on TA of Berangan banana. Vertical bars represented LSD at ($P \leq 0.05$).

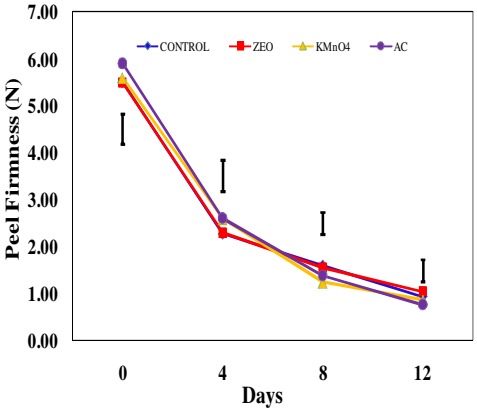

Figure 4. Effects of different ethylene removals on firmness of Berangan banana. Vertical bars represented LSD at ($P \leq 0.05$).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

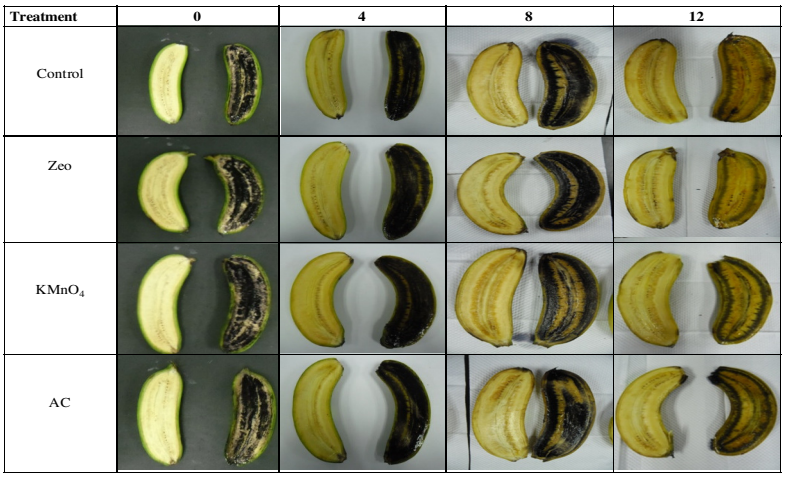

Figure 5. Effects of different ethylene removals on starch pattern in Berangan banana

Conclusions

Climacteric peak of ethylene production was suppressed by the application of ethylene removals, AC. However, the absorption of ethylene by AC was not able to retard peel colour changes and fruit softening. Meanwhile, other quality attributes (SSC and TA) were maintained throughout the 12 day experiment. In conclusion, AC and $KMnO_4$ have the ability to delay climacteric peak in ethylene production, but not the postharvest quality attributes as mentioned above. On the other hand, zeolite was less effective in delaying the ripening of Berangan banana.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Evaluation of Double-Cross Eksotika Papaya Hybrids

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Introduction

The Eksotika papaya was developed by MARDI through a backcross breeding program. This variety was released in 1987 and became Malaysia flagship papaya for export (Chan, 2004). Four years later, MARDI released an improved variety, namely Eksotika II, which had better flesh texture and longer shelf-life. Eksotika II is a hybrid between Eksotika and its sister line, Line 19. Subsequently, the double-cross method was applied to improve the yield and fruit qualities of Eksotika. The cross between two F_1 hybrids or double-cross hybrids (DC) are commonly applied in industrial and vegetable crops such as maize and okra breeding programs (Charles et al. 2013). Involvement of multiple parents in the double cross improvement program will provide a wide genetic base and enhance expression of heterosis.

The objectives of this study were to generate DC hybrids to improve fruit quality of Eksotika and, to evaluate the performance of DC hybrids compared to Eksotika, Eksotika II and Line 19.

Materials and Methods

Double-cross is a new approach in papaya breeding program adopted in MARDI to improve yield and fruit qualities. Four specific steps were involved in this program as shown below:

Step 1- Selection of parental varieties/lines: Four papaya varieties including two commercial local varieties, namely Eksotika and Sekaki, introduced variety PR217 and a sister line of Eksotika, Line 19 were chosen.

Step 2 - Development of F_1 hybrids: Crosses between selected parents were done to generate three combinations of F_1 hybrids viz Eksotika x Line 19, Sekaki x Eksotika and Line 19 x PR217. Hybrid Eksotika x Line 19 is commonly known as Eksotika II.

Step 3 - Development of DC hybrids: The F_1 seeds from each cross were harvested, germinated and planted as F_1 parental plots to generate two DC hybrid seeds namely DC hybrids [Eksotika II X (Line 19 x PR217)] and [(Sekaki x Line 20) X (Line 19 x PR217)]. The two double cross hybrid seeds were harvested and planted together with the Eksotikas. Eksotika, Eksotika II and Line 19 were used as benchmark varieties in this study.

Step 4 - Evaluation of DC hybrids: The two DC hybrids and three other genotypes were planted at a density of 2000 plants/ha. A Randomised Complete Block Design with four replications and 20 plants per replicate were applied. One year crop cycle yield was recorded. Ten fruits per plant from five randomly selected hermaphrodite plants per plot were harvested and analysed. Nine fruit qualities were assessed which were number of days required from maturity index 2 to index 5 (eating stage), fruit weight, incidence of freckles, incidence of brown blotches, flesh thickness, total soluble solids content (^{o}Brix), flesh texture, aroma and anthracnose incidence. Symptoms of freckles, brown blotches and anthracnose were graded into six groups (grade 0 - symptom free; grade5 - most severe) and the incidence indexes were calculated based on Sarip (1994). However, flesh texture and aroma were evaluated through sensory tests. Analysis of variance (ANOVA) was carried out on seven fruit quality traits namely number of days required from maturity index 2 to index 5, fruit weight, freckles index (F.I.), brown blotches index (B.I.), flesh thickness, total soluble solids content, flesh texture and anthracnose index (A.I.).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Results and Discussion

Mean squares for the eight traits of five genotypes indicated highly significant differences (P<0.01) for all traits except for anthracnose index which was not significant. No significant difference was also detected between the four replications.

Duncans Multiple Range Test (DMRT) indicated that both double-crossed hybrids (DC) had longer shelf-life compared to Eksotika, Eksotika II and Line 19 (Table 1). DC (Line 19 x PR217) X (Line 19 x Line 20) and (Line19 x PR217) X (Sekaki x Line 20) required 6.9 and 6.6 days, respectively, from maturity index 2 (harvesting stage) to index 5 (eating stage). In contrast, the Eksotikas ripen much faster, between 4.6 to 5.9 days.

Table 1.Seven fruit quality traits of Eksotikas and double-cross papaya genotypes

	Fruit Quality Traits (Means)						
Genotypes	A	B	C	D	E	F	G
Eksotika	4.6 a	670.8 b	2.23 c	0.85 c	24.6 b	11.33 b	5.3 a
Eksotika II	4.5 a	683.8 b	2.13 c	0.87 c	24.7 b	11.55 b	5.5 a
Line 19	5.9 b	528.9 cd	1.65 b	0.88 c	25.0 b	12.57 bc	6.4 b
(Line19 x PR217) X (Sekaki x Line 20)	6.6 c	743.6 a	1.63 b	0.32 a	32.8 c	11.15 a	6.3 b
(Line 19 x PR217) X (Line 19 x Line 20)	6.9 c	497.8 d	1.39 a	0.59 b	22.9 a	13.57 d	6.9 c

Mean values in the same column with different letters are significantly different (P<0.05) according to DMRT.
Note: A) number of days required from maturity index 2 to index 5 (eating stage), B) fruit weight (g), C) freckles index, D) brown blotches Index, E) flesh thickness, F) total soluble solids content (^{o}Brix.) and G) flesh texture.

DC (Line 19 x PR217) X (Line 19 x Line 20) produced the smallest fruit (497.8 g) compared to the other DC (743.6 g) and Eksotikas (528.9 to 683.8 g). DC (Line19 x PR217) X (Sekaki x Line 20) had bigger fruits most possibly due to the influence of the bigger fruit from the Sekaki parent. DC (Line 19 x PR217) X (Line 19 x Line 20) had the most attractive skinappearence with minimum freckle incidence (F.I. = 1.39). The grouping of DC (Line19 x PR217) X (Sekaki x Line 20) and Line 19 had the second lowest freckle incidence with F.I. of 1.63 and 1.65, respectively. The varieties most susceptible to freckles were Eksotika and Eksotika II with F.I. of 2.13 and 2.23, respectively.

Brown blotch incidence, considered as a physiological disorder, was found to be lesser in both the DC hybrids compared to the Eksotikas. The lowest BI was observed in DC (Line19 x PR217) X (Sekaki x Line 20) (B.I.=0.32) followed by DC (Line 19 x PR217) X (Line 19 x Line 20) (B.I.=0.59), Eksotika (B..I=0.85), Eksotika II (BI=0.87) and Line 19 (BI=0.88). The lowest B.I. occurred in DC (Line19 x PR217) X (Sekaki x Line 20) and this could most likely be inherited from the robust parent, Sekaki.

All three Eksotika genotypes had medium flesh thickness at the broadest part of the fruit, between 24.7 to 25.0 mm. DC (Line 19 x PR217) X (Line 19 x Line 20) had thinner flesh (22.9 mm), while DC (Line19 x PR217) X (Sekaki x Line 20) has the thickest flesh (32.8 mm).

The highest total soluble solid content (13.57^{o}Brix) was detected in DC (Line 19 x PR217) X (Line 19 x Line 20) compared to the other Eksotikas (11.33 to 12.57^{o}Brix). However, DC (Line19 x PR217) X (Sekaki x Line 20) had the lowest TSS of 11.15^{o}Brix.

From sensory test, DC (Line 19 x PR217) X (Line 19 x Line 20) was deemed the most preferable with better flesh texture and aroma compared to the other genotypes. However, all of them were equally susceptible to anthracnose.

The double-cross hybrid (Line 19 x PR217) X (Line 19 x Line 20) can be considered as the most promising amongst the five genotypes evaluated. It has aromatic reddish orange flesh (Royal Horticulture Society Color Chart-N30B). TSS was between 12.5 to 14.5 ^{o}Brix. Fruit size was between 500 to 550 g, which was considered a more desirable size for the export market. The skin was attractive yellow with minimum freckles. It produced higher yield per plant (between 30 to 45 kg) as compared to the Eksotika (28 to 42 kg).

All commercial papaya varieties including potential 'double-crossed hybrid' are susceptible to bacterial dieback disease (BDB). As of now, none of the papaya growing areas in Malaysia can be categorised as BDB free zones. Therefore, extra precautions and attention are necessary in planting papaya in Malaysia.

Conclusions

Double-cross method was found to be suitable for developing improved Eksotika papaya. DC hybrid (Line 19 x PR217) X (Line 19 x Line 20) has better fruit quality than DC hybrid (Line19 x PR217) X (Sekaki x Line 20), Eksotika, Eksotika II and Line 19. It was suggested that DC hybrid (Line 19 x PR217) X (Line 19 x Line 20) can be commercialised in BDB free areas.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Conservation of the Lada Solok (*Capsicum annuum*)

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Introduction

Lada Solok (Figure 1), a chili variety popular in Kelantan and Terengganu, is also known as Cili Putih Kelantan. It is a cultural heritage, used in the preparation of *solok lada*, an accompaniment of *nasi kerabu,* a well-known breakfast dish. Seeds were imported from Thailand or produced as farm-saved seeds, the quality of which is poor. There was no record of local breeding work (Melor, personal communication) and no reference collection existed. The aims of this study were to assess the quality of seeds obtained locally, and to characterize and conserve this variety in the gene bank at MARDI.

Figure 1. A Lada Solok plant at the early bearing stage

Materials and Methods

Plant materials preparation

Plants, flowers, fruits and seeds of Lada Solok, Kulai and MC11 chilli varieties were used in this study. Seedlings were sown in trays containing cocopeat and watered by capillary absorption. After six weeks, seedlings were transplanted into 9" x 12" polybags containing cocopeat:soil ratio (2:1). Fertilizer was given as generally recommended.

Seed quality testing

Germination and purity testing was carried out according to the methods of the International Seed Testing Association (ISTA) (2011) and carried out at room temperature by using the filter paper technique.

Morphological characterization

Plants, flowers, fruits and seeds were used in the characterization of the Lada Solok accession following the format of Asian Vegetable Research and Development Center (AVRDC) (2004) and according to International Plant Genetic Resources Institute (IPGRI) Descriptors for *Capsicum* spp. (1995).

Fingerprinting

Fingerprinting of Lada Solok was carried along samples from Kulai and MC11 chili varieties. DNA extraction, polymerase chain reaction (PCR) amplification and fingerprinting using Amplified Fragment Length Polymorphisms (AFLP) technique were carried out at the Centre for Marker Discovery and Validation (CMDV), according to Shah (2013). Eleven AFLP markers were used to differentiate chili varieties based on the presence or absence of a particular DNA fragment (bp) associated with them.

Results and Discussion

Seed quality

The germination rate was 80%, higher than SIRIM's standard of 65% (SIRIM, 2001). Purity, however, was poor, only at 94%, i.e. below SIRIM's standard of 98%. As a comparison, chili seed lots produced by MARDI normally have a physical purity of 99% and a germination rate of 86% (Amyita et al., 2010). Seed samples were kept as a germplasm collection at MARDI.

Morphological characterization of Lada Solok accession

Morphological characterization (phenotype) data obtained are as shown in Table 1 and in general was found to be quite uniform. However, few plants showed variation in terms of leaf and fruit shape. One plant produced fruits with 3 locules, instead of the normal two. This suggests genetic variation or probably segregation within the studied population (Melor, 2003). A temporary passport data has been created for the accession.

Fingerprinting

Fingerprinting results show that Lada Solok can be easily differentiated from other popular commercial chili varieties, based on presence/absence of DNA fragments associated with each of eleven AFLP markers as illustrated in Figure 2. The overall results are as shown in Table 2. Out of 48 DNA bands produced by the eleven markers used, Lada Solok showed only three bands similar to Kulai and none similar to MC11 varieties.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 1. Morphological characterization of Lada Solok

A.	Vegetative data	
	Leaf pubescence density	Sparse
	Stem pubescence density	Sparse
	Plant growth habit	Erect
	Leaf colour	Green
	Leaf shape	Ovate
	Nodal anthocyanin	Dark purple
	Plant stature	Small
	Branching habit	Sparse
B.	Inflorescence data	
	Pedicel position an anthesis	Pendant
	No. of pedicels per axil	1
	Anther colour	Blue
	Corolla colour	White
	Calyx margin shape	Dentate
	Filament colour	White
C.	Fruit data	
	Fruit wall thickness (mm)	1.43
	No. of locules	2
	Fruit colour at intermediate stage between immature and mature stage	Whitish green
	Fruit colour at mature stage	Red
	Fruit cross-sectional corrugation	Intermediate
	Anthocyanin spots in unripe stage	Absent
D.	Seed data	
	Seed colour	Cream-white
	No. of seeds per fruit	>50

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 2. A summary of presence/absence of DNA fragments specific for each particular marker detected in electrophoresis gel image. (1) Indicates presence and (0) indicates absence.

No	Marker	Size (bp)	Sample		
			LADA SOLOK	KULAI	MC11
1	M-CAC_E-AAC	M379.0	0	1	1
		M378.4	0	1	1
2	M-CAG_E-ACC	M433.9	0	1	1
		M232.3	1	0	0
		M229.1	0	1	1
		M208.8	0	1	1
3	M-CAG_E-AAG	M400.7	1	0	0
		M295.1	0	1	1
		M293.9	0	1	1
		M259.2	1	1	0
		M228.0	0	1	1
4	M-CAG_E-ACA	M406.0	0	1	1
		M370.7	0	1	1
		M369.8	0	1	1
		M241.1	0	1	1
		M221.8	1	1	0
5	M-CAG_E-AAC	M429.5	0	1	1
		M401.6	0	1	1
		M378.6	0	1	1
		M378.0	0	1	1
		M358.8	1	0	0
		M355.3	1	0	0
		M350.7	0	1	1
		M285.8	1	0	0
6	M-CAC_E-AGG	M361.5	1	0	0
		M353.9	0	1	1
		M311.8	0	1	1
		M308.7	0	1	1
7	M0CAG_E0ACG	M481.2	0	1	1
8	M-CAG_E-AAG	M345.9	0	1	1
		M337.5	0	1	1
		M334.6	0	1	1
		M280.6	0	1	1
		M272.1	1	1	0
		M188.4	0	1	1
9	M-CAG_E-AGC	M347.4	0	1	0
		M280.1	0	1	1
		M265.2	1	0	0
		M253.5	0	1	1
		M244.0	0	1	0
		M233.4	1	0	0
10	M-CAG_E-AGG	M402.2	0	1	1
		M387.3	0	1	1
		M386.6	0	1	1
11	M-CAT_E-ACG	M292.8	0	1	1
		M290.4	0	1	1
		M168.8	0	1	1
		M167.9	1	0	0

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

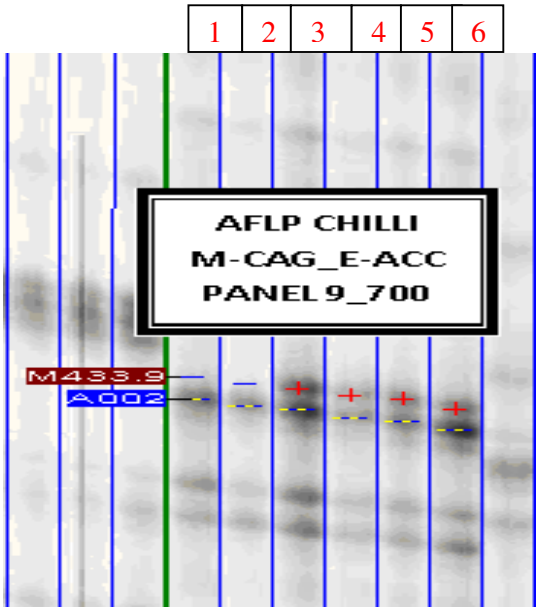

Figure 2. The partial image of a gel obtained using marker M-CAG_E-ACC showing 433.9 kb DNA fragments - absent (-) in Lada Solok (lane 1&2); present in Kulai (lane 3&4 and MC11 (lane 5&6).

Conclusions

Germination of procured Lada Solok seeds was found to be satisfactory high at 80%, but purity was poor, only at 94%, i.e. below SIRIM's standard. Fingerprinting via AFLP using eleven markers, shows that Lada Solok can be easily differentiated from other popular commercial chilli varieties. Evaluation of morphological traits shows that plants were quite uniform. However, variation was observed in terms of leaf shape, fruit shape and locule number. Seeds of Lada Solok are kept as a germplasm collection in the genebank at MARDI. A temporary passport data has been created for this accession while further characterization work is being carried out.

Acknowledgements

The authors wish to thank Muhamad Nuradha Paiman and the staff of the Seed Quality Laboratory for their excellent technical assistance.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Storage Period and Cutting Sizes of Fresh-Cut Wax Apple (*Syzygium samarangense*)

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Introduction

Wax apple (*Syzygium samarangense*) consumption has been increasing nowadays due to its attractive appearance and thirst-relieving properties. Wax apple have become one of the important fruit in market and also receive high demands from Western countries due to their apple-like crispness, watery-sweet, low-acid taste and the aroma of roses (Supapvanich et al., 2011; FAO, 2005).

It is widely accepted that fresh-cut fruits are more perishable than intact fruits (Gil et al., 2006). Supapvanich et al. (2010) reported that the respiration, ethylene production, and membrane degradation increased in fresh-cut fruits and subsequently reduced their usage life in comparison to whole fruits (Portela and Cantwell, 1998). There are many surrounding factors that could affect the retention of fresh-cut fruits quality including lenght of storage, cutting sizes, type of packaging, and storage temperature. However, this study will be focusing on the effect of storage period and cutting sizes on the postharvest quality of fresh-cut wax apples.

Materials and Methods

Syzygium samarangense (red skin wax apple) were bought from a farm in Melaka (Poh Keong Realty Sdn. Bhd) at the maturity stage that is suitable for consumption. The fruits were stored overnight at 12^0C before being processed. Wax apples with similar characteristic (colour, free from disease and physical defects) were selected. The fruits were cleaned using tap water for 5 min and then rinsed with water before being air-dried at room temperature (25 ^{0}C).

The fruits then were prepared according to the treatments: whole (S1), halved (S2), quartered (S3) and 1/8 fruits (S4) and stored in a clear, rectangle plastic container (1000 mL) 260-280 g per container. The fruits were then stored in domestic refrigerator (4 ^{0}C).

Changes in weight loss, firmness, total soluble solid contents, pH, titratable acidity, and total ascorbic acid were monitored on day 0, 3, 6, and 9 using the following techniques:

i. Weight Loss - The weight loss of the fruits was expressed by using this formula. Weight Loss Percentage (%) : (Initial Weight-Evaluation Weight)/(Initial Weight) x100.
ii. Firmness - Fruits firmness was determined using texture analyzer by Instron Universal Testing Machine (Model 5543, Instron Corp, Canton, MA) by measuring the maximum penetration force (N) required for tissue breakage was measured using a 5mm diameter flat probe at the middle of the samples.
iii. Total soluble solid (TSS) - was measured by using a Digital refractometer (Model PR-32, Atago, Japan) by squeezing the fruit's juice onto the prism of the refractometer. Three readings were taken and are expressed as percentage of brix.
iv. Titratable acidity - 10 g of fruits was homogenized with 40 mL distilled water (1:4) in domestic blender and was filtered with cotton in order to obtain clear juice 5 mL of the extract was titrated with 0.1N NaOH with the presence of drops of phenophthalene indicator until the colour of the juice changes its colour from white/very pale pink into slightly pinkish/ slightly darker pink. The volume NaOH used for the titration was taken and the TA of the extract will be defined as percentage of citric acid.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

(Titre x 0.1 N x vol.made up (50 mL)x 64 g (equivalent weight of citric acid)x 100)/(Weight of the sample (10g)x vol.used for titration (5mL)x 1000).

v. pH - the same juice used for TA was used for pH measurement using pH meter (Model CRISON GLP 21, Barcelona, Spain).

vi. Ascorbic acid content - 10 g of fruits flesh was macerated and homogenized in 40 mL of 3% metaphosphoric acid (HPO_3). The mixture was filtered using cotton. 5 mL of the filtrate was titrated with 2,6-dichlorophenol-indophenol (DCPIP) until the filtrate turned slightly pinkish. The volume of the dye used was recorded. Amount of ascorbic acid can be determined using the formula below:

(Vol.of titre x dye factor (0.1)x vol.made up (40 mL)x 100)/(weight of the sample (10g)x vol.used for titration (5 mL))

Results and Discussion

Water loss percentage was observed to be the highest in D9 ($p \leq 0.05$). There was a significant interaction between the loss of water and the cutting sizes of the fresh-cut wax apple. Wax apples being stored as whole (S1) lost the least amount of water in comparison to S2, S3, and S4. For all cutting sizes, it was observed that the water loss percentage was less than 1.6%. This is due to the fact that the fruits were kept in plastic containers that help to maintain the high relative humidity during the storage time.

Generally, it was observed that the firmness of wax apple on D9 was significantly lower than their initial firmness on D0. The whole wax apples (S1) was found to be softer than half (S2), quarter (S3) and 1/8 (S4) ($p \leq 0.05$). The retention of firmness of fresh cut products is depending on the cell softening enzymes activities. The loss of water also plays important roles in maintaining the turgidity of the fresh cut products that affects the firmness of the products. According to Adel Kader (2004), answering a postharvest question, water can be lost from fresh cut fruits in two forms which are liquid form and vapor form.

It was observed that the total soluble solid content decreased from D0 to D9. For D0, the TSS of freshly processed wax apples was around 7-8 □Brix and decreased significantly irrespective cutting sizes from D0 to D9. This result was found to be slightly different with the study of wax apples by Supapvanich *et* al. (2011) in which the TSS decreased from day 0 to day 4 but then remained constant throughout the experimental period. However, in a study conducted by Rosnah et al. (2012) on intact wax apple, TSS was also found to be decreasing after 4 days of the study being conducted till the last day (Day 19). Similar results were reported by Mao et al. (2006) and Perkins-Veazie and Collins (2004) on fresh-cut watermelon. There are several possible causes of the reduction in TSS including as the results of the respiration process (Mao et al., 2006) conversion of sugar to alcohol (Rosnah et al., 2012), and biosynthesis of anthocyanin which is induced by sugars (Supapvanich et al., 2011) leading to the increase of redness in wax apple.

For titratable acidity, there was a significant interaction between storage days and cutting sizes ($p \leq 0.05$). The titratable acidity increased significantly from D0 to D9 for S2, S3, and S4 but not for S1. It was observed that for S1 the TA decreased from 0.20% to 0.18%. Similar situation were reported by Gil et al (2006) on his study on the comparison of fresh-cut and whole pineapple. In the study, it was observed that the TA of whole pineapple decreased from 0.64% (initial) to 0.60% during the last day of the study (D9). The increase of TA for fresh-cut wax apple is similar to the study by Mao et al. (2006) on fresh-cut watermelon. The range of TA was found to be in the similar range with TA in the study conducted by Rosnah et al. (2012) which is lower than the range found in the study made by Supapvanich et al. (2011). This is probably due to the difference in weather, climates, and practices of the wax apple farm, Malaysia and Thailand, respectively, that leads to the differences in biochemical composition of wax apples.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 1. Effect of Cutting Sizes on weight loss, firmness, total soluble solid, titratable acidity, pH value, and ascorbic acid content.

Day	Treatment	Weight Loss (%)	Firmness (N)	Total Soluble Solid (□Brix)	Titratable Acidity (%citric acid)	pH	Ascorbic Acid Content (mg/100g)
0	S1	0.00	19.66a	7.6a	0.20a	4.53a	5.42a
	S2	0.00	20.77a	7.9a	0.16b	4.52a	5.56a
	S3	0.00	20.75a	7.4a	0.17b	4.51a	5.56a
	S4	0.00	19.95a	7.6a	0.21a	4.42b	5.00a
3	S1	0.65c	20.57a	7.3ab	0.20a	4.45a	3.91 a
	S2	1.32a	18.88a	6.7b	0.20a	4.47a	3.39 b
	S3	1.18ab	18.84a	8.1a	0.19a	4.49a	3.78 ab
	S4	0.93bc	20.14a	7.6ab	0.21a	4.43a	3.39 b
6	S1	0.51b	16.84b	7.1a	0.19a	4.36b	4.03a
	S2	0.97a	17.88ab	6.4a	0.19a	4.41ab	3.61a
	S3	0.92a	19.83a	7.1a	0.15b	4.45a	3.89a
	S4	0.82a	19.85a	7.2a	0.19a	4.42ab	3.75a
9	S1	1.16a	17.14b	6.0a	0.18b	4.37a	3.23a
	S2	1.53a	18.32ab	5.9a	0.22a	4.37a	3.10a
	S3	1.35a	19.46a	6.8a	0.22a	4.42a	2.83a
	S4	1.45a	19.15ab	5.9a	0.22a	4.39a	3.36a
F-test value							
Storage Days		*	*	*	*	*	*
Fruit size		*	ns	ns	*	ns	ns
Fruit size x Days		ns	ns	ns	*	ns	ns
LSD Value		0.138	1.158	0.579	0.014	0.041	0.293

[a]*Means (n= 3) in each column followed by the same letter at each time do not differ significantly at P ≤ 0.05. ns,* non-significant or significant at p≤0.05, respectively*

There was no significant interaction between storage days and cutting sizes in determining ascorbic acid content. However, it was observed that the concentration of ascorbic acid decreased significantly throughout the period of storage ($p \leq 0.05$). Results in Table 1 showed that S1 had lost the least amount of ascorbic acid in comparison to S2, S3, and S4. This result is similar to the result reported by Beaulieu and Lea (2007) in which ascorbic acid was lost throughout 7 days of storage. During storage and processing, there is a tendency for ascorbic acid level to decrease (Veltman et al., 2000) due to the degradation of ascorbic acid that could occurs either aerobically or anaerobically (Matei et al., 2009). Similar incident was also observed for pH. It was observed that the pH values had increased from D0 to D9 irrespective to the cutting sizes.

Conclusions

From this experiment, it can be concluded that storage days does affect the physico-chemical of wax apples regardless of their shapes (cutting sizes) during storage days but not to the extent that reduce the qualities of fresh-cut wax apples. Hence it is appropriate for the wax apple producers and retailers to trade wax apples as fresh-cut products. The fresh-cut wax apples may be treated with the technologies available in order to maintain or increase the acceptability of the products.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Antibrowning Treatments on Decored Green Roselle (*Hibiscus sabdariffa* var. UKMR-3) Prior to Chill Storage

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Introduction

Roselle (*Hibiscus sabdariffa* L.) is a relatively a new crop in Malaysia and is commonly known as 'asam paya', 'asam susur' and 'asam kumbang'. In 2009, three new varieties of roselle had been introduced by National University of Malaysia (UKM) which was UKMR-1, UKMR-2 and UKMR-3 using mutation breeding (Mohamad et al., 2009). Among these varieties, UKMR-3 has its own specialities because it is green in colour. However, this variety is still under-utilized and the products that can be made from green roselle (UKMR-3) are still limited. Green roselle has more obvious browning problem compared to red roselle which make it less acceptable for processing and made into food products. Appearance, flavour, texture and nutritional value are four attributes considered by consumers when making food choices. Appearance which is significantly impacted by color is one of the first attributes used by consumers in evaluating food quality.

Browning may occur because of polyphenol oxidase (PPO) enzyme in the chloroplast which rapidly oxidize phenolic compound in the fruit tissues to form *o*-quinones. These chemicals (PPO Enzyme) interact with amino acids from proteins or they self-assemble to form brown polymers (Stefan, 2007). These problems can be overcomed using antibrowning treatments which can be divided into two categories which are physical and chemical treatments. This study was done to determine the effect of different antibrowning treatments on the physico-chemical properties of green roselle and to determine the best antibrowning treatment that can prolong the storage life of green roselle.

Materials and Methods

Sample Preparations: The harvested green roselle (*Hibiscus sabdariffa* var. UKMR-3) calyces were brought to Postharvest Technology Laboratory to be processed. This process must be done quickly to avoid enzymatic browning before antibrowning treatment is carried out. The calyces were first decored which involved the removal of the seed capsule from its calyces. Decored calyces was then washed and allowed to dry. Then, the calyces were treated with the antibrowning treatments by dipping it in solutions of Calcium chloride – 1 % (w/v), DETANO (Nitric oxide) – 0.01 %(w/v) and 4-Hexylresorcinol – 0.01 %(w/v). For each treatment, 3 replications were made and calyces dipped in distilled water were used as control. In this experiment, two types of physical treatments were also applied, i.e. hot water and steam blanching. All the treated calyces were then packed in Polypropylene (PP) plastic bag with holes to avoid anaerobic respiration and stored in the cold room at 5 ± 1 °C.

Physico-chemical Analyses: The L* a* b* color of each roselle were determined by measuring L* a* b* values at 3 different sites on each roselle. Lightness value, L*, indicates how dark/light the sample is which varies from 100 (white) to 0 (black), a* value indicates the redness/green color with values varying from +60 (red) to -60 (green) and b* is the grade of blueness/yellowness which is also varying from +60 (yellow) to -60 (blue). Browning score of 0 to 3 were established prior to the analyses. The browning score were based on physical observation of browning in the green roselle calyces. Texture measurements of roselle calyces were done using TA.XTplus texture analyser (Stable Micro System) using P/2 stainless steel probe. The pH of the treated samples was also measured using pH meter at every 2 day intervals.

Chlorophyll a content was determined by extracting treated calyces with acetone followed by reading the absorbance using UV-Vis spectrophometer (100% acetone used as blank), at the wavelengths of 662 nm. Ascorbic acid content was determined by using colorimetric technique as described by Jagota and Dani (1982). Roselle juice was treated with folin reagent before taking absorbance at 760nm. The amount of ascorbic acid in the roselle juice was then calculated based on the ascorbic acid standard curve.

Experimental Design and Statistical Analysis: The experiments were carried out according to completely randomized design (CRD). All the analyses parameters were done in 3 replicates for each roselle samples. The data were analyzed using one way analysis of variance (ANOVA) and differences of means among treatments were determined for significant at $P<0.05$ statistical SPSS software.

Results and Discussions

Colour: There were significant differences ($p<0.05$) among all the treatments. In retaining the L*, a* and b* values throughout the 8 days of storage, the 4-hexylresorcinol was observed to be the lightest in color L* among all which showed significant ($p<0.05$) higher L* value. However, the roselle treated with steam blanching showed the significant ($p<0.05$) lowest in lightness. Meanwhile, for color a*, roselle treated with steam blanching showed significantly ($p<0.05$) the highest value amongst all. Roselle treated with calcium chloride showed negative a* value due to the roselle green color. For b* value, there were significant differences ($p<0.05$) with DETANO showed significant ($p<0.05$) higher intensity of yellow color. Most authors use the decrease in lightness to evaluate the extent of browning. A browning study on cut surfaces of apples showed that the L and a tristimulus values were linear or occasionally bilinear with log time and related to the extent of browning (Sapers and Douglas, 1987).

Browning score: There were significant differences ($p<0.05$) among all the treatments where roselle treated with DETANO showed significantly ($p<0.05$) lowest browning score throughout the 8 days of storage. However, roselle treated with both steam and hot water blanching showed the higher browning score indicating faster browning. Based on this result, it was clearly showed that DETANO can be used to prolong the postharvest of green roselle. Browning of the cut surfaces of fresh cut slices of apple was inhibited by dipping in a solution of the nitric oxide donor (NO-donor) compound, diethylenetriamine nitric oxide (DETANO) and thus postharvest life was extended (Leshem, 2000).

Texture: There were significant differences ($p<0.05$) among different treatments shown in Figure 3. Roselle treated with steam and hot water blanching showed significant ($p<0.05$) the least firm texture compared to other treatments. This is because blanching process has slight cooking effect on the roselle which causes the texture to be softer. Blanching can result in undesirable softening of vegetable tissues. However, calcium can be added to reduce the softening.

pH Value: There were significant differences ($p<0.05$) among all the treatments. Roselle treated with steam blanching and DETANO showed significant ($p<0.05$) higher pH value. However, roselle treated with no treatment showed the lowest pH value. Browning control may be possible provided that the dipping solution is acidic with pH at least 3.5 (Maurice et al., 2000).

Cholorophyll a content: There were significant differences ($p<0.05$) among all the treatments. Control and 4-hexylrecorcinol showed significantly ($p<0.05$) the highest value of chlorophyll a. From Figure 5, roselle treated with both steam and hot water blanching showed significanty ($p<0.05$) the lowest chlorophyll a value. Chlorophyll a absorbs light within the violet, blue and red wavelengths while mainly reflecting green. This reflectance gives chlorophyll its green appearance. The degradation of chlorophylls will also result in discoloration in green roselle.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Ascorbic acid: From Figure 6, there were significant differences ($p<0.05$) among difference treatment for day 0 and day 2 while the rest of the days 4, 6, and 8 showed no significant different. Throughout the experiment, roselle treated with 4-hexylresorcinol showed the significantly ($p<0.05$) the highest value of ascorbic acid while steam blanching showed the significantly ($p<0.05$) the lowest value of ascorbic acid. The differences in values of ascorbic acid might differ mainly because of two reasons which are destructive sample and type of treatments.

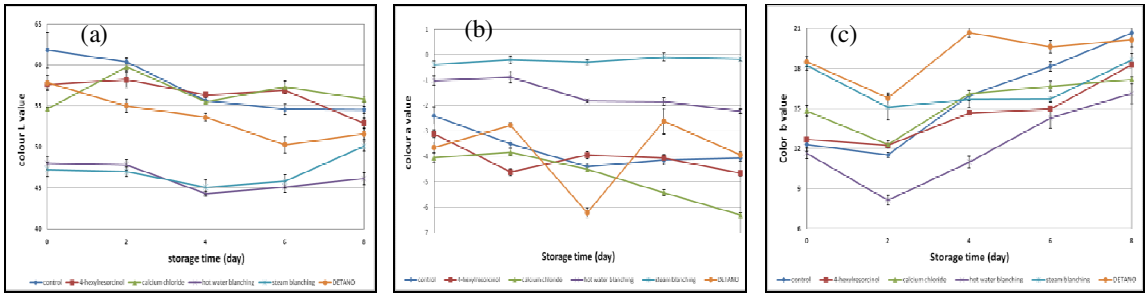

Figure 1: The color of decored green roselles treated with different antibrowning treatments. (a) L* value; (b) a* value; (c) b* value

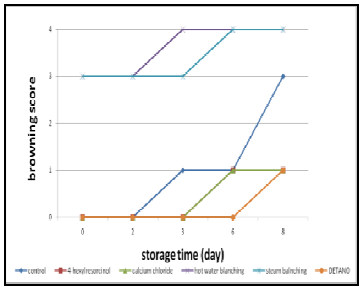

Figure 2: Browning score of decored green roselle treated with different antibrowning treatments

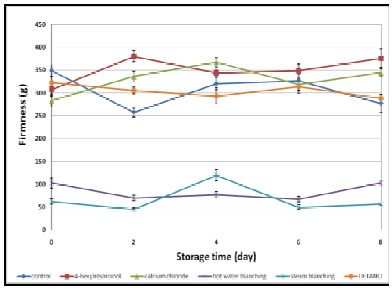

Figure 3: The firmness measurement of decored green roselle treated with different antibrowning treatments.

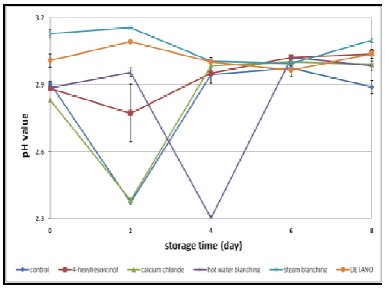

Figure 4: The pH value of decored green roselle treated with different antibrowning treatments

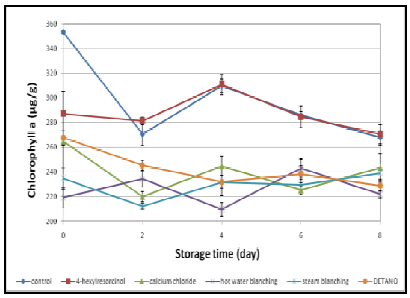

Figure 5: The chlorophyll a content of decored green roselle treated with different antibrowning treatments.

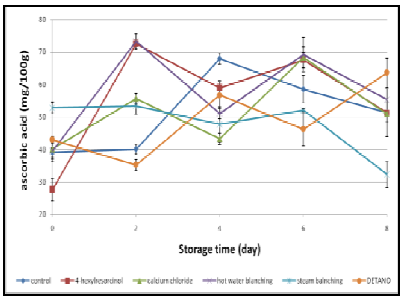

Figure 6: The ascorbic acid value of decored green roselle treated with different antibrowning treatments

Conclusions

Most of the treatments had significant effect in preventing enzymatic browning in green roselle except for hot water blanching and steam blanching. DETANO and calcium chloride had the highest value compared to others but DETANO can be classified as the best treatment because it can prolong the shelf life of the green roselle up to 8 days based on the browning score.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Starch-Based Edible Coating in Maintaining the Quality of Chok Anan Mangoes

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Introduction

Chok Anan mango is one of the mango varieties that gained high demand in Malaysia. However, Chok Anan mango is easily susceptible towards deterioration and spoilage as it is a climacteric fruit. The shelf life of mangoes ranges from 4 to 8 days at ambient temperature (Carrillo-Lopez et al., 2000). Hence, more research needs to be carried out in order to prolong the shelf life and maintain the quality of Chok Anan mango. Recently, edible coatings on fresh produced fruits have received great attention as it helped to reduce postharvest losses. Edible coatings (polysaccharides, proteins and lipids) provide a selective barrier against moisture and gases; thereby delayed fruit ripening, retarded weight loss and retained fruit firmness (Baldwin et al., 1999). Starch is one of the most selective choices of raw materials used in fruits coating (Kittur et al., 2001; Bibi and Baloch, 2012) because of its odorless, tasteless, colorless, impermeable to oxygen, biodegradable and safe to be consumed characteristics (Pareta and Edirisinghe, 2006). Due to the probable global food shortage and increasing price of other traditional starch (wheat and soybeans), tapioca starch is viewed as an alternative source used in coating solutions (FAO, 2004). Besides, starch from sago palm also gained more attention nowadays as this underutilized palm has been an extremely sustainable plant with the ability to thrive in most soil conditions (Dharmesh, 2013). In Malaysia, sago palm is found abundantly in Sarawak and is also one of the highest productivity among the starchy crops in the world (Flach and Michiel, 1997). Despite of having almost the same characteristic with tapioca starch, sago starch is easy to gelatinize, high in viscosity and undergo low syneresis (Takahashi, 1986). However, no study has been carried out on mango coating using tapioca and sago starch. Tapioca and sago starch were mostly applied in edible film making (Flores et al., 2007) and mangoes were usually coated with other sources such as Chitosan (Kittur et al., 2001) and carnabau wax (Baldwin et al., 1999). Therefore, the aim of this study was to determine the effect of tapioca-sago edible coating on the postharvest quality of Chok Anan mangoes for 13 days storage.

Materials and Methods

Chok Anan mango fruits of uniform size (200-300g), shape, color, free from any blemish and decay at the mature green stage (index 2) were harvested from Sui Yan orchard, Kuala Bikam, Ipoh. Starches with different ratios of tapioca (T) to sago (S) starches i.e. F1 (50%T:50%S), F2 (75%T:25%S) and F3 (25%T:75%S) were dissolved in distilled water. The flour mixture was stirred and heated until 90±2°C. Then, 1% (w/v) of palm oil and 0.5% (w/v) of glycerin were added and stirred continuously for 3 minutes followed by addition of 0.5% (w/v) potassium sorbate until dissolved. After that, the coating solutions were homogenized and left it to cool down to room temperature. The fruits were washed, sanitized using sodium hypochlorite solution, rinsed and dried prior to coating. Then, the fruits were dipped in the coating solutions for 2 min and drip off for 1 min. After that, the coated Chok Anan mango fruits were dried, packed in the corrugated box and stored at ambient temperature (23± 2°C) for 13 days. Uncoated fruits were used as the control. The weight loss percentage of Chok Anan mangoes were calculated as the fresh weight change at each sampling time divided by the initial weight of the mangoes. The skin color of Chok Anan mangoes evaluation (lightness (L*), redness (a*), yellowness (b*), chroma (C*) and hue angle (h°)) was determined using Minolta CR-310 colorimeter while fruit firmness using a texture analyzer. Soluble solids concentration (SSC) of mango juice was determined using a digital refractometer while titratable acidity (TA) was determined by titrating mango juices against 0.1N NaOH using phenolphthalein as

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

the indicator. All the analysis was carried out every 2 days for 13 days storage. Two-way analysis of variance was conducted by using SPSS (Version 17.0) statistical software package to test the significance of coating formulations and storage times on the qualities of mangoes. Separation of means was performed with the Duncan's multiple range test (alpha=0.05).

Results and Discussion

As shown in figure 1, there was a significant interaction between coating formulations and storage periods on the weight loss of Chok Anan mangoes. Tapioca-sago coatings reduced the weight loss of mangoes throughout the 13 days. This was explained by previous research that coatings block all lenticels and stem-end scar on the fruit (Dhalla and Hanson, 1988) to prevent moisture evaporated out to the environment. However, throughout the 13 days of storage, only coatings with 50% and 75% of sago starch (F1 and F3) showed effective results in reducing the weight loss of Chok Anan mangoes. This finding showed significantly different compared to F2 and control ($p \leq 0.05$). This may due to the higher composition of amylose in the sago starch which is responsible for the formation of coherent and effective strong coating (Rindlav-westling et al., 1998) hence lower the water vapor permeabilities.

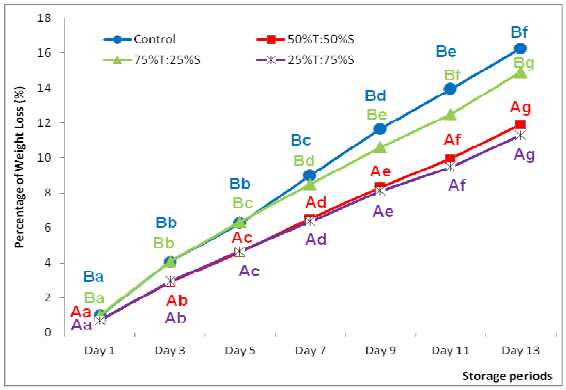

Figure 1 :Effects of various edible coatings formulations and storage periods at ambient temperature on the weight loss (%) of Chok Anan mangoes

a-g Mean values with same small letters showed no significant difference for each formulations between storage periods ($p>0.05$)

A-B Mean values with same capital letters showed no significant difference between coating formulations for each storage periods. ($p>0.05$)

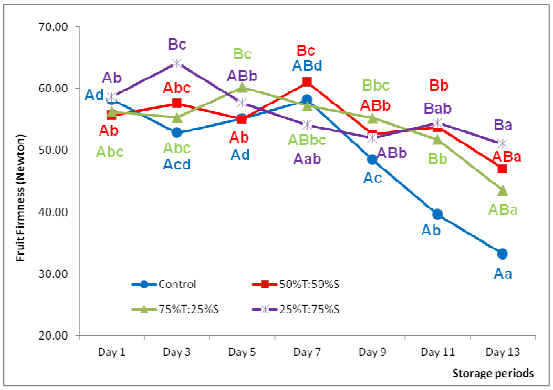

Figure 2 :Effects of various edible coatings formulations and storage periods at ambient temperature on the fruit firmness (N) of Chok Anan mangoes

a-d Mean values with same small letters showed no significant difference for each formulations between storage periods ($p>0.05$)

A-B Mean values with same capital letters showed no significant difference between coating formulations for each storage periods. ($p>0.05$)

The interaction effect on firmness between edible coatings and storage periods on Chok Anan mangoes were significant ($p \leq 0.05$) (Figure 2). As the storage period prolonged, the fruit firmness decreased which may be due to the advance breakdown of pectin and hence decreased the fruit firmness. No significant difference was recorded on fruit firmness in all coated Chok Anan mangoes. However, coatings with a higher percentage of sago starch (75%) was shown to delayed texture softening in Chok Anan mangoes significantly compared to control (Figure 2). This showed that coating played a role in modified the internal atmosphere of Chok Anan mangoes. Less oxygen to the coated fruits may delay the degradation of cell wall components and cell membranes by pectinesterase, polygalacturonase and other enzymes which negatively affected the tissue rigidity in mangoes, and hence delayed shrinkage and fruit softening during the storage period (Paliyath and Dennis, 2008).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 1: Effect of various edible coatings formulations and storage periods of color development (L*, a*, b*) of Chok Anan fruits

Day	L*				a*				b*			
	Control	Formulation A	Formulation B	Formulation C	Control	Formulation A	Formulation B	Formulation C	Control	Formulation A	Formulation B	Formulation C
1	52.26^{Aa}	51.46^{Aab}	50.87^{Aa}	50.91^{Aab}	-15.18^{Ba}	-16.41^{Aa}	-16.52^{Aa}	-15.99^{ABa}	30.01^{Aa}	31.54^{Aa}	31.78^{Aa}	29.60^{Aa}
3	51.38^{ABa}	50.00^{Aa}	50.77^{ABa}	51.91^{Bab}	-16.18^{Aa}	-16.57^{Aa}	-16.01^{Aab}	-16.11^{Aa}	30.01^{Aa}	32.01^{Aa}	32.83^{Aa}	31.27^{Aab}
5	51.62^{Ba}	49.28^{Aa}	52.23^{Ba}	49.16^{Aa}	-15.79^{Aa}	-15.55^{Aa}	-15.12^{Aab}	-15.26^{Aa}	28.96^{Aa}	30.25^{ABa}	33.02^{Ba}	31.14^{ABab}
7	53.85^{Ba}	50.45^{Aa}	50.68^{Ba}	49.99^{Aab}	-14.66^{Ba}	-16.50^{Aa}	-15.88^{Aab}	-16.49^{Aa}	32.50^{Aa}	31.91^{Aa}	31.98^{Aa}	32.09^{Aab}
9	59.81^{Bb}	49.43^{Aa}	50.68^{Aa}	51.49^{Aab}	-9.30^{Bb}	-16.33^{Aa}	-15.95^{Aab}	-16.32^{Aa}	53.89^{Bb}	32.03^{Aa}	32.35^{Aa}	32.61^{Aabc}
11	68.98^{Bb}	50.23^{Aa}	50.83^{Aa}	52.70^{Ab}	-7.17^{Cc}	-17.03^{Aa}	-14.56^{Bb}	-16.35^{Aa}	62.15^{Bc}	32.15^{Aa}	33.57^{Aab}	34.80^{Abc}
13	71.70^{Bc}	61.98^{Ab}	52.95^{Aa}	52.20^{Ab}	-7.92^{Cc}	-7.55^{Bb}	-15.30^{Aab}	-15.10^{Aa}	59.39^{Bc}	32.83^{Ab}	36.77^{Ab}	36.37^{Ac}

a-c Mean value with same small letters showed no significant difference for each coating formulations between storage periods ($p>0.05$)
A-C Mean value with capital letters showed no significant difference between coating formulations for each storage periods ($P>0.05$)

The skin color of Chok Anan mango was significantly ($p<0.05$) affected by the interactions of coatings formulations and storage periods. Control fruits showed an extreme change ($p<0.05$) in color development after one week storage compared to all coated mangoes. On day 13, uncoated Chok Anan mangoes were fully ripened and turned to yellow (L*=71. 70, hue°=82. 44 and chroma= 59.92) (Table 1 and 2). Retardation of color development coated Chok Anan mangoes could be attributed by slower rates of respiration and ethylene production which in turn delayed the ripening process.

Table 2: Effect of various edible coatings formulations and storage periods of color development (C* and hº) of Chok Anan

Day	hue angle (hº)				Chromatocity (C*)			
	Control	Formulation A	Formulation B	Formulation C	Control	Formulation A	Formulation B	Formulation C
1	116.85^{Acd}	117.51^{Ab}	117.57^{Ab}	118.41^{Ab}	33.66^{Aa}	35.56^{Aba}	35.84^{Ba}	33.66^{Aa}
3	118.40^{Ad}	117.42^{Ab}	116.15^{Aab}	117.27^{Ab}	34.10^{Aa}	36.05^{Aa}	36.57^{Aa}	34.10^{Aa}
5	118.67^{Bd}	117.22^{ABb}	114.70^{Aab}	116.15^{ABab}	33.02^{Aa}	34.02^{Aba}	36.35^{Ba}	33.02^{Aa}
7	114.34^{Ac}	117.04^{Bb}	116.44^{ABab}	117.25^{Bb}	35.69^{Aa}	35.93^{Aa}	35.72^{Aa}	35.69^{Aa}
9	94.31^{Ab}	117.92^{Cb}	116.36^{Bab}	116.64^{Bb}	53.13^{Bb}	35.96^{Aa}	35.99^{Aa}	53.13^{Bb}
11	83.42^{Aa}	98.10^{Ba}	113.56^{Ba}	115.84^{BCab}	62.56^{Bc}	36.83^{Aa}	36.62^{Aa}	62.56^{Bc}
13	82.44^{Aa}	116.15^{Ab}	112.88^{Ba}	113.03^{Ba}	59.92^{Bc}	53.88^{Ab}	39.94^{Ab}	59.92^{Bc}

a-c Mean value with same small letters showed no significant difference for each coating formulations between storage periods ($p>0.05$)
A-C Mean value with capital letters showed no significant difference between coating formulations for each storage periods ($P>0.05$)

The increment of SSC resulted in the decrement of acidity in Chok Anan mangoes during ripening (Figure 3 and 4). Coated Chok Anan mangoes significantly slowed down the biochemical changes as compared to control ($p\leq0.05$). This result was confirmed by previous studies that starch coating control the changes in SSC and TA (Kittur et al, 2001). After 9 days storage, there were drastic increased in biochemical changes in uncoated Chok Anan mangoes which indicated the fruit has ripen (Figure 3 and 4). This phenomenon was due to the starch degradation by the activities of amylase which converted starch and acid to sugar content. In mango, glucose, fructose and sucrose are the major forms of simple sugars that contributed to the high SSC (Kittur et al, 2001). Coating with 75% of sago starch were the most effective coating formulations in retarding the increased of SSC among other coating formulations (Figure 3). This may probably due to the protective oxygen barrier which reduced oxygen supply on the fruit surface, hence inhibited respiration and slower hydrolysis of carbohydrate to sugar. For acidity, all coated Chok Anan mangoes showed no significant difference ($p>0.05$). However, coating with 75% of sago starch evidently remained the high acidity in Chok Anan mangoes (Figure 4).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

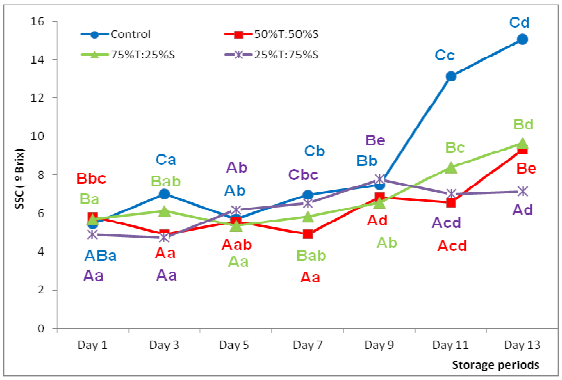

Figure 3 :Effects of various edible coatings formulations and storage periods at ambient temperature on the total soluble solids of Chok Anan mangoes

a-d Mean values with same small letters showed no significant difference for each formulations between storage periods (p>0.05)
A-B Mean values with same capital letters showed no significant difference between coating formulations for each storage periods. (p>0.05)

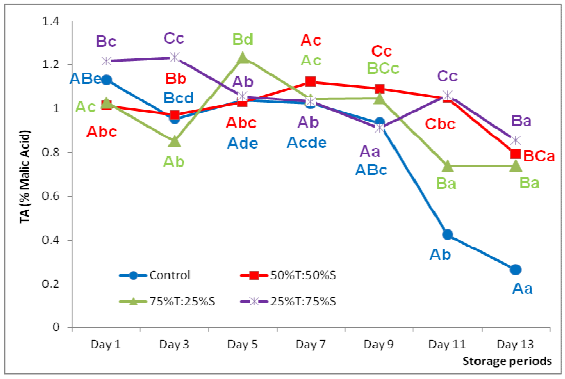

Figure 4 :Effects of various edible coatings formulations and storage periods at ambient temperature on the titratable acidity of Chok Anan mangoes

a-c Mean values with same small letters showed no significant difference for each formulations between storage periods (p>0.05)
A-B Mean values with same capital letters showed no significant difference between coating formulations for each storage periods. (p>0.05)

Conclusions

The study indicated that coating the fruits with the highest percentage of sago starch (F3) has the potential to maintain fruit qualities by retarding the moisture loss, retaining fruit firmness and delaying the color and biochemical changes effectively hence prolonging the shelf life of Chok Anan mangoes.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Nutrient Content Analysis of Rust-Affected Jackfruits

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Introduction

Jackfruit remained traditionally a minor and less important fruit crop in the 1970s and 1980s. However, its hectarage has increased from 3,133 ha in 2005 to 3,559 ha in 2010 with the production of 17,624 to 19,516 matric tonne, respectively. The steady increase has been attributed to several factors such as improved cultural management, varietal selection, post harvest handling, new market outlets and government initiatives. In the Economic Transformation Programme (ETP), Agriculture National Key Economic Areas (NKEA) and Entry Point Projects (EPPs), the government aims to double agriculture sector contribution to gross national income (GNI) and upgrading capabilities to produce fruits for premium markets (Bahagian Perangkaan Jabatan Pertanian, 2010). Jackfruits have become one of the important fruits in the Entry Point Projects (EPP) besides papaya (Eksotika), pineapple (MD2), rockmelons (KR), starfruits (B10) and banana (Cavendish).

Early research in jackfruits showed that most emphasis were focussed on pests and disease control (Sariah, 1999), varietal selection (Palaniappan and Fui, 2001) and recently minimally process studies in post harvest (Latifah, 2007). However, fruit quality problems particularly rusty fruit flesh incidence occurred in some varieties. There are three varieties commercially grown namely Mastura (J35), Mantin (J29) and Tekam Yellow (J33); the latter has mostly been affected by fruit pulp quality. Sariah (1999) confirmed this disorder was not caused by pathological diseases such as *Erwinia* or *Phytopthora* but due to nutrient imbalance. Reports showed that rusty in fruit pulp of jackfruits occurred during wet spells. The fruit pulp hardened and its flesh changed from yellow to brownish yellow that makes the fruit inedible, less crunchy, bitter and not visually accepted to customers as such fruits are not marketable. This has caused losses to growers and the problems still remain unresolved although jackfruit has been considered a versatile crop.

The understanding of nutrient contents in both soil and plant as well as correct fertilization and agro-management procedures will help to reduce existing problems that could boost the jackfruit industry sustainability. Therefore, this paper will discuss nutrient content of rusty pulp incidence to ascertain what elements that are involved in this phenomenon.

Materials and Methods

Three commercial jackfruit varieties were studied in relation to rusty incidence namely Subang, J29 (Mantin) and Tekam Yellow (J33). Parameters such as fruit weight, flake weight w/o seeds / fruit, flake numbers, recovery rate and percentage of rust were recorded.

Rust affected and non-rust affected fruits of J33 variety were collected from Rofken Farm and Saujana Farm, in Lanchang, Temerloh, Pahang, respectively. The samples were then sent to the lab and separated into different parts. The fruit flake (pulp), undeveloped perianth, pith and endocarp (skin) were oven-dried, ground and stored at room temperature for nutrient analysis using autoanalyser.

Leave samples from J33 trees obtained from both farms were used for foliar analysis. Soil pH, conductivity and soluble P were taken at soil depths of 15 cm (6in) and 30 cm (12 in).

Eight elements were analysed namely nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), boron (B) and zinc (Zn). The standard of nutrient elements was

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

based on SRM 1547: Peach leaves (NIST); SRM 1573a: Tomato leaves (NIST) (Certified Reference Material).

A complete randomized design was adopted and data was analysed using SAS procedures. A T- test using "PROC TTEST' method was plotted to test means significance.

Results and Discussions

Results showed that among the three varieties, J33 variety had 38.5% rust incidence compared to none both in Subang and J29 (Mantin). However, fruit weight, flake weight w/o seeds / fruit, flake numbers and recovery rate showed not much difference (Table 1).

Table 1. Fruit characteristics in three jackfruit varieties

Variety / Parameter	Tekam Yellow (J33)	Mantin (J29)	Subang
Fruit weight (kg)	15.11	22.69	18.12
Flake weight w/o seeds / fruit (kg)	7.77	11.03	9.18
Flake numbers	198	209	141
Recovery rate (%)	44.28	49.16	42.77
Percentage of rust (%)	38.46	0.00	0.00

*n=10; *,**, at p <0.05, 0.005, respectively and ns denotes non-significant*

Four elements were found to be significantly less in rust-affected flake namely P, K, Cu and Fe except Mg (Table 2). Rust-affected perianth also showed significantly less in Cu. Meanwhile, both affected and non-affected pith and affected and non-affected endocarp had no element difference. At farm level, four elements namely P, Cu, Al and Mn were found to be higher in Rofken Farm compared to Saujana Farm although Fe and Mn were found to be significantly low (Table 3). Soil pH, conductivity and soluble P had no significant difference in the farms (Table 4).

Deductions could be made that rust-affected fruit flakes and the farms (Rofken) had common elemental uptake namely P, Fe and Cu. However, Fe showed singly consistent and significantly less in both in rust-affected flake and Rofken Farm. Therefore, it could be demonstrated that Fe is the main element that occurred 'deficient' in rust–affected fruit and at farm level.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 2. Nutrient contents in non-affected and affected rust incidence in flake, perianth, pith and endocarp of jackfruits in J33 variety

Element	P (%)	K (%)	Mg (%)	B (ppm)	Al (ppm)	Ca (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Na (ppm)	Zn (ppm)
Non- Rust Affected Flake	1.51	0.31	0.15	21.14	nd	17.63	14.07	31.41	15.16	105.55	2.23
Rust Affected Flake	0.79	0.19	0.89	13.58	nd	8.05	8.18	20.69	14.80	105.34	nd
Significance level	**	**	**	ns	ns	ns	*	*	ns	ns	ns
Non- Rust Affected Perianth	1.29	0.35	1.69	23.69	nd	64.44	10.00	49.36	23.49	98.10	10.69
Affected Perianth	0.92	0.29	0.82	17.48	nd	21.89	7.34	34.95	38.97	103.99	1.94
Significance level	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns
Non-Rust Affected Pith	1.93	0.67	0.88	31.74	nd	76.26	15.37	49.12	35.29	106.63	34.71
Affected Pith	1.48	0.53	0.67	18.85	nd	46.62	11.57	44.75	72.11	106.91	14.70
Significance level	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Non-Rust Affected Endocarp	1.71	0.40	1.09	44.35	35.62	37.64	37.95	57.48	38.12	111.83	6.54
Affected Endocarp	1.25	0.36	1.24	31.03	5.49	19.16	29.91	41.04	25.39	128.32	3.83
Significance level	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*n=2, *,**, at p <0.05, 0.005, respectively and ns denotes non-significant.*
Note: Nitrogen was not analysed due to machine failure

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 3.Nutrient contents in two jackfruit farms cultivated with J33 varieties in Lanchang, Temerloh Pahang

Location	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Cu (ppm)	Al (ppm)	Mn (ppm)	Na (ppm)
Saujana Farm (Non-Rust Affected)	2.45	3.02	1.94	0.12	0.23	87.90	10.30	7.97	180.71	125.17
Rofken Farm (Rust –Affected)	2.34	4.80	2.09	0.11	0.24	71.39	22.94	13.11	92.84	129.17
Significance level	ns	**	ns	ns	ns	*	**	*	**	ns

n=10; *,**, at $p<0.05$, 0.005, respectively and ns denotes non-significant

Table 4. pH, conductivity and soluble P in two jackfruit farms in Lanchang, Temerloh

Location	pH	Conductivity (µs/cm)	pH	Conductivity (µs/cm)	soluble P (ppm)	
	15 (cm)		30(cm)		15(cm)	30(cm)
Saujana Farm (Non-Rust Affected)	5.0	57.05	4.53	38.67	40.59	19.97
Rofken Farm (Rust Affected)	5.5	98.17	4.86	47.97	111.17	58.39
Significance level	ns	ns	ns	ns	ns	ns

n=10; *,**, at $p<0.05$, 0.005, respectively and ns denotes non-significant

Conclusions

Tekam Yellow (J33) has highest rust incidence compared to Subang and Mantin (J29) varieties as shown by earlier findings. The edible portion of jackfruits, in particular the flake or fruit pulp was highly influenced by macro- and micro-nutrients particularly P, K , Fe, Mg and Cu that might cause pulpy rust. However, inedible portions such as pith and endocarp were not influenced by nutrient uptake except perianth (Cu). Fe was found to be the main element that occurred 'less' in both rust–affected fruit and at farm level (Rofken Farm). The uptake of nutrients is believed to be affected by environmental conditions like rainfall. More results needed to be sought to ascertain the relationship of nutrient imbalance between plant-soil in order to provide proper corrective measures and cultural practices to overcome rust incidence.

Acknowledgments

Mohd Safidin bin Kashim and Dr.Rahman Milan for their collaboration work in this project. This project was funded by e-Sciencefund Project No.RH5053SF10.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

CHAPTER 3

ECOPHYSIOLOGY AND STRESS BIOLOGY

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effects of Girdling Technique on Flowering of Mango cv. Chok Anan

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Introduction

There are several methods to induce flowering of mango such as through hormonal influence, cultural manipulation, or combination of both techniques. Common practices by farmers in Malaysia is application of commercial plant growth retardants namely Paclobutrazol (PP-333), also known as Cultar®. It can be applied directly through soil drenching or by spraying onto plant canopy. The popularity of this product among the growers is probably due to its wide range of efficacy and long-lasting response. According to Kulkarni and Hamilton (2001), mango trees applied with Paclobutrazol promote greater flowering when compared with Morphactin and is reflected with greater numbers of fruits at harvest. In fruit production system, yield is important so that the farmer could sustain or increase their income every year. Flowering and fruit set are the most critical of all events occurring after establishment of a tree crops (Davenport, 2009). Furthermore, branch girdling is another cultural technique that is useful to improve earliness and flowering intensity. It is widely used in China, Myanmar, Indonesia and other Asean countries to stimulate early flowering and to force plant to bear larger fruit. Although Winston and Wright (1986) concluded that effect of girdling to promote flowering is rather inconsistent, there is still possibility that adoption of girdling would help especially when application of paclobutrazol becomes less effective. Branch girdling technique is yet to be established in Malaysia and there are several aspects that need to be considered. Therefore, the objective of this trial was to determine the girdling frequency that promotes flowering of mango cv. Chok Anan and its effects on yield and fruit quality.

Materials and Methods

The experiment was carried out at Station MARDI Bukit Tangga, Changlun in Kedah. Twenty five healthy and uniform mango trees cv. Chok Anan grown under high density planting (HDP) system were selected for this study. The treatments consisted of four girdling frequencies; 1, 2, 3, 4 times and plants that are not girdled as control (T1, T2, T3, T4 and T5). Girdling was done using a girdling knife on secondary branches at one week interval for each treatment and it was replicated 5 times. All trees have been applied with Paclobutrazol (PBZ) at 25% active ingredient (8 mL/L) in November 2012. Concurrent with girdling treatment, soluble NPK blend at 12:5:35 +TE (Boron, Copper, Manganese and Zinc) and also effective microbes (EM) at 1:30 ratio were applied every 2 weeks until flower panicle emerged. During the experiment, all cultural practices followed standard agronomic procedure for mango production. Data collection involved in this study were plant height using tangent height gauge, trunk cross sectional area (TCSA), date of flushing and flowering, number of flushing and flower panicle, yield, yield efficiency and total soluble solids. The analysis of variance (ANOVA) was analyzed using PROC GLM of the Statistical Analysis System (SAS) Institute, Cary, USA and Least Significance Difference (LSD) ($p \leq 0.05$) was used for means values comparison when treatment effects were significant.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Results and Discussion

Growth and flushing

The plant height, number of flushes and flushing date after girdling are listed in Table 1. The results show that the mango trees which have been selected to undergo the treatment are considerably uniform statistically with co-efficient variation value between 7.28-20.21. The plant heights ranged between 2.7-2.9 m. New shoots were observed 5 days after the 1st girdling for all treatments except for ungirdled which was delayed by 19 days. However, there was no significant difference between the numbers of flushes for each treatment. This shows that girdling technique together with foliar sprays would result in earlier flushing of mango trees. This result is in agreement with Pandey (1989), that girdling effects was similar to defoliation which resulted in flushing by altering the cytokinin:auxin ration in buds.

Table 1: Growth performance of mango cv. Chok Anan after girdling.

Girdling frequency	Plant height	No. of flushes	Flushing date
	— m —		
1	2.92 a	133 a	27-Jan-13
2	2.84 a	132 a	27-Jan-13
3	2.72 a	163 a	27-Jan-13
4	2.74 a	184 a	27-Jan-13
Ungirdled	2.92 a	122 a	10-Feb-13
Mean	2.83	147	
C.V	7.28	20.21	

* Means with similar letter are not significantly difference ($p \leq 0.05$) by LSD test among treatments.

Flowering pattern

It was observed that all girdled tree were able to promote flower panicle emergence by as early as 28-42 days after girdling compared to the control (52 days). Furthermore, the mango trees which were being girdled 2 and 3 times produced the highest number of flower panicles at 42 days (60 panicles) and at 52 days (45 panicles), respectively. Observation on day 60 depicted that the number of flower panicles emerged were almost uniform ranging from 16-22. On day 71, only T3 showed an increment in flower panicle emergence whereas for other treatments there was a reduction. Jose (1997) reported that girdling treatment at 60 and 75 days before application of potassium nitrate resulted in higher percentage of flowering and advanced harvest as early as 23 days compared to control. Urban et al. (2009) stated that flowering of mango cv. Cogshall occurred 15 days earlier in the girdled than in ungirdled treatment.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

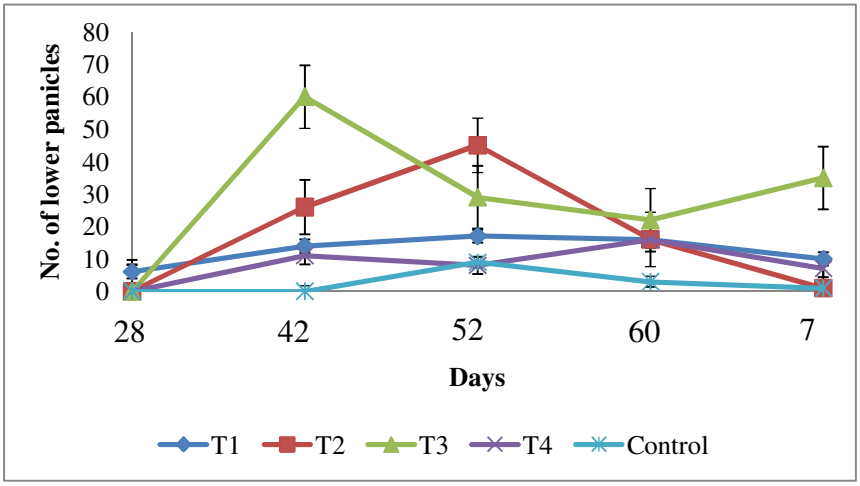

Figure 1: Girdling effects on numbers of flower panicles emergence at 28, 42, 52, 60 and 71 days starting from 1st girdling. Vertical bars represent mean (±S.E) of five replications ($p \leq 0.05$).

Yield and fruit quality

Effects of girdling frequency on yield and fruit quality were listed in Table 2. Harvesting was done twice on 23 June 2013 and 2 July 2013 for T1, T2, T3 and T4 except for T5 only once on 2 July 2013. There were significant different ($p \leq 0.05$) between treatment with fruit weight of the second harvest, total yield and yield efficiency. The fruit weights at first harvest were ranged from 309.72-366.67 g and 266.94-396.67 at second harvest. The highest total yield was obtained from T2 (3.38 kg tree^{-1}) meanwhile the lowest yield was observed from ungirdled trees (1.75 kg tree^{-1}). The results revealed that adoption of girdling technique significantly influenced the yield and yield efficiency of mango cv. Chok Anan. It was reflected by increasing the number of flush emergence after girdling which later turned into reproductive shoots. Sharma et al. (2011) reported yield obtained from Chausa mango resulted from girdling of primary branches and secondary branches was at par with chemical application and that may be attributed to higher proportion of shoots which flowered.

Table 2: Effects of girdling frequency on fruit weight, total soluble solid, trunk cross sectional area, total yields and yield efficiency of mango Chok Anan cultivar.

Girdling frequecy	1st Harvest		2nd Harvest			TY^{H1+H2}	YE
	Fruit weight	TSS	Fruit weight	TSS	TCSA		
	— g —	—°Brix—	— g —	—°Brix—	—m^2—	— kg tree^{-1} —	
1	342.14 a	15.6 a	278.33 b	18.0 a	4.10 a	3.01 ab	0.73 a
2	309.72 a	15.9 a	266.94 b	18.0 a	3.59 a	3.38 a	0.94 a
3	327.50 a	14.9 a	360.00 a	18.5 a	4.00 a	3.35 a	0.84 a
4	366.67 a	17.4 a	366.36 a	17.7 a	4.17 a	3.10 ab	0.74 a
Ungirdled	n.a	n.a	396.67 a	18.6 a	3.63 a	1.75 b	0.48 b

* Means with similar letter are not significantly difference ($p \leq 0.05$) by LSD test among treatments; TSS: total soluble solid; TCSA: trunk cross sectional area: TY^{H1+H2}: Total yields of 1st harvest and 2nd harvest; YE: yield efficiency; n.a: not available

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Conclusions

Girdling technique can be practiced to enhance early flowering of mango cv. Chok Anan. Furthermore, frequency of girdling up to 3 times will prolong flowering initiation until 10 weeks. Girdled mango trees are able to produce higher yield and with staggered harvest (2 times) compared with ungirdled trees. At the same time, the mango fruit quality in term TSS values was not affected with the various girdling treatments.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Allelopathic Effects of *Dioscorea hispida* Dennst. Tubers towards Growth of Test Plants *via* Laboratory and Field Bioassays

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Introduction

Knowledge in phytochemical will lead to knowledge of allelopathy, but both are two different fields. This allelopathic knowledge has been widely applied on crop cultivation system to increase the yield of plant, for example in fruit production, tuber production, and faster-germination and higher-aerial growth crop (Gao et al., 2009). Manikandan and Jayakumar (2012) have mentioned the main aspect of allelopathy, which was related with secondary metabolites in plants that are also known as allelochemicals. The phenolic compounds presence in *D. hispida* was described by Sudawadee and Baker (2009), such as the caffeic acid, chlorogenic acid and methyl protocatechuate.

In previous study of *Dioscorea hispida*, the root extract had more allelopathic effects compared to other parts of plant (Nornasuha et al., 2012). In order to optimize growth and development of roots for secondary metabolite production, optimum irrigation frequency is necessary. The wild *D. hispida* tuber water extract was revealed to inhibit germination and growth of mustard and stimulated growth of maize (Nornasuha et al., 2012), but the cultivated *D. hispida* with constant irrigation frequency applied has never been studied. Thus, the aim of this study is to determine the allelopathic effects of *D.hispida* at different irrigation frequencies.

Materials and Methods

Dioscorea hispida Dennst. tubers were collected at Ladang Karas, Merchang, Terengganu, Malaysia, at location N 05^{o} 02.125' E 103^{o} 14.767' and each tuber was weighed and planted in a polyethylene bag at the Nursery of Universiti Sultan Zainal Abidin. A Complete Randomized Design (CRD) with six plants for every treatment was arranged at a distance of 0.3 m from each plant and 0.7 m between rows. The treatments were 1) watered at one day interval (T1); 2) watered every day (T2/control); 3) watered at two day interval (T3); 4) watered at five day interval (T4); and 5) watered at six day interval (T5). The volume of water for each plant receives was 442 ml for every irrigation which was calculated base on wether broadcast (Muhamad Azhar et al. 2011). All *D. hispida* plants were harvested at nine months growth which was the maturity period of *D. hispida*. This harvesting process was selected based on comparison of harvesting time as suggested by Craufurd et al. (2001) and Behera et al. (2009).

The *D. hispida* tubers were water extracted by using modified method from Nornasuha et al. (2012). The treatments were 10 mL of 12.5 g/L of previous experiment which *D. hispida* had been under irrigation frequency and indicated as T1, T2, T3, T4 and T5, while negative and positive controls were distilled water and wild tuber water extract, respectively. The design was Completely Randomized Design (CRD) with three replicates and was repeated twice. Each replicate consist of ten seeds of *Zea mays* F1 hybrid (Big Fruit (926)) and *Brassica parachinensis* (Calxin (JB-777)) in both bioassays. The percentages of germination, radicle length and fresh weight of laboratory bioassay plant species were observed and recorded after seven days of sowing.

In field bioassay, the experimental design was (CRD) with three replicates that consist of 30 crops per replicate. The treatments used were T1 and T4 (because they showed the highest significant difference compared to control on radicle length at laboratory bioassay). A concentration of 12.5 g/L water extract was sprayed immediately after sowing by using a hand sprayer, at 10 mL, to each base of bioassay crops for every week until harvest. The yield parameters were measured at harvest (nine week after sowing for maize and four week after sowing for mustard) on six randomly selected plants from a total of 30 plants bioassayed for each replicate.

All data collected were evaluated with one-way analysis of variance (ANOVA) by using SPSS (version 17.0, IBM) software. The laboratory and field bioassay comparison tests were determined by the Dunnett test.

Results and Discussion

Germination rate, radicle length and fresh weight of maize and mustard

Table 1 shows all treatments (irrigated tuber water extracts) insignificantly increased the germination rate as compared to control (distilled water and wild tuber water extract) for both bioassay plants. This result suggested that *D. hispida* tuber aqueous extract at concentration 12.5 g/L contained some stimulating allelochemicals resulting in the increased germination of maize and mustard.

Allelopathic effects of T1 *D. hispida* aqueous extract showed significant differences in radicle length of maize at 4.79 ± 0.34 cm or 3.26 cm increment as compared to control (Table 1). This result was consistent with Nornasuha et al. (2012). Then, in this recent study, water extract of *D. hispida* tuber probably contain compounds which can stimulate radicle length. According to D'Abrosca et al. (2001), compounds such as furofuran lignans and cyanogenins have stimulating effects on radicle elongation of *Raphanus sativus*. On the other hand, caffeic acid can enhance the lignification of the roots of soybean (Bubna et al., 2011). Table 1 also shows that aqueous extract of *D. hispida* tuber had significantly stimulated radicle elongation in mustard seeds. This was showed by T4 which had significantly highest radicle length that was 0.54 cm more elongated than from control (distilled water).

The results from this study was however inconsistent with Nornasuha et al. (2012) which stated that at 12.5 g/L concentration, the aqueous extract of *D. hispida* tuber had significantly reduced the radicle length of *Brassica nigra* (mustard). According to Verma and Rao (2006), findings on allelopathic effects of *Ageratum conyzoides* L. showed both inhibitory and stimulatory influences on radicle length (cm) of different varieties of *Glycine max* (L.) Merrill. Although they were similar species and only differ in varieties, the allelopathic effects were also different on them. Therefore, the allelopathic effect of *D. hispida* proved to posses different interaction mechanism. Although the targeted crops were from similar genus which was *Brassica* spp., but they were different in species which were *Brassica parachinensis* (test species in this study) and *Brassica nigra* (test species that was studied by Nornasuha et al. (2012)). On the other hand, this study was consistent with Hassan and Samy (2007) who found stimulation in radicle length of cucumber, fenugreek and alssana seeds due to the influence of the *Calotropis procera* aqueous extract at 5% concentration compared to control.

Besides that, tuber water extract showed insignificantly reduced and increased of maize fresh weight but increased mustard fresh weight as compared to control (Table 1). Therefore, these results also suggested that *Dioscorea hispida* tuber may have allelopathic effect on fresh weight of maize and mustard.

Table 1. Allelopathic effects of 12.5 g/L *D. hispida* tuber water extract according to irrigation regime on *Z. mays* and *B. parachinensis* as compared to distilled water (control). The second control is the wild tuber water extract.

Bioassay Plants	Parameters	T1	T2	T3	T4	T5	Control tuber
Maize	germination rate (%)	17	15	3	8	28	2
	Radicle length (cm)	3.26**	1.39	1.85*	1.13	1.54	1.37
	Fresh weight (g)	0.018	-0.005	-0.004	0.048	-0.022	0.032
Mustard	germination rate (%)	5	9	14	10	10	5
	Radicle length (cm)	0.42	0.14	0.18	0.54**	0.06	0.45*
	Fresh weight (g)	0.0020	0.0004	0.0019	0.0055	0.0015	0.0103*

+ Denote promoter (than distilled water control); - Denote inhibitor (than distilled water control).

Allelopathic effects of Dioscorea hispida tuber water extract on field bioassay test plants

The tuber water extract of T1 had significantly increased maize height by 5.39%, total biomass weight by 25.27% and kernel number by 47.78% as compared with the control (Figure 1, 2 and 3). Meanwhile, tuber water extract of T4 significantly increased the mustard total biomass weights by 59.08% and number of mustard leaf by 35.68% when compared to control (Figures 4 and 5). Allelochemicals in *D. hispida* tubers improved maize and mustard growth and yield perhaps through chemical interactions which regulated high growth hormone production. These results were consistent with Sarajuoghi et al. (2012), who revealed that allelopathic compounds of rapeseed stimulate maize growth and yield. Besides that, they also stated that rye covered crop increases maize grain yield by 7.89% and plant height by 10.24%. Other researchers have reported that alfalfa (*Medicago sativa*) produce substance called triacontanol, meanwhile, soybean (*Glycine max*) and groundnut (*Arachis hypogaea*) are stimulatory to the growth and yield of maize (Zakaria and Razak, 1993). According to Einhellig and Leather (1988), allelochemicals like cinmethylin and methoxyphenone may also be adapted as yield stimulants.

These results suggest that tuber aqueous extracts of T1 and T4 contain optimum allelopathic compounds which increased yields of maize and mustard (Table 2). Both results were supported by the earlier laboratory experiment where it was stated that similar irrigation frequency of cultivated *D. hispida* would affect physiological characteristics of mustard with regards to allelopathic traits in *D. hispida.*

Table 2. Allelopathic effects of *D. hispida* tuber water extract on maize and mustard at field bioassay.

Bioassay plant	Parameter	Control	T1	T4
Maize	Plant height	186.56 ± 2.18^{b}	196.61 ± 3.73^{a}	-
	total biomass weight (g)	1596.00 ± 91.13^{b}	1999.28 ± 139.57^{a}	-
	kernel number	4.00 ± 0.34^{b}	6.00 ± 0.45^{a}	-
	kernel weight	469.06 ± 45.34^{a}	577.33 ± 37.14^{a}	-
Mustard	total biomass weight (g)	93.94 ± 15.39^{b}	-	149.44 ± 15.30^{a}
	Number of leaf	24.61 ± 2.53^{b}	-	33.39 ± 2.51^{a}

Value were mean ± standard error.
Values bearing different letters in the same column were significantly different ($P \leq 0.05$) or ($P \leq 0.01$).

Conclusions

Bioassay test in laboratory showed different effects on the germination and growth of maize and mustard seeds according to irrigation treatments assigned to each species. Meanwhile, the highly significant

effect was shown by T1 and T4 for maize and mustard seeds, respectively, against distilled water. These laboratory results had been supported by allelopathic effects that had been carried out in the field. Then, there was significant difference obtained through mustard and maize growth which was deductible for treatment and control. Decisive test conducted at the field had dismantled allelopathic properties of *D. hispida*, in which aqueous extract of 12.5 g/L concentration of *Dioscorea hispida* tuber that got irrigated by one day interval (T1) and five day interval (T4) had shown a growth factor stimulation of maize and mustard, respectively. Possibility that growth regulator found in the aqueous extract of *D. hispida* can be commercialized if specific study of phytochemical compositions can be carried out. This was agreed upon by Einhellig and Leather (1988) who stated that the work of discovering a plant growth regulator is substantially more valuable, perhaps by a factor of 10-100 times, than for a new herbicide.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The Comparison Studies of Slope Stability between Natural and Planted Slope Coverage

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Introduction

Bioengineering has been known as an ecological strategy to enhance slope stability with regard to natural and man-made hazards. In this technique, vegetation will be used in maintaining the soil stability through the enhancement of mechanical and hydrological aspects of soil (Mitsch and Jørgensen, 2003). The potential advantages of this technique are effective and environmental-friendly in solving slope instability problems, particularly shallow landslides.

Thus, this study focuses on the effect of natural and planted vegetation cover on plant community changes and slope stability, in five treatments; i.e., low and medium natural vegetation cover, and also low and medium planted vegetation cover and bare soil as a control. This experiment is also aimed to investigate the correlations between slope stability with different mechanical and hydrological factors; i.e. soil water content (SWC), soil shear strength and leaf area index (LAI).

Materials and methods

Experimental design

Study site has been chosen along the Guthrie Corridor Expressway, Selangor for the natural and man-made slope. The experimental set-up consisted of five treatments with three replications respectively with 5.0 m x 5.0 m size, covering relatively well the heterogeneity in the vegetation. This plot size is commonly for vegetation descriptions in similar habitats (Prach and Pyˇsek, 2001). Five treatment plots were studied: low (10N) and medium (50N) natural vegetation cover and also low (10MM) and medium planted vegetation cover (50MM) and bare soil as a control. The plant species that were planted on the slope is tabulated (Table 1).

Table 1: Species that have been established at low and medium vegetation cover

Low vegetation cover (10%)	Medium vegetation cover (50%)
Axonopus compressus	*Axonopus compressus*
Melastoma malabathricum	*Melastoma malabathricum*
Portulaca grandiflora	*Portulaca grandiflora*
	Lantana camara
	Chrysopogon zizanioides
	Ixora coccinea

In natural vegetation cover plots, three species (*Brachiaria paspoloides, Melastoma malabathricum and Nephrolepis biserrata*) in low vegetation density plots and six species (*Axonopus compressus, Melastoma malabathricum, Ageratum conyzoide, Imperata cylindrical, Lycopodium squarrosum* and *Brachiaria paspaloides*) were observed.

Transplanting

The transplanting of selected species in planted slope coverage plots was conducted using a Microclimate Plant propagation Technique with a modified soil depth (Normaniza and Barakbah, 2011). In order to establish a condusive environment for the plants to grow, plant supplements such as NPK fertilizer (15 g/hole), sphagnum moss (15 g/hole) and rock-phosphate (15 g/hole) were applied at the beginning of the experiment.

Species diversity, soil water profiles, shear strength, leaf area index (lai) and erosion rate

Species diversity and frequency were observed randomly by throwing the steel quadrates (1 m x 1 m) diagonally across each plot in three random subsamples through time (Normaniza and Barakbah, 2011). The species diversity was observed every month, throughout six months of observation. In terms of soil erosion rate, The Gerlach sampling field method (Bochet and Garc´ıa-Fayos, 2004) has been used for runoff and sediment losses collection. Soil water profiles include soil water content (SWC), soil field capacity (SFC) and soil saturation level were also determined in six months. The soil samples were sampled using cylindrical soil cores (11 cm in diameter; 100 cm depth) using a soil coring machine (Eijelkamp Agrisearch Equipment, Model Cobra, The Netherlands) at three replications per treatment. The soil samples from the two locations were oven dried at 85 ^{0}C to a constant weight. SWC was calculated as follows :[(fresh weight – dry weight)/ fresh weight] X 100%. Whilst, SFC was determined by pouring excess water into a container filled with soil so that the soil becomes supersaturated. The excess water was drained out through small holes at the bottom of the container. Once the water stopped dripping, the saturated soil were weighed (SW) and dried in the oven at 85 ^{0}C to obtain a constant weight (DW). The saturation level of the soil was calculated as SWC /SFC x 100%.

Shear strength was recorded using the field inspection vane tester (Eijkelkamp Agrisearch Equipment, model 14.05, The Netherlands), which can provide values ranging from 0 to 260 kPa. To measure the soil shear strength, the readings were taken manually at 10 cm and 30 cm of soil depth for each treatment. These measurements were taken every month, for six months of experimental periods. Lastly, the Leaf Area Index (LAI) was measured using a leaf area meter (AccuPAR-LP80, UK) monthly, throughout six months of observation.

Results and Discussion

Plant community changes

In six months, species diversity has been increased in planted slope coverage, either in low or medium vegetation cover plots. In planted medium vegetation cover, the plant diversity has been slowly increased about 33%, comprising shrubs and grasses and 33% of plant diversity increment has been observed from low vegetation cover plots (Figure 1). The result implies that in a case where there is cultivation such as the human induced vegetation, a number of species is expected to share same opportunity for colonization. For example, in the planted slope coverage, the faster establishment of trees will increase the growth rate of other species and is likely to speed up regrowth towards a stable structural stage (Morana *et al.,* 2000).

However, there is no increment of plant diversity recorded in both low and medium vegetation cover of natural vegetation plots. The result may reflect the invaded species that consequently dominate the environment and form self-perpetuating ecosystem. The species, like ferns, may also exhibit allelopatic characteristics which do not allow the regeneration of natural forest (Shono et al., 2007). In this case, a vast expanse of *Dicranopteris linearis* ferns has been observed throughout the study period. In terms of LAI (Figure 2), all treatments have shown an improvement of LAI value throughout six months of

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

observation. The figure also showed that natural 50% slope coverage has recorded the highest value of LAI compared to the other treatments which were 44.16%, 77.16% and 67.01% higher than treatment 10% natural slope coverage, 10% planted coverage, and 50% planted coverage, respectively. This high value was presumably contributed by the thick coverage established from *Dicranopteris linearis* at the site.

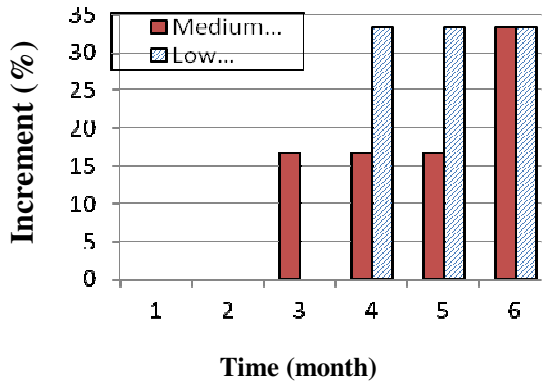

Figure 1. Percentage of diversity increment in low and medium vegetation density of planted slope coverage.

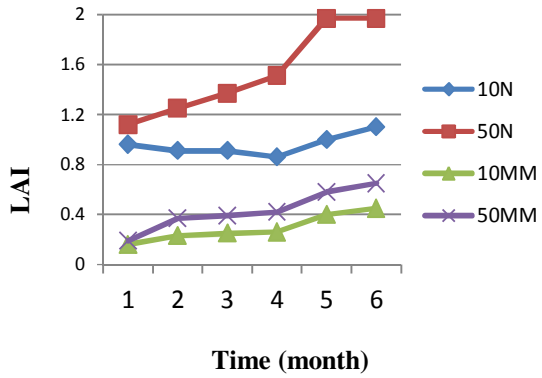

Figure 2: LAI values for each treatment

The erosion rate was found to be negatively correlated with the shear strength (r = -0.83). This implied that as the shear strength value increased, the rate of soil erosion decreased. As mentioned before, the shearing resistance was mainly contributed by the roots of the vegetation. In this study, the presence of shrubs, legumes and trees have contibuted to the increasing of the soil shear strength value as a result from extensive root system below the ground. These various plant sizes and density can play significant role in stabilizing slope stability, particularly on mechanical factors. On the other hand, the erosion rate had showed a positive trend with soil water content (r = 0.82). In this case, as a result from the influx of new species observed on the slope area, soil water was gradually diminished due to the increase in vegetation density and complexity. In addition to that, a dense root system below the ground anticipated by the positive growth of leave coverage above the ground could help to reduce the soil water through the soil-plant-atmosphere continuum (SPAC), which consequently can avoid the super-saturated condition of the slope (Egeli and Pulat, 2011).

In contrast, the erosion rate was found to have negative correlation with LAI (r = -0.73) (Figure 3). This value indicated that as the LAI value increases, the possibility for the erosion rate to be decreased is higher. This is possible as the leaf area increased; it will contribute to higher vegetation cover at the slope area. This high slope coverage will consequently lead to intercept the rainfall velocity that usually becomes a main cause of soil erosion especially in the slope area.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

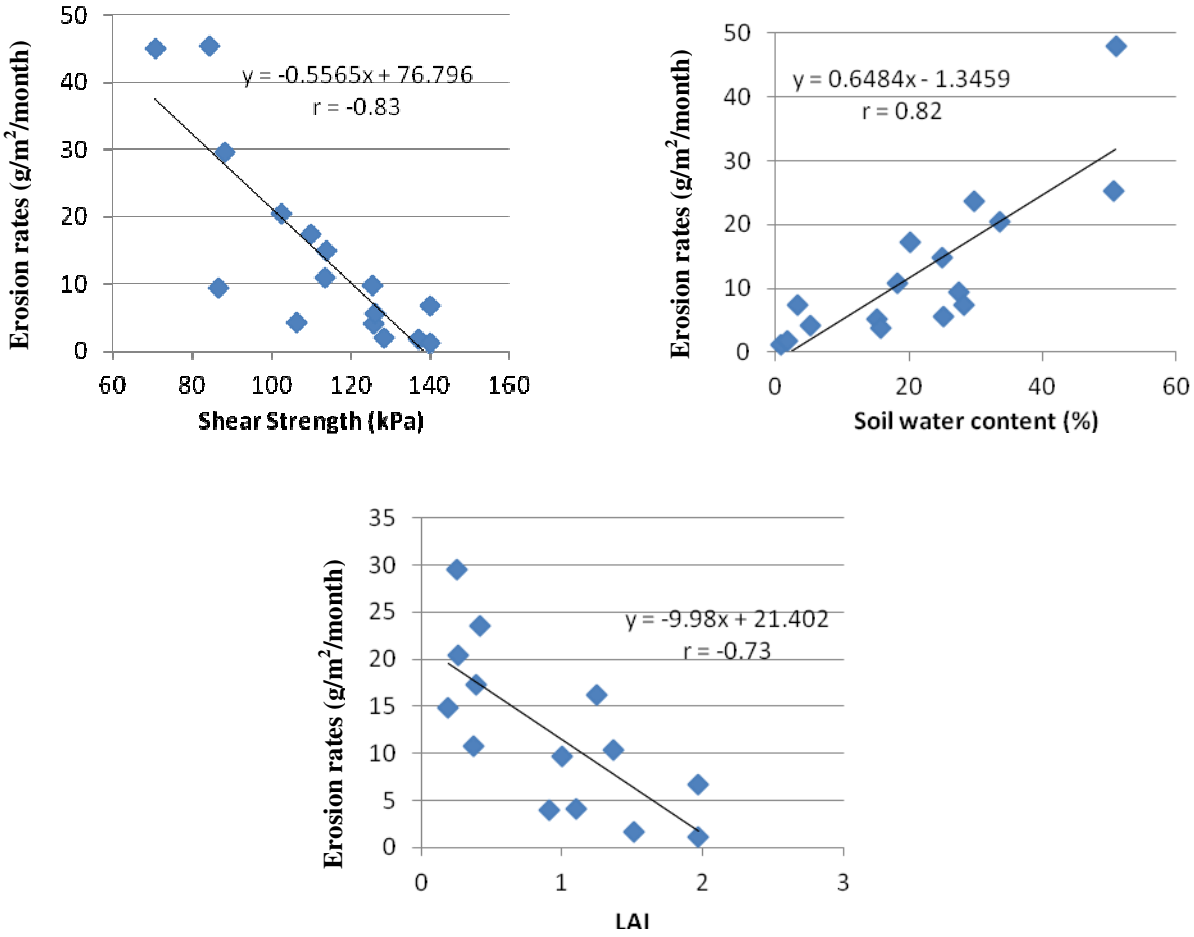

Figure. 3. Correlations between soil erosion rates and other factors

Conclusions

Within 6 months, the planted vegetation coverage has showed a higher plant communities growth; plant diversity and leaf area index. The enhancement of plant diversity (natural and planted) has reduced the soil saturation level and improves the soil shear strength. A strong positive correlation between soil water with erosion rates and negative correlations between shear strength and LAI with erosion rates have been observed in this experiment.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effects of Irrigation Frequency on Agro-Morphological and Physiological Parameters of *Zea mays* L.

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Introduction

Zea mays L. (maize) is originated from Mexico or Central America (Watson and Dallwitz, 1992) and brought to Malaysia in early 16th century (MARDI, 1992). It can be grown at temperature ranges between 5-45 °C but for the higher production yield, it must be grown at temperature between 30-35 °C and 10-15 °C during the sunny day and night, respectively (Department of Agriculture of Peninsular Malaysia, 1988). In addition, this crop also requires 500-800 mm of total water volume for optimum yield production (MARDI, 1992). However, on certain types of maize grown in certain areas, the maize requirement for water is different based on the rainfall distribution, plant size, soil texture and topography (Department of Agriculture of Peninsular of Malaysia, 1988).

The requirement of irrigation on maize is very crucial at certain growth stages. During flowering and seed formation, water application is critically very important but, during crop maturity, only low water application is needed. Flowering has been found to be the most sensitive stage to water deficit, with reductions in biomass, yield and harvest index (Rhoads and Bennet, 1990; Otegui et al., 1995; Pandey et al., 2000). Thus, this study aims to investigate the effects of irrigation frequency on agro-morphological and physiological parameters of *Z. mays*.

Materials and Methods

Two maize varieties (F1 Sweet Maize Hybrid and F1 Supersweet Maize Hybrid) from Thailand were used. F1 Sweet Maize Hybrid was produced by the Advanced Company, while F1 Supersweet Maize Hybrid was produced by the Leckat Corporation Sdn. Bhd. This experiment took place at the Research Plot of the Universiti Sultan Zainal Abidin, Gong Badak Campus, Kuala Terengganu, Terengganu, under a waterproof shade house which covers approximately 19.67 m^2 often closed surface area. Five irrigation treatments for each variety were set up by using Randomized Complete Block Design (RCBD) with four replicates. There were 40 plants planted and each plant was sown in a 40 cm diameter earthen pot that allows irrigation water to drain out form its bottom. The treatments were, irrigated once every two days, 500 mL per day (T1) as the control; once a day, 1000 mL per day (T2); two times a day, 2000 mL per day (T3); three times a day, 3000 mL per day (T4); and once in every three days, 333.33 mL per day (T5).

Oktem et al. (2003) had recommended that once every two days irrigation frequency, with 100% ET water application by a drip system, would be the optimal irrigation for sweet corn grown in semi-arid regions; thus that is why it became the control treatment in this study. The system used was a drip irrigation system that was connected to a power supply. The automatic timer was attached to the power supply, while each pump was controlled by a separate timer according to the specific watering frequency or irrigation time in a week which had been setup.

The parameters of agro-morphological (height and number of leaves of maize plants) and physiological (chlorophyll content and temperature of leaves) of the growth stages of maize were observed and

recorded. The height of maize plants was measured using measuring tape while number of leaves was recorded manually. Besides that, the chlorophyll content was measured using Chlorophyll Meter (SPAD-502 plus, Konica Minolta, Japan), while the leaf temperature was measured once in every week by using the Infrared Thermometer (62 Mini, Fluke, USA). The data for all parameters were recorded weekly and analysed by using SPSS 17 software, and subjected to One-Way and Two-way Analysis-of-Variance (ANOVA) at 0.05 level of significant.

Results and Discussion

Based on Figure 1a, for variety A, each treatment increased the plant height uniformly until week 6, and in treatments T3, T4, and T5 the increments were higher compared to other treatments that have only smaller increment. Lauer (2003) found that decreasing water supply during vegetative growth periods resulted in delays of development of stem and leaf cells thus reducing plant height and leaf area. As compared to variety B (Figure 1b) the increment of maize plant's height was slower except for T4. At week 6 (tasselling stage), T4 showed more increment than other treatments. Based on estimation for irrigation requirement of maize, each maize plant at T4 received 3000 mL per day of irrigation water. The frequencies yield adequate moisture which was suitable for maize growth. This result was in agreement with Elzubeir and Mohamed (2011), who found that the plant height was reduced as the irrigation intervals increased due to water stress which produces short plants.

The trends in number of leaves were presented to evaluate the effects of irrigation treatments throughout growth period in relation with the vegetative stages. Cakir (2004) found that a reduced root development has resulted from a stress maize plant during the vegetative stage, which can caused problem in deep water intakes. Based on Figures 2a and 2b, the trend in variety B showed more uniform as compared with variety A. In this study, the variation in irrigation treatments did not affect the number of leaves produced due to uniformly trends. Sulewaska (1990) stated that leaf area and leaf area index increase as the plant population increases.

In Figures 3a and 3b, both varieties showed the non-uniform trends. The chlorophyll content (CCI) was important during week 7, in which the maize plants were ready for fruit formation and the photosynthesis rate became critically important. At this stage, the plant should efficiently absorb nutrient from the soil especially nitrogen to produce more chlorophyll in the leaves in order to fasten the photosynthesis. The amount of CCI is also related with the amount of nitrogen applied. Furthermore, chlorophyll content of leaf is an indicator of photosynthetic capability of plant tissues (Wright et al., 2000; Nageswara et al., 2001). During tasselling stage, the CCI in all treatments tremendously increased, showing that the crops were ready to produce or form the cobs.

Based on Figure 4a and 4b, the trends showed by both varieties were nearly in similar pattern. The initial leaf temperature was higher but decreased in weeks 1 and 3 (vegetative stage). After that, they increased and became nearly flat in week 4, ear shoots were starting to form known as V10 stage. At this stage, water and nutrients applications were very scarce. Two weeks before tasselling stage (at week 6), the temperature should be at similar pattern to ensure that the photosynthesis will occur normally and not physiologically disturbed by leaf temperature. Besides that, Mebrahtu et al. (1991) mentioned that as leaf temperature exceed optimum values, the photosynthesis rate declined. They also stated that the internal factors involved were increased photorespiration rates and dark respiration, also decreased electron transport rates and photophosphorylation while the external factor was the diffusion of gaseous from leaf surfaces. Based on the observation, the leaves temperature in this study has not exceeded weekly recorded air temperature, thus ensured the photosynthesis rate of all maize plants was not affected by leaf temperature.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

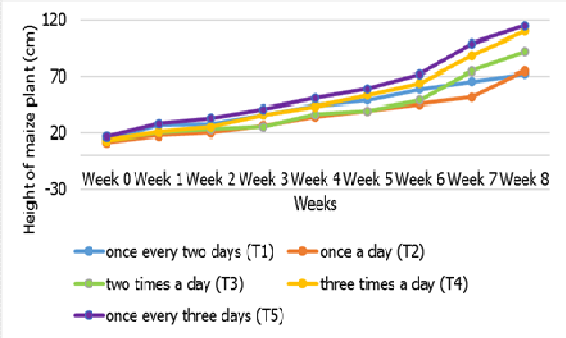

Figure 1a. Means of height of maize plant against weeks of varieties A.

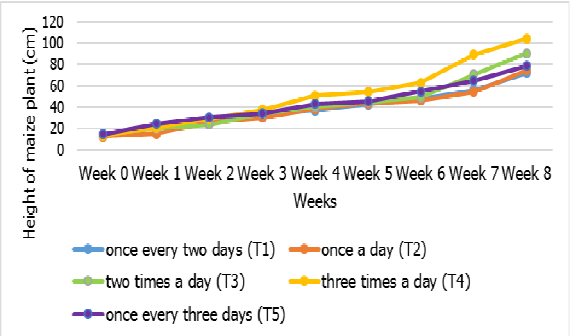

Figure 1b.Means of height of maize plant against weeks of varietiesB.

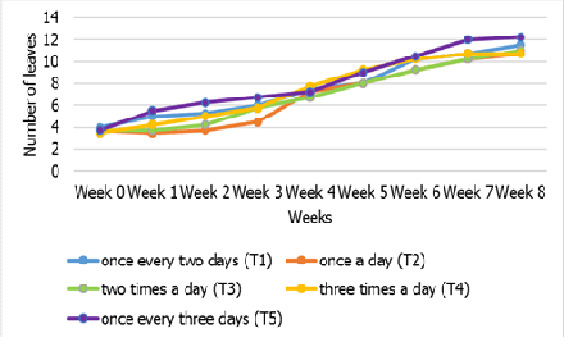

Figure 2a. Means of number of leaves against weeks of varieties A.

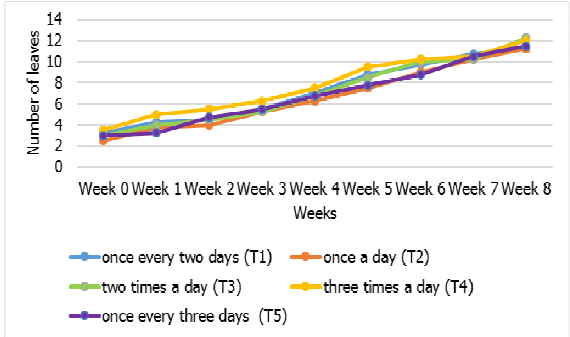

Figure 2b.Means of number of leaves against weeks of varieties B.

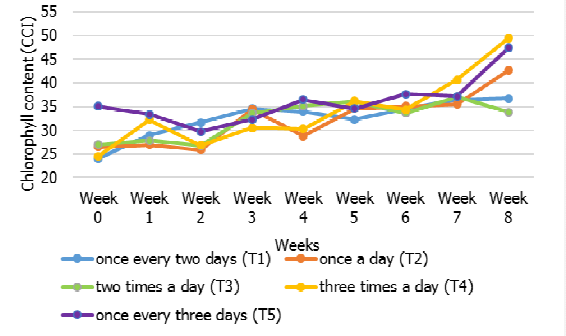

Figure 3a.Means of chlorophyll content against weeks of varieties A.

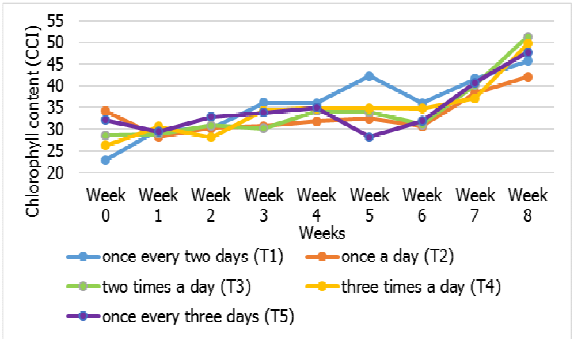

Figure 3b. Means of chlorophyll content against weeks of varieties B.

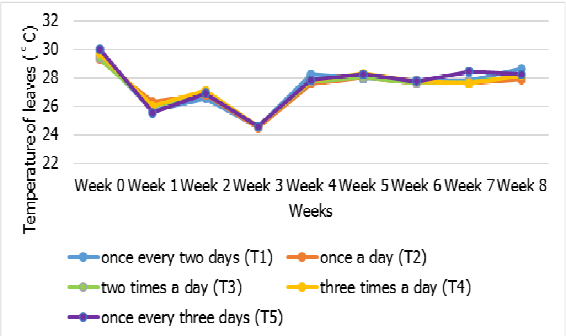

Figure 4a. Means of temperature of leaves against weeks of varieties A.

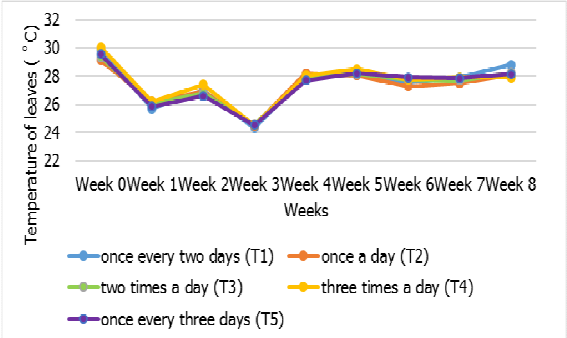

Figure 4b.Means of temperature of leaves against weeks of varieties B.

Conclusions

For varieties A and B, T5-treated plant showed the optimal growth for sweetcorn grown in Malaysia. This treatment showed better result not only compared with control (T1) but also with other treatments. Instead, by applying this treatment, water consumption can be saved from previous applications. On the other hand, different growth stages have different effects towards maize physiology and growth which can influence the potential yields. This was due to different requirements for irrigations at different growth stages.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The Impacts of Gibberellin and Indole-3 Butyric Acid on the Physiological, Mechanical and Chemical Properties of Root of *Leucaena leucocephala* and *Peltophorum pterocarpum*

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Introduction

Slope plant helps to stabilize the masses of soil via hydrological and mechanical means. The effects of vegetation on soil depend on the overall root physiology, chemical composition and its hydrological and mechanical functions. The cellulosic composition in root has been found highly resistant in tension and played important role for lodging resistance (Genet et al., 2011). Phytohormones (Gibberellin and Indole-3 Butyric Acid) are claimed to enhance cellulosic composition of roots (Xie et al., 2011). Additionally, phytohormones application on plants provides a tremendous potential in increasing plant growth, root initiation and plant adaptability (Sharifi and Hassan, 2010). A well adapted plant generally promoted the performance and functions of root systems such as water absorption, anchorage and soil-root interaction.

Plant roots increased soil shear strength by providing soil-root interaction and improving soil cohesion (Stokes et al., 2009). The fiber elongation mobilizes the tensile resistance in the roots. However, high cellulose content in root will enable the plant to keep attached in the soil and to prevent slippage (Genet et al., 2005). In addition, field and laboratory studies showed that plant roots especially fine roots efficiently penetrate the soil and improved soil-root interaction (Stokes et al., 2009). Thus, soil shear strength depends on the root profiles and the cellulosic composition of a single root. The specific aim of this experiment was to assess the impact of Gibberellin and Indole-3 Butyric Acid on physiological, mechanical and chemical properties of roots.

Materials and Methods

Plant materials and phytohormone treatment

In this study, *Leucaena leucocephala* (LL) and *Peltophorum pterocarpum* (PP) were tested against Gibberellin (GA_3) and Indole-3 Butyric Acid (IBA). Hormone of 20 mg L^{-1} of GA_3 and IBA were prepared individually. Homogenous seeds of LL and PP were dipped into 100 ml of GA_3 and IBA hormone mixture (mixed 1:1) and regarded as hormone treated. Whilst, those that seed dipped into 100 ml of distilled water were considered as control. Germination process was carried out in a growth chamber (temperature, 24 ± 2 ºC and light intensity 400 µE m^{-2} s^{-1}). The petri dishes were moistened daily with hormones and the process was continued for 15 days.

Plants grown in the wooden shear box

After 15 days, the seedlings were transferred into the customized (300 mm × 300 mm × 300 mm) wooden shear boxes. Slope soil (volume: 27,000 cm^3) was filled into the box and each seedling was grown at the center of the box. Seedlings treated in phytohormones were considered as treated plants and seedlings treated in water were considered as non-treated plants. Plant samples as well as the control (bare soil) were arranged in a completely randomized design (CRD) with three replications under

prevailing glasshouse conditions (temperature of 21 – 32 °C, average 12 h photoperiod, maximum PAR of 2100 µE m^{-2} s^{-1} and relative humidity of 60 - 90%). The plants were irrigated once every two days interval to avoid the water stress condition. After eight months, each plant was cut off near the base of plant stem and was preserved to measure biomass.

Laboratory shear box test

The soil shear strength was performed using a modified (300 mm × 300 mm × 150 mm) direct shear box machine. The normal stresses of 10, 20 and 30 kPa were applied and the cohesion factor and angle of friction were calculated from the Coulumb's equation: $\tau = \sigma \tan \theta + c$ (O'Loughlin, 1974).

Root Profiles

The root profiles were analyzed by using the WinRHIZO Pro Software at the end of experiment. This software was used to assess the total root length and root volume.

Root chemical analysis

Root holocellulose and alpha-cellulose content were measured by Wise et al. (1946) and Genet et al. (2005 and 2011) methods.

Statistical analysis

The two way ANOVA was applied to evaluate the significant difference among means. The significant ($p<0.05$) difference among means was compared using the Fisher's Least Significant Difference (LSD).

Results and Discussion

Physiological parameters of the species studied

Root profiles were differed significantly between species and treated and non-treated plants (Table 1). Treated *L. leucocephala* had a 57% higher root length than non-treated *L. leucocephala.* The root volume of treated *L. leucocephala* and *P. pterocarpum* were increased by 66 and 50%, respectively, than non-treated plants at 7.5 cm soil depth. Treated *L. leucocephala* showed the highest number of root tips followed by treated *P. pterocarpum,* non-treated *L. leucocephala* and non-treated *P. pterocarpum* plants. This result indicated that hormonal treatment was effective in promoting root length, volume and tips of species studied.

Table 1: Root profile of the species studied

Plant species	Treatments	Root length (cm)	Root volume (cm^3)	Root tips (No.)
L. leucocephala	Non-treated	1543 ± 37b	12.5 ± 0.4b	1449 ± 32c
	Treated	2434 ± 225a	20.8 ± 0.1a	2112 ± 53a
P. pterocarpum	Non-treated	926 ± 15c	7.9 ± 0.4c	918 ± 25d
	Treated	1389 ± 23b	11.9 ± 0.3b	1746 ± 28b

Means with different letters within the same column were significantly different ($p < 0.05$).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Mechanical characteristics (soil shear strength, cohesion factor and angle of friction) of plants

The shear strength of root permeated soils subjected to different normal load or pressure was affected by the hormonal treatments. The Table 2 showed that root permeated soils (both treated and non-treated) had a higher shear strength than control soil. Amongst the treatments, hormonal treated plants of the species studied exhibited the highest shear strength and residual strength at both soil depths. At 7.5 cm of soil depth with the normal pressure of 10 kPa, hormonal treatment increased the shear strength of *L. leucocephala* by 35% and 384% as compared to the non-treated *L. leucocephala* and control soil, respectively. Under 10 kPa normal load, the shear strength of treated *L. leucocephala* and *P. pterocarpum* were 60.6 and 54.9 kPa, respectively, at the 7.5 cm soil depth. Whereas, under 30 kPa of normal load, the shear strength of treated *L. leucocephala* and *P. pterocarpum* were increased by 26.8 and 24.7%, respectively, than the 10 kPa of normal load. Similar results were observed in non-treated and control soil. This was due to the presence of plant roots in which root-penetrated soils may not fail in stress. This result pointed out the fact that the increment of shear strength was mainly due to the increased root profiles where phytohormone was known to enhance the root system (Saifuddin et al., 2013).

Table 2: Shear strength and residual strength of plant species at different normal pressure and soil depth.

			Treated		Non-treated	
Species studied	Soil depth (cm)	Normal pressure (kPa)	Shear strength (kPa)	Residual strength (kPa)	Shear strength (kPa)	Residual strength (kPa)
Control	7.5	10	-	-	12.5	5.6
		20			21.5	10.5
		30			28.6	21.7
Control	22.5	10			11.2	5.1
		20			20.4	10.5
		30			28.1	19.6
L. leucocephala	7.5	10	60.6	23.7	44.6	18.4
		20	67.1	27.4	52.6	22
		30	76.9	33.3	59.7	28
	22.5	10	49.5	21	38.1	16.1
		20	53.2	25.3	40.4	18.5
		30	58.3	28.4	47.6	21.4
P. pterocarpum	7.5	10	54.9	18.2	35.1	14.6
		20	65.4	24	44.8	17
		30	68.5	27	56	20
	22.5	10	43.2	17	29.9	12.4
		20	51.8	21	34.3	14
		30	55.2	25	38.6	18

On the other hand, the results implied that root-penetrated soils had a higher cohesion factor than the root-free or control soils (Figure 1). Additionally, treated plants exhibited a higher cohesion factor than the non-treated plants. At 7.5 cm soil depth, the cohesion factor of treated *L. leucocephala* was enhanced by 62% than non-treated *L. leucocephala*. Similarly, at 7.5 cm soil depth, cohesion factor was enhanced by 102% in treated *P. pterocarpum* than those of non-treated *P. pterocarpum*. Among the root penetrated soil, the angle of friction was higher at 7.5 cm soil depth than 22.5 cm soil depth. For both species, angle of friction was lower in root penetrated soil (22.5 cm soil depth) than the control soil. This result implied that the presence of plant roots does not have positive effects on the angle of friction. Normaniza et al.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

(2008) documented that roots tend to increase the cohesive component while it did not affected on the angle of friction.

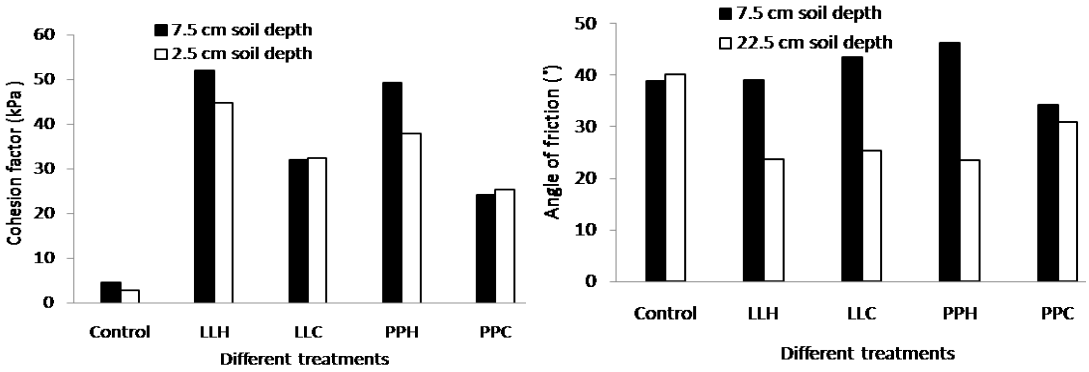

Figure. 1: Values of cohesion factor and angle of friction of plant species at 7.5 and 22.5 cm soil depth. Control: Bare soil; LLH: Treated *L. leucocephala*; LLC: Non-treated *L. leucocephala*; PPH: Treated *P. pterocarpum* and PPC: Non-treated *P. pterocarpum*.

Chemical composition of plant roots

The holocellulose and alpha-cellulose content of *L. leucocephala* was significantly higher than that of *P. pterocarpum*. Additionally, holocellulose and alpha-cellulose content of treated plants were significantly higher than non-treated plants (Table 3). A positive correlation (r = 0.86) was observed between root holocellulose content and soil shear strength of the species studied, implying that a high holocellulose content of roots improved soil shear strength (Figure 2). Likewise, root alpha-cellulose content and soil shear strength was positively correlated (r = 0.85), indicating that an increased in root alpha-cellulose content would increase soil shear strength. It has been reported that high cellulosic composition ***of roots*** improved the mechanical effects on soil, thus enhanced the shear strength of soil (Stokes et al., 2009). Genet et al. (2011) documented that the resistance of a root to failure in stress was controlled by root cellulosic composition. Thus, the roots of *L. leucocephala* have given a higher soil shear strength than *P. pterocarpum* when horizontal displacement or stress was applied on soil.

Table 3: Holocellulose, alpha-cellulose and lignin in plant root.

Treatments	Treatments	Holocellulose (%)	Alpha-cellulose (%)
L. leucocephala	Non-treated	70.6 ± 1.1b	44.5 ± 0.4b
	Treated	79.6 ± 0.5a	50.1 ± 0.5a
P. pterocarpum	Non-treated	56.7 ± 0.5d	31.3 ± 0.2d
	Treated	67.5 ± 0.1c	41.5 ± 0.3c

Means with different letters within the same column were significantly different ($p < 0.05$)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

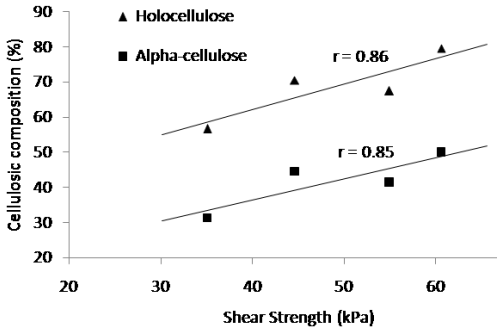

Fig. 2: Relationship between soil shear strength and root cellulosic composition.

Conclusions

In conclusion, phytohormone approach significantly enhanced root growth and improved the physiological, mechanical and chemical properties of root of the species studied which in turn, led to the enhancement of the soil-root reinforcement capacity of potential slope plants.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

CHAPTER 4

BEST PRACTICES AND CURRENT TECHNIQUES

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Propagation of *Musa acuminata* cv. Berangan by Using Quartering Technique

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Introduction

Musa acuminata is the largest herbaceous flowering plant. The plant grows in tropical and subtropical regions and is normally tall, up to 2-9 m in height. It is fairly sturdy and its main stem is pseudostem that grows from a corm. Each pseudostem can produce a single bunch of fruits and it will die after fruiting. *Musa acuminata* is of extraordinary significance to human societies. It produces the fourth most important food in the world today after rice, wheat and maize and is one of the main fruits in international trade (Zhang et al., 2005).

Musa acuminata has recently been commercially mass propagated by tissue culture. However, the natural propagation means using suckers or pieces of the rhizome must not be neglected and should continue to be studied for increased and uniform growth performance. There are three types of suckers, i.e. maidenhead, sword sucker, and water sucker. Sucker used for propagation is taken when it has diameter of 5 to 20 cm. The best sucker is young sucker emerged when the tree is about five to eight months after planting (Cristofori et al., 2010). Young new emerging plants from the parent plant are also traditionally dug up and planted to produce new plants.

Propagation of *M. acuminata* using quartering technique was attempted recently with occasional success. With this technique, the corm from young sucker is cut into four to six cuttings, depending on the size of the corm, and the cuttings are used to produce new plants. Auxins have commonly been used in enhancing rooting with cuttings (Ricci et al., 2001; Chatterjee et al., 2008; Tian et al., 2008; Guo et al., 2009; Mukta and Sreevalli, 2010; Balestri et al., 2012; Perilli et al., 2012). This study, hence, aimed to determine the effectiveness of several common auxins in producing new plants with cuttings of *M. acuminata* using quartering technique.

Materials and Methods

Test material

Corms of *M. acuminata* cv Berangan obtained from an orchard in Kuala Selangor in March 2012 were cut into cuttings and used as test materials. Corms of 6-10 cm with newly emerged suckers or sucker buds were chosen. For this study, corms from maidenheads or sword suckers were used as such corms are generally better in rooting capability. Each corm was cut into four to six cuttings depending on its size.

Location of study

Experimentation on propagation of *M. acuminata* using quartering technique was carried out at rain shelter of Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia.

Experimental procedures

Sand Medium Preparation

Sand sized 0.2 to 2.0 mm was used as medium to plant the corm cuttings. It was first sieved to remove the fine sand as it will cause insufficient aeration in the medium. The sand was washed with water until it became clear in colour to remove any dissolved materials in it as osmotic dissolved materials will affect rooting of the cuttings. Then, the cleaned sand was dried under direct sunlight until it was completely dry before spraying with insecticide and left again for another day under direct sunlight prior planting. This procedure was aimed to prevent the cuttings from being affected by ants or termites.

Preparation of Treatment Suspension

Benomyl and auxin suspensions were freshly prepared for this experiment. Auxins of indole-3-butyric acid (IBA), naphthalene-1-acedic acid (NAA) and indole-3-acetic acid (IAA) at concentration of 500 mg/L were used in this study for enhancing rooting with the cuttings. IBA, NAA and IAA suspensions were prepared by dissolving 0.25 g respective auxin in drops of 1N NaOH and then topped up to 500 mL with distilled water in conical flasks. Then, 0.25 g benomyl was added into each treatment suspension.

Preparation of Corm Cutting

All the corms were cleaned from all roots and dirts and the sucker buds were then removed from the corms. Then, cuttings of 4-5 cm in length were made from each corm by cutting the corm vertically. Sometimes, up to six cuttings could be obtained from a bigger corm depending on the corm size.

Treatment and Planting Procedure

The corm cuttings were treated with auxin suspensions respectively prior to planting in the sand medium. The cuttings were totally immersed in the treatment suspension for 30 minutes. The control cuttings were treated similarly with distilled water added with the same amount of benomyl. After 30 minutes, the cuttings were planted in sand at a depth of 5-6 cm in perforated plastic trays. Experimental area was covered with commercial shade netting to give only 30% relative light intensity (RLI) to the cuttings. Watering was carried out when necessary to keep the medium moist but not water logged at all time.

Data Collection and Statistical Analysis

Data on corm cuttings' rooting were collected weekly. The number of roots per cutting and length of individual root were also recorded weekly. This experiment was based on a Completely Randomize Design (CRD) with 10 replicates for each treatment. Descriptive statistics of the growth parameters were calculated.

Results and Discussion

According to Figure 1, control cuttings showed relatively higher rooting capability as compared to auxin treated cuttings. Among the auxin treatments studied, only IBA was effective for rooting of the cuttings. No rooting was observed with NAA and IAA treated cuttings. Lower concentrations of NAA and IAA may be attempted as rooting was recorded with distilled water treatment. Concentration of 500 mg/ L with IBA was acceptable for corm cuttings but this concentration of NAA or IAA was probably too high for the under study corm cuttings. Both control and IBA treated cuttings showed increased rooting

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

percentages at day 21 and no further rooting increment was observed thereafter. Control treatment had 40% rooting while those treated with 500 mg/L IBA had 30% rooting at 21 days after planting. Unrooted cuttings and those treated with IAA and NAA eventually died with failure of rooting.

Despite relatively lower rooting percentage, cuttings treated with IBA had higher mean root number as compared to control cuttings (Figures 1 and 2). For IBA treated cuttings, the mean number of roots at 7 days after planting was 4 and further increased to 7.33 at 21 days after treatment and showed no further new root emergence thereafter. For the control cuttings, there was a mean root number of 1.33 at 7 days after planting but only increased slight to 1.75 at 21 days after planting and also showed no further increment in this parameter thereafter.

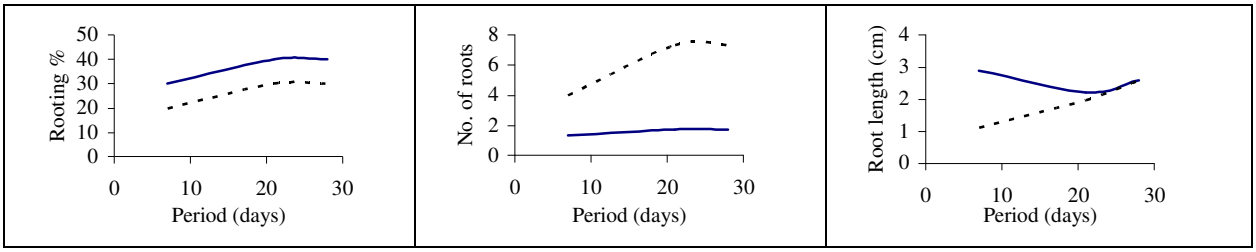

Figure 1. Rooting percentage (left), root number (centre) and length of roots (right) with control (—) and IBA treatment (···)

In spite of the higher mean number of roots with IBA treatment, the control cuttings showed longer roots initially (Figures 1 and 2). The mean root length was 2.9 cm at 7 days after planting with the controls while those treated with IBA was 1.13 cm. However, mean root length for IBA treatment increased with prolonged period and was 2.6 cm and comparable to that of the control cuttings at 28 days after planting. No adventitious shoots emerged with all rooted cuttings within the observation period of 28 days.

The results in this experiment show that *M. acuminata* could be rooted by quartering technique. Lower rooting percentage found in this study was attributed to contamination of the parent materials collected from the field. Corms collected from field may be hosts to some microorganisms such as fungi (Finér et al., 2011). Microorganisms will alter the physiology of the cuttings and result in rooting failure (Hacke and Sperry, 2001).

IBA was found effective in enhancing the development of adventitious roots with quartering technique in propagation of this fruit species. IAA or NAA alone at 500 mg/L was not recommended for rooting of the cuttings. Lower concentrations or combined auxins may be attempted for rooting of these cuttings in future studies (Lecompte and Pages, 2007). Environmental factors, e.g. surrounding temperature and relative humidity coupled with appropriate RLI must also be studied to enhance root development of these cuttings.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Figure 2. Rooted cuttings; control (top 3 figures) and IBA treatment (bottom 3 figures)

Adventitious shoot growth was not observed in the present study. It may be attributed to the subsequent nutrition status of rooted cuttings and hormonal changes within these cuttings. Proper growth of shoots is of equal importance to ensure the success of the quartering technique in rapid propagation of *M. acuminata*.

Conclusions

Rooting and growth of roots with *M. acuminata* corm cuttings obtained with quartering technique were different due to auxin treatment. The control cuttings and those treated with IBA showed only 30-40% rooting over a propagation period of 1 month. When compared to the controls, IBA treated cuttings eventually produced relatively more roots and longer roots. No adventitious shoot growth was observed in this study. Some other treatments, e.g. altering the nutrition status (e.g. application of fertilizer) or subsequent exogenous application of cytokinin, may be studied for enhancing growth of the shoots.

Acknowledgements

The authors would like to thank Faculty of Plantation and Agrotechnology, UiTM, for financial assistance and permission to use the rain shelter for this experimentation.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Enhancing Efficiency at the Distillation Process by Using Estimated Virtual Exhaustive (EVE) Technique

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Introduction

Distillation is the process of heating a liquid until it boils, then condensing and collecting the resultant of the hot vapours. The distillation word is taken from the Latin 'de-stillare' for 'drip or trickle down' giving mean that the separation of a liquid by evaporation and condensation. Mankind has applied the principles of distillation for thousands of years. Distillation was probably first used by ancient Arab chemists to isolate perfumes (Aboutabl et al., 1986). The current generations owe a great debt to the Arabian alchemist (and physician) Ibn Sina who is also known as Avicenna, who lived between 980-1037 AD, since he was the first alchemist to perfect steam distillation. His process was so good that it stayed unchanged for a couple of hundred years (Zaoui et al., 2002; Majeed, 2005).

Commercially, distillation has a number of applications. It is used to separate crude oil into more fractions for specific uses such as transport, power generation and heating (Jarullah et al., 2011). Water is distilled to remove impurities, such as salt from seawater (Zhang et al., 2012). Air is distilled to separate its components- notably oxygen, nitrogen, and argon for industrial use (Gao et al., 2011). Production of essential oil extracted from selected crops will give concentrated hydrophobic liquid containing volatile aromatic compounds from plants like Citronella *(Cymbopogon nardus)* and Lemongrass (*Cymbopogon citratus*). In the industries, the oil from Citronella (Figure 1) and Lemongrass (Figure 2) are used for making toiletries and repellents (Khalid and Kiong, 2010; Matasyoh et al., 2011; Wei and Wee, 2013).

Figure 1. Citronella

Figure 2. Lemongrass

In the modern technique of extraction, essential oils are generally extracted by distillation, often by using steam. They are used in perfumes, cosmetics, soaps and other products, for flavouring food and drink, and for adding scents to incense and household cleaning products (Sallehhudin et al., 2012). However, there is no established technique to determine the commercial end-point of the distillation process.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Thus, the objective of the study is to determine the estimated virtual exhaustion (EVE) time of distillation for selected crops (*Cymbopogon nardus* and *Cymbopogon citratus*).

Materials and Methods

Plants were planted at the MARDI Kuala Linggi Station which is located at the border of Negeri Sembilan-Melaka state of Malaysia. Citronella (*Cymbopogon nardus*) and Lemongrass (*Cymbopogon citratus*) planted (maturity at eight months old) were harvested at 50 kg, respectively. The crop was chopped using chipping machine (7 Horse Power) Model SFC 1840 supplied by Mareqsue Sdn. Bhd. The chopped crop was loaded into the distiller vessel of fabricated distiller and closed tightly (Figure 3).

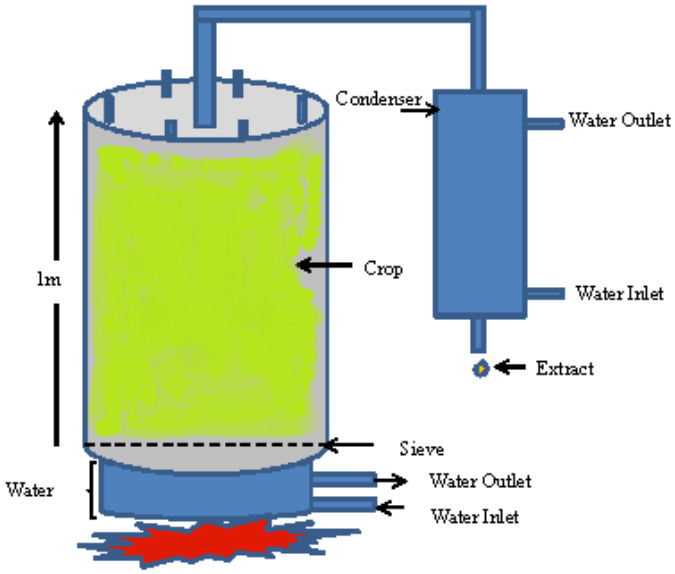

Figure 3. Fabricated distiller

The burner was fired and the time between the fire started and the first drop of the extract was recorded. Each of every six minutes, the extracts were collected for a minute and allowed to separate, by forming a layer of oil-water interphase. The volume of water and oil was recorded, respectively. The process was continuously performed until a constant reading of oil (±0.1 mL) was recorded. The recorded data was analysed *via* simple mathematical equations.

Results and Discussion

The equation below was used to determine the progress of oil volume at respective time.

$$v_n = \frac{(x_{to} + (x_{to} + \Delta t))}{2}(\Delta t - 1)$$

$$v_{n+1} = \frac{(x_{to} + (x_{to} + \Delta t))}{2}(\Delta t - 1) + v_n; \quad n = 6,12,18, \ldots\ldots\ldots$$

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Where,
V = progress volume of oil at respective time.
X_{t0} = volume of oil collected at t_0
x_t = volume of oil collected at different time (minutes)
Δt = time interval (minutes)

From the results obtained after analysing the datasheet, the graph of oil volume progress versus distillation time was plotted for each crop (Figures 4 and 5).

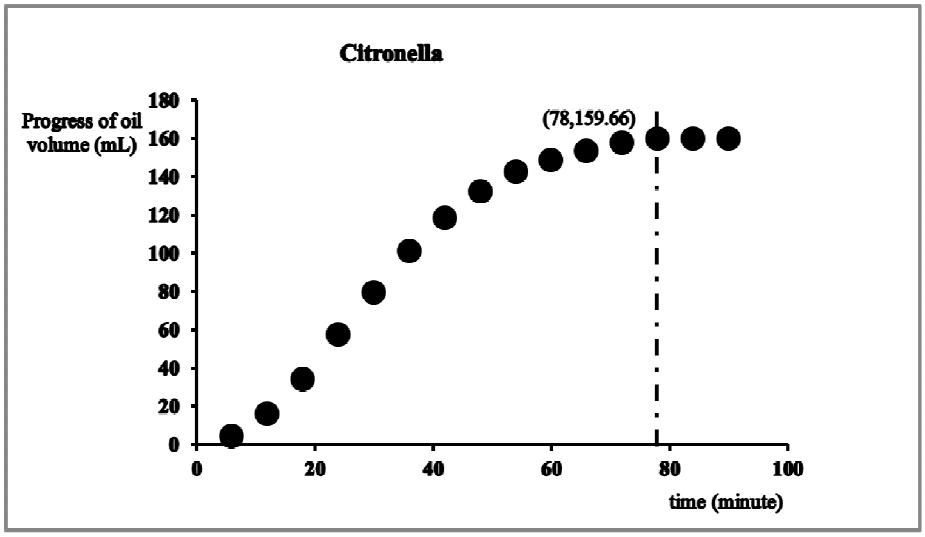

Figure 4. Progress of oil volume at different distillation time for Citronella

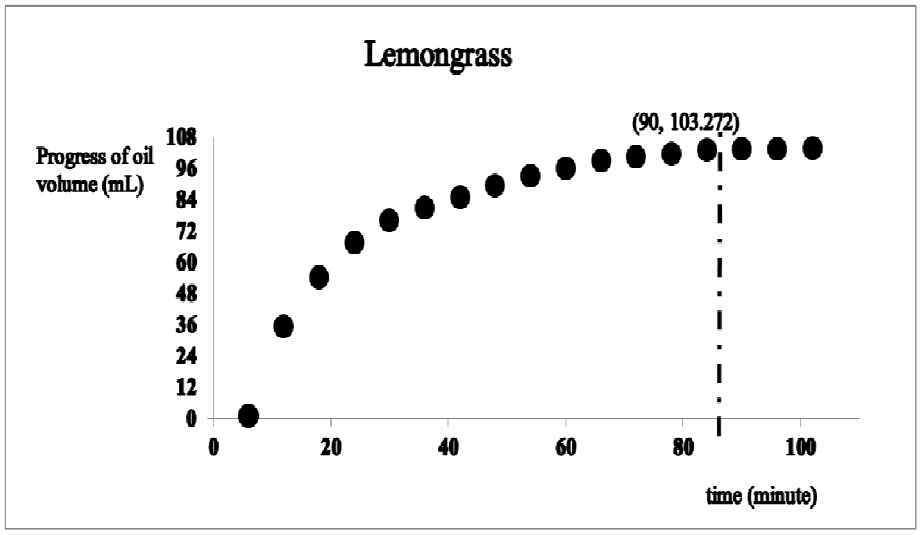

Figure 5. Progress of oil volume at different distillation time for Lemongrass

The graphs plotted showed the commercial end point for each crop. It was found that the EVE for Citronella is 78 minutes and Lemongrass is 90 minutes.

Conclusion

The technique is extremely useful for reducing operational cost where virtual exhaustive points are determined and applicable for distilling all types of crop.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Physico-Chemical Properties of Different Parts of Roselle (*Hibiscus sabdariffa* var. UKMR-2) Plant after Drying and Oxidation Processes

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Introduction

Roselle *(Hibiscus sabdariffa L)* is one of the exploited food crops with nutritional and food industry processing potential. Utilization of roselle as food products is still considered new. Roselle is locally known as 'asam susur', 'asam paya', and 'asam kumbang' (Wong et al., 2003). This crop was processed to be used as healthy juice due to its high contents of vitamin C and anthocyanins that was found in the calyces. The abundant pigments in roselle are responsible for the red color and are the main source of its antioxidant capacity (Tsai et al., 2002). There is still lack of utilization and no study was done on the other parts of roselle plant. In this study, the potential of roselle has been studied so that new food products in dry form can be produced from this plant.

According to Mohd Esa (2010), the young leaves and tender stem are eaten raw in salads and chutney which are also added to curries and some Malaysian dishes as seasoning. However, the drying process may result in some changes in roselle quality and nutritional properties. The drying requirement for locally grown roselle has not been fully investigated. This study was aimed to determine the physico-chemical properties and sensory acceptability of calyces, leaves, and shoots of roselle variety UKMR-2 plant after drying with and without oxidation processes. Up until now, most of the scientific study has only focused on the calyces of roselle and also no study done on the usage of different parts of the plant like shoots and leaves to be made into drinks.

Materials and Methods

The calyces, shoots and leaves of UKMR-2 roselle were used as raw materials. The harvested calyces were decored first then all the samples were washed with distilled water to remove dirt. All the plant parts were then divided into 2 parts. The first part was drying after oxidation process where all the plant parts were ground in rock grinder for 1 minute. Then, the ground samples were then left at room temperature (27 ± 2 ^{o}C) for 2 hours (for oxidation process). The oxidized samples were then dried in an oven at 100 ^{o}C for 20 minutes. Another part was drying without oxidation where the washed calyces, leaves and shoots were straight away dried in the oven at 100 ^{o}C for 2 hours until the moisture content was less than 3%. Then the dried samples were ground and ready for analyses.

Color measurements were measured using Minolta Chromameter CIE Lab. The infusion color vs time was measured every 30 seconds intervals until 2.0 minutes. Total anthocyanins content of roselle extract was determined colorimetrically according to the procedure described by Du and Francis (1973) where the absorbance of the mixture was measured at 525 nm and 700 nm. The concentration of the anthocyanin pigments is calculated based on Beer- Lambert Law using delphinidin 3-glucoside as the main anthocyanin pigment. Ascorbic acid content in all the samples were then determined using method by Jagota and Dani (1982). A standard curve was prepared using various concentration of standard ascorbic acid (0-60 µg/ml) and absorbance was measured at 760 nm.

The determination of pH value was measured using a pH meter that was calibrated using pH buffer solutions of 4.0 and 7.0 according to the method of (Association of Official Analytical Chemists, 2000). Total phenolic content of dried roselle samples were determined based on current protocols in Food Analytical Chemistry (2001) using a Folin-Ciocalteu method using gallic acid as the standard, and absorbance measured at 765 nm, expressed as gallic acid equivalents in mg/100 g dried samples.

The dried samples of different parts of roselle plant after drying with and without oxidation process were made into drinks and served to 30 panelists who were randomly selected from students and staffs of Universiti Malaysia Terengganu for sensory evaluation. The panelists' acceptability and preferences were determined for the attributes of color, aroma, taste, after taste and overall acceptability. The panelist evaluated the samples based on their hedonic score of 1 to 7 for every attribute which indicated very much unacceptable and very much acceptable, respectively.

All analyses were done in triplicate and were statistically analyzed using 2-way ANOVA at 5% level of significance which was determined by Tukey test using SAS 9.3V.

Results and Discussion

Color: The L*, a* and b* values was observed to be higher in the directly dried samples for all parts except calyx which showed significant ($p<0.05$) lower in lightness as compared to oxidized dried calyces. However, there were significant differences ($p<0.05$) in L*, a* and b* values among all the samples where directly dried shoot showed significantly ($p<0.05$) the highest in L* and b* values. Meanwhile calyx for both directly dried and oxidized dried showed significantly ($p<0.05$) the highest a* value due to its red color. According to (Al-Kahtani and Hassan, 1990), roselle calyces contained natural constituents of organic acid such as malic acid, citric and 3-indlyl acetic acids which played an important role in giving brilliant red color of the sample.

Infusion Color vs Time: Statistically there were significant differences ($p<0.05$) in b* values for infusion color vs. time among all the roselle parts, where extract from calyx showed significant ($p<0.05$) decrease in yellow color after 30 seconds infusion time while leaf and shoot showed increasing in the intensity of yellow color. Basically, for color L*, a* and b*, calyx just needs 30 seconds to fully infuse. The polar character of anthocyanins makes them soluble in several types of polar solvents such as water, methanol, ethanol and acetone (Abou-Arab et al., 2011).

Anthocyanins: The anthocyanins values showed significant differences ($p<0.05$) among all the samples. The calyx showed significantly ($p<0.05$) the highest value in anthocyanins content, while dried shoot showed significantly ($p<0.05$) the lowest for both drying processes. Comparing between two drying processes, the directly dried roselle parts showed significant ($p<0.05$) higher value than its oxidized dried samples except leaf. Degradation rate of anthocyanins increases with increasing solid content during heating. Oxygen and heat have been reported as the most important factors affecting the destruction of anthocyanins (Jackman and Smith, 1996).

Ascorbic Acid: There were significant differences ($p<0.05$) in ascorbic acid content among all the three parts where dried calyx showed significantly ($p<0.05$) the highest value compared to dried leaf and shoot. Dried calyx contained the most significant value of the ascorbic acid content for both drying process as these results were consistent with the fact that calyces are rich in vitamin C, anthocyanin and malate (Mohd Nazil, 2011). Nevertheless, among all the samples, the directly dried samples seem to show higher value than the oxidized dried samples. When oxidized, ascorbic acid does tend to be destroyed as it involves transfer of an electron to form the free radicals (Grocery, 1996). Grocery (1996)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

has reported that the heat and light can also accelerate the process, while factors such as pH, oxygen concentration, and water activity strongly influence the rate of reaction.

pH: There were significant differences ($p<0.05$) in pH values between the roselle parts and the drying methods. Dried leaf showed significantly ($p<0.05$) the highest pH values followed by dried shoots where dried calyx gave the lowest values. Oxidized dried samples were higher pH value than directly dried samples which showed significantly ($p<0.05$) less acidic. It is well known that roselle is characterized by its sour taste. pH value is a very important factor affecting the extraction of anthocyanins indicating that at lower pH value, anthocyanins yields was the highest (Abou-Arab et al., 2011).

Total Phenolic Content: It was observed that total phenolic value showed significant differences ($p<0.05$) between roselle parts. Dried calyx showed significantly ($p<0.05$) the highest total phenolic content, followed by dried shoots and then dried leaf. Since phenolic compounds are antioxidants, they are subject to oxidation during storage and processing of foods (Rickman et al., 2007).

Sensory Acceptability: There were significant differences ($p<0.05$) among all the dried roselle parts. Tea (extract) made from dried calyx appears to be the most acceptable in all the attributes of aroma, color, taste, after taste and overall acceptability based on the responses from 30 untrained panelists from UMT. Oxidized samples gave higher acceptability score as compared to directly dried samples especially for oxidized calyx. Based on this result, it is clearly shown that color becomes the most important quality attributes which affect people's acceptance since it gives the first impression of the food quality. Roselle calyces appear to be good and promising sources of water soluble red colorants that could be utilized as natural food colorants (Abou-Arab et al., 2011).

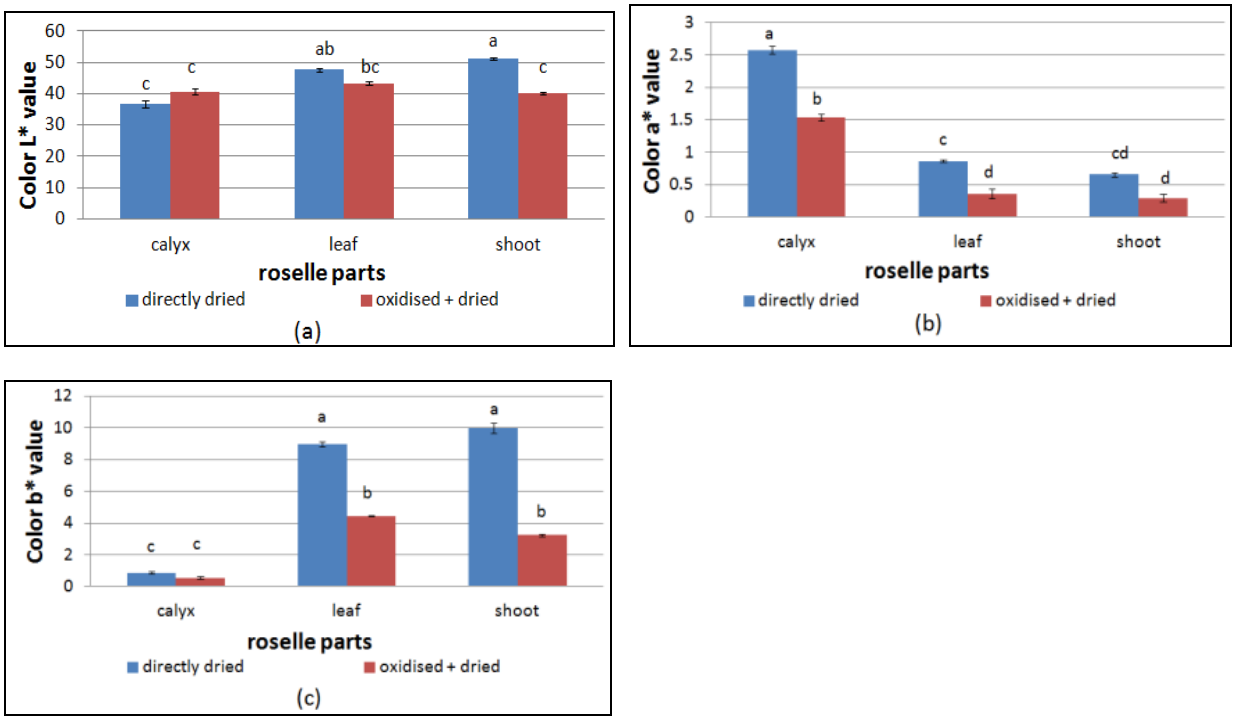

Figure 1: The color of directly dried and oxidized dried roselle parts. (a) L* value; (b) a* value; (c) b* value.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

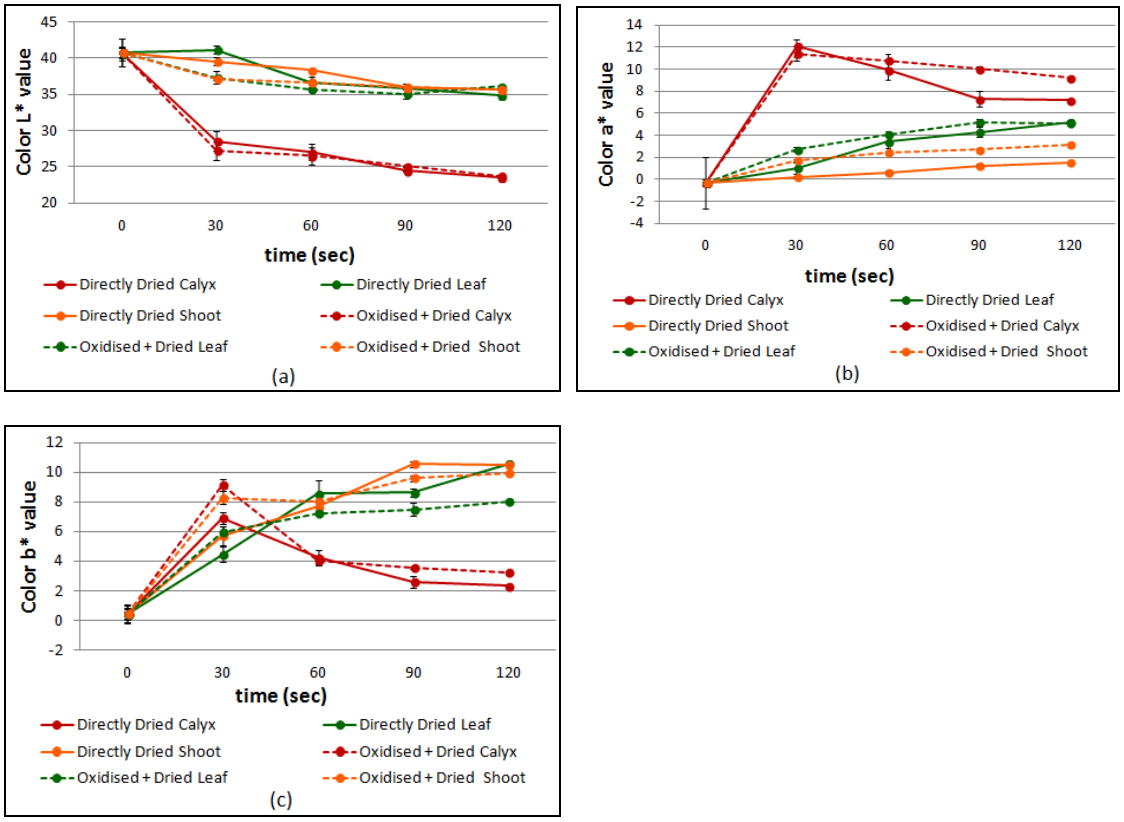

Figure 2: The infusion color vs time of directly dried and oxidized dried roselle parts. (a) L* value; (b) a* value; (c) b* value.

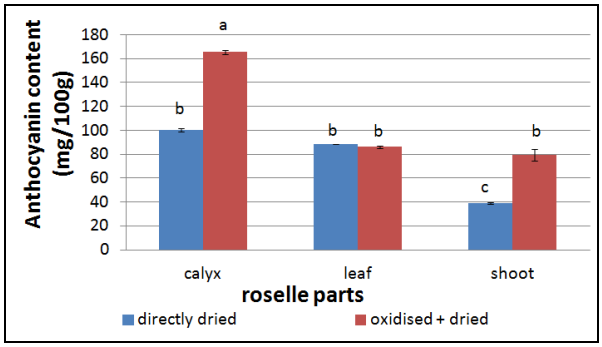

Figure 3: The anthocyanins content of both directly dried and oxidized dried roselle parts

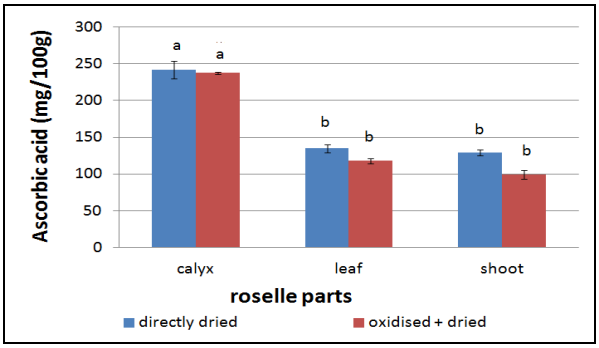

Figure 4: The ascorbic acid content of directly dried and oxidized dried roselle

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

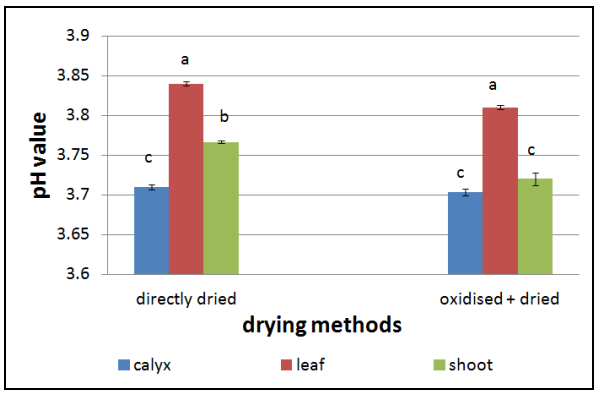

Figure 5: The pH value of both directly dried and oxidized dried roselle parts

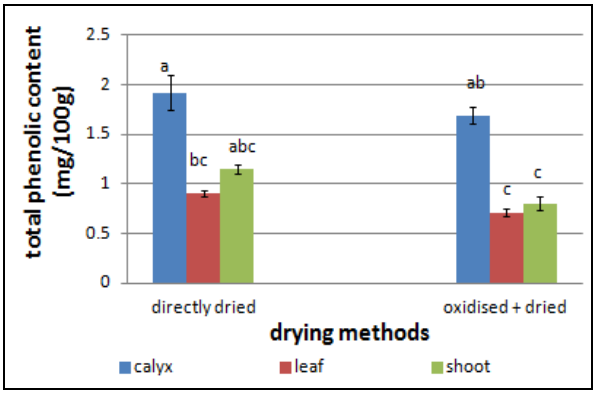

Figure 6: The total phenolic content of directly dried and oxidized dried roselle parts

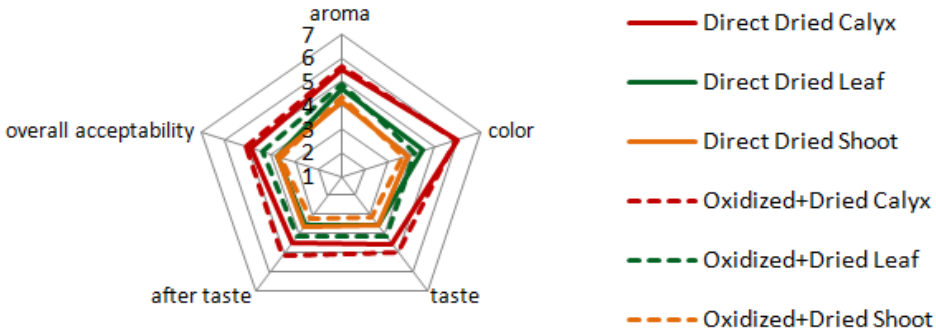

Figure 7: The sensory acceptability of roselle drinks made from directly dried and oxidized dried roselle parts

Conclusions

Among different roselle parts, dried calyx had better physico-chemical properties compared to the dried leaf and shoot where dried calyx contained high anthocyanin, ascorbic acid and total phenolic contents which contributed to the attractive red color. When comparing between directly dried and oxidized dried processes, directly dried samples showed higher values in most of physico-chemical characteristics especially ascorbic acid, pH and total phenolic content. However, anthocyanin was higher in oxidized dried samples. From the sensory evaluation, it can be concluded that the most acceptable roselle drinks was from oxidized dried calyx as it obtained significant higher acceptability score in all the attributes. Meanwhile, directly dried and oxidized dried shoot were considered not acceptable for roselle drink.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Morphological Description for Kunyit Hitam (*Kaempferia parviflora)* and Breaking Bud Dormancy with BAP and Ethephon Treatments

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Introduction

Kaempferia commonly known as peacock gingers are less notable members of the Zingeberaceae family with a number of 60 species distributed from India to Southeast Asia (Sirirugsa, 1992; Larsen, 2006). Some *Kaempferia* spp. found in Malaysia include *K. galanga, K. pulchra, K. elegans, K. parviflora, K. angustifolia.* These low growing, understory plants have short fleshy rhizomes, tuberous roots, leaves erect or apprised to the soil and inconspicuous flowers usually white or violet (Kiew, 1980; Kuehny *et al.,* 2002). Its functional uses include ornamental gingers, food source and medicinal use. Among the *Kaempferia* species*, K. parviflora* has a high medicinal value. Recognition of *K. parviflora* rhizome's high potential for the development of various health products has prompted various pharmacological researches (Chivapat *et al.,* 2010). Despite the tremendous demand for the rhizomes of *K. parviflora*, there is a scarcity of its planting materials in Malaysia. This is due to sluggish natural regeneration of *K. parviflora* through rhizome and a long dormancy period of almost 6 months. Sporadic sprouting of rhizomes also effect cultivation and rhizome production. Based on the problems discussed and prospects, the present study aims to describe the morphological features of native *K. parviflora* and break the bud dormancy using BAP and Ethephon treatments at various concentration.

Materials and Methods

Morphological description was carried out by evaluating 24 qualitative and quantitative parameters of *K. parviflora* collected from Taman Herba UPM in 3 replications. The bud dormancy study was carried out in two separate experiments to study the effects of BAP and Ethephon in breaking bud dormancy of *K. parviflora.* In experiment A, six different levels of BAP at 0, 50, 100, 150, 200 and 250 mg/L applied, while in experiment B, six different levels of Ethephon concentrations at 0, 150, 300, 450, 600 and 750 mg/L were used. Both BAP and Ethephon were applied by soaking the rhizomes into the growth regulators for 30 minutes. The rhizomes were placed on moistened cotton in flasks under 25±2 °C and 16 hours daily illumination with 1,000 lux of white fluorescent light for 24 days. Data on days to visible bud, percentage of bud sprouts, shoot length and rhizome weight gain was collected. The data was analyzed by Statistic Analysis Software (SAS) version 9.1 and means were compared by Duncan's Multiple Range Test (DMRT).

Results and Discussion

A number of 24 qualitative and quantitative morphological traits were observed and documented from *K. parviflora.* Preliminary observation shows leaf morphology and rhizome characteristics were distinct traits that can be used for future identification. *Kaempferia parviflora* can be described as follows;

- *Kaempferia parviflora* is a herb, 30-40 cm tall. Its leaves are 1 to several; blades ovate or oblong, slightly unequal sided, apex acute, base subcordate, adaxial surface yellow green, abaxial surface green, petiole 17 x 0.5 cm, leaf scales 7 cm long, margin undulated and red tinted. The rhizome is subglobose with several succulent roots in a fascicle. Its interior flesh is purple with an exterior of brownish skin. Its inflorescence is enclosed by two innermost leaf-sheaths.

In studying the effects of BAP and Ethephon on breaking bud dormancy of *K. parviflora*, BAP gave a better performance in comparison to Ethephon. In the first experiment, as the concentration of BAP increased, the number of eye bud breaks reduced except for BAP treatment at 150 mg L^{-1} which had a significant effect on number of days to visible bud. BAP at 150 mg mg L^{-1} gave the earliest time for visible bud appearance as compared to control and other concentrations. There were significant differences for number of shoots among treatments. However no significant differences were observed among treatments for shoot length and rhizome weight gain (Table 1).

Table 1. Effects of BAP on days to visible bud, percentage of bud sprouts (%), shoot length (cm) and rhizome weight gain (g)

Treatment (mg L^{M})	Days to visible bud	Number of buds	Number of shoots	Shoot length (cm)	Rhizome weight (g)
0	23.0 a	0.25 b	0.00 b	0.00 a	0.00 a
50	8.0 b	1.25 b	1.00 ab	1.70 a	1.70 a
100	11.0 b	1.00 b	0.25 b	0.80 a	0.80 a
150	5.0 b	2.75 a	1.75 a	1.70 a	1.70 a
200	12.0 b	1.00 b	0.50 b	1.75 a	1.75 a
250	15.0 ab	0.25 b	0.25 b	0.68 a	0.68 a

Means with similar letter are not significantly different at P<0.05

In the second experiment, the number of eye bud emergence also reduced as the concentration of Ethephon increased. There were significant effects for days to visible buds and shoot length (cm). Ethephon at 150 mg L^{-1} gave the most number of eye buds and longest shoot length (cm) while Ethephon at 300 gave the earliest time to visible eye buds (Table 2).

Previous studies show that BAP at a concentration of 100 mg L^{-1} was successful in breaking bud dormancy in *Curcuma cordata* and *Curcuma alismatifolia* (Arimuna *et al.,* 2000; Thohirah *et al.,* 2010). Futurani and Nagao (1986) reported that increasing concentrations of Ethephon increased the number of shoots in *Zingiber officinale*. Treatment at 750 mg L^{-1} Ethephon produced the highest number of shoots, shoot length and rhizome weight in comparison with untreated rhizome, these results are opposite to second experiment which showed that Ethephon at a lower concentration of 150mg/l gave a better result.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 2. Effects of Ethephon on days to visible bud, percentage of bud sprouts (%), shoot length (cm) and rhizome weight gain (g)

Treatment (mg L^{-1})	Days to visible bud	Number of buds	Number of shoots	Shoot length (cm)	Rhizome weight (g)
0	23.0 a	0.25 a	0.00 a	0.00 b	0.44 a
150	13.0 ab	2.25 a	1.25 a	1.30 a	0.94 a
300	8.5 b	1.50 a	1.50 a	1.05 ab	0.34 a
450	15.5 ab	1.00 a	1.00 a	0.48 ab	0.02 a
600	12.5 ab	1.50 a	1.50 a	0.40 ab	1.34 a
700	13.0 ab	1.25 a	1.25 a	0.35 ab	0.22 a

Means with similar letter are not significantly different at P<0.05

Conclusions

In conclusion, BAP was superior to Ethephon in promoting dormant bud break in *K. parviflora*. BAP treatment recorded a shorter number of days to bud emergence, most number of buds, most number of shoots and longer shoot length. BAP at 150 mg L^{-1} is a recommended concentration to break bud dormancy of *K. parviflora* rhizomes.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The Influence of Plant Diversity on the Rate of Natural Succession Process at Different Slope Coverage

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Introduction

Nowadays, hills are being cut usually for urban development and transportation system. Hence, the ecosystem will be affected when vegetation is destroyed. The disturbed slopes need longer time in order to stabilize the ecosystem where the natural succession process may take place. Natural succession is a process of natural changes in plant community through space and it is very time consuming. Usually, pioneer species will occupy the exposed soil during early stages of succession where the pioneer species have higher resistant towards insufficient nutrients, water and extreme condition. The pioneers are usually fast-growing plant species where they can improve the soil by preparing the nutrients and allow other species into the ecosystem (Dalmacio, 1991). After several years, other plant species such as grasses, legumes, shrubs and trees may move to the ecosystem until it reaches stabilization which is also known as climax ecosystem.

It is anticipated that plant diversity (Pohl et al., 2009; Genet et al., 2010) and good pioneers (Normaniza et al., 2009) are amongst the crucial aspects which can enhance the succession rate. Hence, the objectives of this study are to determine the plant diversity in different slope coverage and to assess the rate of natural succession process.

Materials and Methods

Experimental set-up

The experimental plots, each of 5 m x 5 m, were set up with 3 different coverage; 0% (Treatment A), 10% (Treatment B) and 50% (Treatment C) in three replications at Guthrie Corridor Expressway, Sungai Buloh, Selangor.

Ecological experiment

A quadrate of 1 m x 1 m in size was thrown randomly three times at each plot. The plant species were identified (Ahmad Azly, 1988; Wee, 2005) and recorded. The number of individual species at all treatments were counted by following a modified Braun-Blanquet cover class scale where >75% cover = 6, 50-75% = 5, 25-50% = 4, 5-25% = 3, 1-5% = 2, and individual cover = 1 (Shono et al., 2006). The plant diversity at each treatment, was evaluated by using Simpson's Index. The leaf area index (LAI) at each treatment was assessed by using PAR/LAI Ceptometer (AccuPAR LP-80, Decagon Devices, Inc.). The rate of natural succession was measured by calculating the number of species influx at each treatment over time. All parameters were taken once in three months for six months of observation.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Soil water profile

The soil water profile such as soil water content (SWC), soil field capacity (SFC) and saturation level (SL) were measured. Cylindrical soil cores (11 cm in diameter; 45 cm of soil depth) were sampled using a soil coring machine (Eijelkamp Agrisearch Equipment, Model Cobra, The Netherlands).

(1) Soil Water Content (SWC)
The soil water content (SWC) was determined by oven-drying the soil sample at about 85°C in the oven to obtain dry weight (DW). Before that, the fresh weight (FW) of soil sample was taken. The soil water content was calculated by using a formula:

[(FW-DW)/FW x 100]

(2) Soil Field Capacity (SFC)
The soil field capacity was determined by pouring excess water into a container filled with soil so that the soil was supersaturated. The excess water drained out through small holes at the bottom of the container. Once the water stop dripping, the saturated soil was weighed (SW) and oven dried at 85°C to obtain a constant weight (DW). SFC was calculated by using a formula:

[(SW-DW)/SW x 100]

(3) Saturation level (SL)
Saturation level is the ratio of Soil Water Content (SWC) and Soil Field Capacity (SFC). This measurement is important to determine the risk of slope failure.

[(SWC/SFC) x 100]

Results and Discussion

Generally, there were 13 plant species found at the study site. The distribution of species showed that *Dicranopteris linearis, Lycopodium* and *Melastoma malabathricum* were present in all treatments throughout the observation (Table 1). In terms of species richness, treatment A recorded the lowest value of 6, whilst treatment C had the highest. This result can be explained by plant diversity value 0.68, 0.77 and 0.75 for treatment A, B and C respectively (Figure 1). All treatments can be classified as moderately diverse and there was no significant difference as expected. It is anticipated that the difference will be prominent beyond 6 months of observations. The rate of succession was low with only 30% increment happened during 0 to 3 months of observation and there were no changes until 6 months of observation in all treatments.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 1. Plant species found in treatment A, B and C for 6 months of observation

Species	Treatment A	Treatment B	Treatment C
Dicranopteris linearis	/	/	/
Stenochlaena palustris	x	x	/
Nephrolepis biserata	x	/	/
Lycopodium	/	/	/
Lygodium flexuosum	/	x	/
Melastoma malabathricum	/	/	/
Acacia mangium	x	/	x
Macaranga gigantia	x	/	x
Ageratum conyzoides	/	/	/
Asystasia sp.	x	x	/
Imperata cylindrical	x	x	/
Grass A	/	x	x
Grass B	x	/	x
Total (Species Richness, R)	6	8	9

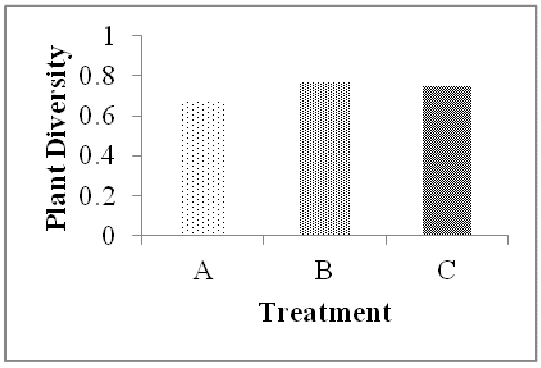

Figure 1. Simpson's Index for Treatment A, B and C

One estimate of a crop's ability to capture light energy is the LAI, which is defined as the proportion of leaf area per unit of land area (Campillo et al., 2010). As the plant diversity increased, LAI would be greater (Figure 2). This increased the slope coverage. When LAI value is higher, the photosynthetic rate would be greater, especially at slope with higher coverage.

Higher plant diversity would provide greater root systems such as root length density (RLD) (Normaniza and Barakbah, 2006) and its distribution, thus, can reduce the SWC on slope and reduce the SL (Figure 3). However, there was positive relationship resulted between the correlation of plant diversity and SWC as shown in Figure 4. The slope location of treatment A is exposed to the sun where evaporation occurs directly from the soil, hence, the SWC and SL is much lower as compared to treatment B and C with higher plant diversity.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

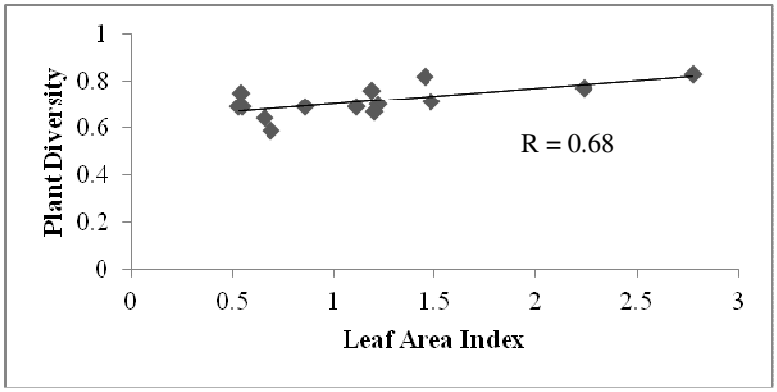

Figure 2. The relationship between plant diversity and LAI

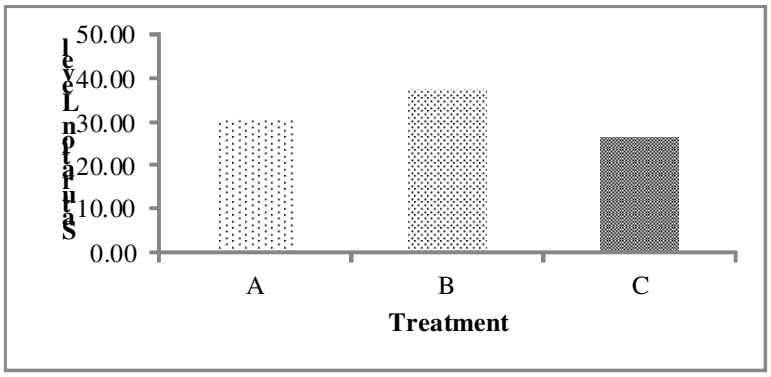

Figure 3. The saturation level of Treatment A, B and C for 6 months of observation

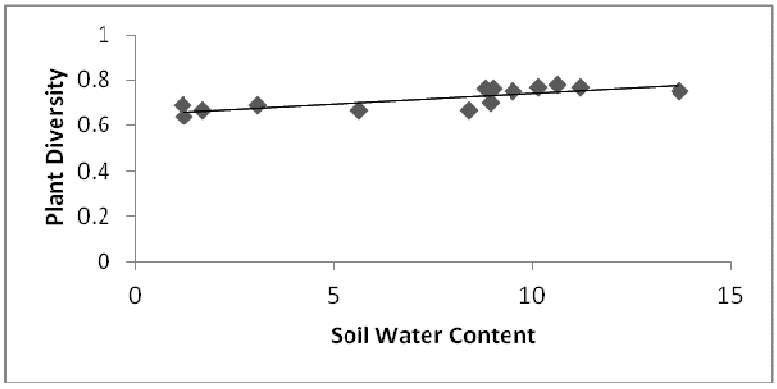

Figure 4. The relationship between plant diversity and soil water content (SWC)

Conclusions

As conclusion, the plant diversity in all treatments has no significant difference and the succession rate increased by 30% within 3 months of observation.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Seed Dormancy Breaking for *Andrographis paniculata* (Burm. F.) Nees (Hempedu Bumi)

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Introduction

Andrographis paniculata, from Acanthaceae family is also commonly known as hempedu bumi or 'King of Bitters'. Andrographolide, from *A. paniculata* is one of the major diterpenoids with important medicinal benefits that can be extracted from its roots, aerial part of mature twig and mainly from leaves. *Andrographis paniculata* can also be used to boost the body immunity and has anti-inflammation, antiviral and antioxidant properties. This species is distributed along tropical Asia, South-East Asia which has hot and humid climatic conditions (Parashar et al., 2011; Benoy et al., 2012; Kumari et al., 2012; Talei et al., 2012).

Seed dormancy is an internal condition that obstructs seeds from germinating under favourable thermal, gaseous and hydric condition (Diego and Roberto, 2010). Examples for seed dormancy are physical, morphological, physiological, morphophysiological and combination of physiological and physical (Baskin and Baskin, 2004). Since the extracts are found mainly from mature leaves, it is important to find out the shortest time from seed propagation to maturity. Seed propagation of this plant faces problems such as poor germination and seed dormancy.

Materials and Methods

Seed collection

Mature pods of *A. paniculata* accession of "Harapan" were collected from mother plants grown in experimental plots in Ladang 2, University Putra Malaysia. The mature pods were put inside paper bag and the pods were left for natural dehiscence. All the seeds obtained from dehisced pods were collected and appropriate amount for seed dormancy breaking tests were taken.

Experimental design

Three sets of experiments were conducted in a Completely Randomized Design (CRD) with different treatments. There were four replicates per treatment with 25 seeds for each replicate. Petri dishes were lined with two layers of filter paper as the germination media. The filter paper was moistened with distilled water every three to four days to prevent the seeds from drying. Germination was performed at room condition (25±2 °C, 50% relative humidity).

Seed dormancy breaking techniques

The seed dormancy breaking techniques for the three sets of experiments included:

First Experiment:
1) Control
2) Water soaking for 24 hours

3) Sand paper scarification
4) Hot water treatments (40 °C and 50 °C for 10 min; 60 °C for 5 min)
5) Gibberellic acid (GA) soaking 24 hours (25, 50, 75, 100 mg/L GA)
6) Concentrated sulphuric acid (H_2SO_4) soaking (3 and 5 min)

Second Experiment:
1) Control
2) Water soaking for 24 hours
3) Sand paper scarification
4) Hot water treatments (40 °C and 50 °C for 10 min; 60 °C for 5 min)
5) GA soaking 24 hours (25, 50, 75, 100 mg/L GA)

Third Experiment:
1) Water soaking for 24 hours
2) Sand paper scarification
3) Soaking in 1% Tween 20 for 24 hours
4) Soaking in 1% washing powder solution for 24 hours
5) Soaking in 1% dishwashing liquid for 24 hours

Observation

The number of days for germination initiation and cumulative germination percentage were recorded. Data were analyzed statistically by using SAS (Version 9.10) for analysis of variance (ANOVA) and Least Significant Difference (LSD) test.

Results and Discussion

Results obtained (Table 1) showed that the seed germination percentage for *A. paniculata* can be improved by applying seed dormancy breaking techniques. From Table 2, the results showed that there was significant difference between the treatments. The highest germination percentage was 97% after scarification by using sand paper. For the seeds soaked in water and 1% Tween 20, they showed the shortest period to germination (2.25 days). However, H_2SO_4 treated seeds (soaking for 3 and 5 min) gave negative results.

The highest germination percentage for the first experiment was found with the seeds soaked with 25 mg/L GA. The result was significantly different with 100 mg/L GA soaking, sand paper scarification and H_2SO_4 soaking treatments. For the second experiment, sand paper scarification gave the highest germination percentage which was significantly different from other treatments. By comparing first and second experiments, the results were different due to the seeds themselves. The days to germination was the longest (13 days) for seeds soaked with 100 mg/L GA. However, there was no significant difference in second experiment for the days to germination (Table 1). By using mechanical scarification like sand paper, scarification can remove or soften the hard seed coat attributed on *A. paniculata* (Kumar et al., 2012; Talei et al., 2012).

Germination percentages for hot water soaked seeds (50 °C for 10 min and 60 °C for 5 min) were not significantly different with 25 mg/L GA soaking treatment in the first set experiment. However, in second experiment, hot water treatments were not effective for enhancing seed germination as compared with first experiment. Talei et al. (2012) mentioned that the delay in germination may be related to prohibitor factors presented during seed coat softening (Table 1).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Soaking in 1% dishwashing liquid resulted in lowest germination percentage while sand paper scarified seeds had the highest germination percentage (Table 1). The results of days to germination were contrary when comparing the seed soaked in water for second and third experiment. Other than hard seed coat, a layer of hydrophobic substances may be present on the outermost of seed; such phenomena can be found in soy bean seed and *Fagus sylvatica* seed (Shao et al., 2007; Riederer and Miiller, 2008). The waxy layer of the seed coat can be removed by sand paper scarification and chemical treatments. Detergent (dishwashing liquid and washing powder) and emufsifier (Tween 20) are surfactants (Schramm, 2000). In this study, we suspect the outermost layer of *A. paniculata* seed contains wax. While by comparing the result in third experiment, although the days to germination was the shortest for detergent and emulsifier treated seeds, as compared to sand paper scarified seeds, but the germination percentage for the later was the highest.

Table 1. The effect of different seed dormancy breaking techniques on germination percentage and days to germination for three sets of experiments

Treatment	Germination Percentage			Days to Germination		
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3
CRT	76^{ab}	6^{bc}	-	5.75^{b}	6.67^{ab}	-
SP	47^{c}	29^{a}	97^{a}	5.00^{b}	4.00^{ab}	3.50^{a}
40HW10	73^{b}	6^{bc}	-	5.75^{b}	5.00^{ab}	-
50HW10	79^{ab}	4^{bc}	-	5.00^{b}	5.00^{ab}	-
60HW5	87^{ab}	9^{b}	-	5.00^{b}	5.00^{ab}	-
H2O	81^{ab}	4^{bc}	45^{b}	5.75^{b}	8.00^{a}	2.25^{b}
1%TWEEN	-	-	57^{b}	-	-	2.25^{b}
1%WASHP	-	-	47^{b}	-	-	3.00^{ab}
1%DISH	-	-	25^{c}	-	-	3.25^{ab}
$25GA_3$	89^{a}	1^{c}	-	5.00^{b}	3.00^{b}	-
$50GA_3$	76^{ab}	3^{bc}	-	5.00^{b}	4.00^{ab}	-
$75GA_3$	87^{ab}	6^{bc}	-	5.00^{b}	3.67^{ab}	-
$100GA_3$	19^{d}	3^{bc}	-	13.00^{a}	4.00^{ab}	-
$3H_2SO_4$	0^{e}	-	-	-	-	-
$5H_2SO_4$	0^{e}	-	-	-	-	-

Means followed by different superscripted letters are significantly different from one another for each column (each experiment) at the probability level of p = 0.05 by LSD. The treatments were: CRT = control, SP = sand paper scarification, 40HW10 = hot water soaking (40 °C for 10 min), 50HW10 = hot water soaking (50 °C for 10 min), 60HW5 = hot water soaking (60 °C for 5 min), 1% TWEEN = soaking in 1% Tween 20 solution, 1% WASHP = soaking in washing powder solution, 1% DISH = soaking in 1% dishwashing liquid, 25/50/75/100 GA3 = soaking in 25/50/75/100 mg/L GA, 3/5H_2SO_4 = soaking in concentrated H_2SO_4 for 3/5 min.

Conclusions

Sand paper scarification resulted in highest germination percentage while soaking in 1% Tween 20 gave the shortest days to germination. The seed dormancy of *A. paniculata* is mainly due to its hard seed coat. It makes the seed impermeable to water and gases that are required for cellular activities before it germinates. Softening or removing the seed coat by using mechanical scarification is more effective as compared to chemical scarification to improve seed germination.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effects of Different Concentrations of Naphthaleneacetic Acid and 6-Benzylaminopurine on Callus Induction of *Capsicum frutescens*

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Introduction

Capsicum frutescens belonging to Solanaceae family is also known as "chilies". It can be further classified into division of Magnoliophyta, class of Magnoliopsida, and order of Solanates (Heiser and Smith, 1953). *Capsicum frutescens* var. *bird's eye chili* possesses valuable economic status as commercial crop, mainly in the group of spices and vegetables. However, *Capsicum* spp. plants have also very high medicinal value and special secondary metabolite content such as capsaicin, capsaicinoids, capsinoids, quercetin, and luteolin (Materska and Perucka, 2005). These compounds are used as medicine to treat many types of disease such as to prevent joint pain, improved metabolism, and act as anticancer and antioxidant (Luo et al., 2011). This species is also used in clinical as medicine for various degenerative diseases such as heart disease, cancer and osteoporosis (Ruanma et al., 2010).

Plant calluses can be defining as a group of undifferentiated cells that grow due to effect of plant hormone secretion. In natural environment, the callus growth may be due to secretion of natural hormone such as auxin and cytokinin, but callus inductions were rare and may be induced due to certain environmental stress and special occurrence such as scarce of nutrients. In recent years, the benefits of callus have been noticed to trigger many plant studies, especially somatic embryogenesis, micropropagation and plant regeneration (Evans et al., 1981). Therefore, many recent studies were done in callus induction of *Capsicum* spp. by using plant growth regulators. Hypocotyl, cotyledon and leaf explants of *Capsicum annum* have been induced to form callus tissues, either with single hormone or combination of hormones such as indole-3-acetic acid (IAA), 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 6-benzylaminopurine (BAP) (Rodeva et al., 2006; Ashrafuzzaman et al., 2009; Aniel et al., 2010; Andrzej and Tomasz, 2011). However, the studies using foliar explant with naphthaleneacetic acid (NAA) and BAP in certain ratio focusing to achieve high growth rate, early initiation day and better morphology are still inadequate.

Therefore, the main objective of this study was to focus on effect of NAA and BAP in certain ratio for inducing high yield, fresh and healthy callus morphology and texture within short period. Callus condition can be an important factor that greatly affects further research studies such as plant regeneration of *Capsicum* spp. which is known to be challenging due to recalcitrant morphogenic nature caused by inability of plant cells, tissues and organs to respond to *in-vitro* culture (Kothari et al., 2010). The successfully induced callus must not only be useful for plant regeneration but also provide clean stock that is free from contamination and plant disease. These obtained calluses can also become very useful tools for other related studies, such as micropropagation, somatic embryogenesis, protoplast fusion, plant hybrid from mixed callus culture, and organogenesis (Castellar et al., 2011).

Materials and Methods

Explant sample preparation and sterilization procedures

About one week old young foliar explants of *C. frutescens* var *bird eyes chili* were taken from germination trays and cut to approximately 5 mm^2. The cut explants were sterilized by using 15%

sodium hypochlorite (NaOCI) with 2-3 drops of tween 80 for 5 min. Then, the explants were rinsed with distilled water 3 times before subsequent sterilization with ethanol 70% for 2 min. Finally, the explants were rinsed with distilled water five times.

Media preparation

Murashinge & Skoong (MS) medium was prepared by adding 4.4 g/L MS medium powder, 30 g/L sucrose, 1 g/L myo-inositol, 4 g/L gelrite into distilled water. The media pH was adjusted in between 5.7 to 6.0 after plant hormones were added respectively. Seven different treatments of NAA:BAP (0.0:2.5, 2.5:0.0, 2.5:2.5, 2.5:5.0, 5.0:2.5, 2.5:10.0 and 10.0:2.5 mg/L) were studied. The medium was then autoclaved at 121 °C at 15 psi for 20 min.

Callus culturing procedure and data collection

Three sterilized foliar explants were then cultured in one petri dish containing treatment medium. Each treatment consisted of 20 replicates. All cultures were incubated with 16 h light and 8 h dark photoperiod under 25 ºC for one month. The data on day of callus initiation were recorded. The changes of both callus and explants morphology were observed. The growth of callus was scaled based on macroscopic observation.

Results and Discussion

In this experiment, *C. frutescens* foliar explants in MS medium treated with NAA and BAP were successfully induced to produce calluses in one month's period. Callus induction was not observed with control treatment. Single treatment of NAA or BAP and combination of NAA and BAP at different concentrations produced callus of different morphology.

Low concentration of single hormone BAP (2.5 mg/L) successfully induced high callus growth. However in this treatment, the callus morphology became excessively brownish and it took longer time (days 13) for callus to initiate (Figure 1). Single hormone NAA (2.5 mg/L) was able to produce better callus morphology with yellowish callus with early callus initiation period (days 8) as shown in Figure 1. However, the callus growth was very poor as compared to single hormone BAP.

The ratio 1:1 of combination of both NAA and BAP at 2.5 mg/L (Table 1) induced better callus morphology with pure whitish color and soft and friable texture, with moderate callus growth rate (9 days) as compared to single hormone NAA or BAP. The combination of growth regulators showed synergistic effects as compared to single hormone treatments which were also shown in previous studies of callus induction of *C. annum* using cotyledon explant (Yang et al., 2000).

Double increment in concentration of BAP (5.0 mg/L) over NAA (2.5 mg/L) not only produced the highest callus growth within the shortest time (5 days) but it also successfully induced pure white, friable, and soft callus texture [Table 1 and Figure 2(e)]. BAP is cytokinin known to boost up the shoot formation in plant (Gunay and Rao, 1978). However, the presence of this hormone alone was able to produce high amount of callus, instead of shoot induction, in this study.

BAP hormone may highly trigger cell to rapidly undergo cell division, instead of cell differentiation. BAP hormone is also known to have very low toxicity to plant cell (Roderick, 1992). Therefore, double concentration of BAP can be used with high compatibility in inducing callus of *C. frutescens*. A very high ratio of BAP at 10.0 mg/L combined with 2.5 mg/L NAA was also able to initiate callus earlier (days 6) as in Figure 1. However, the morphology of callus was less formidable and transparent in color

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

with soft and friable texture. This showed that excessive BAP affected the cell growth and can become toxic to plant cell [Figure 2(g)].

The situation was different with NAA, where higher concentration of this hormone caused opposite effect towards successful callus growth. Both high ratio of auxin at 2:1 and 4:1 with 5.0 mg/L and 10.0 mg/L NAA respectively inhibited efficient callus growth (Table 1). The callus that was successfully induced was also hard and non-friable, which made it difficult to be used for further plant studies [Figure 2(f) and (h)].

Apart from that, high concentrations of NAA also increased the time to induce callus, which happened only in the third week (Figure 1). This shows that the high concentration of NAA may cause this plant hormone to acts as inhibitor towards callus growth, rather than to induce it. It was also shown that NAA may not be compatible to *C. frutescens* and can become highly toxic in cell by blocking the cell channel, expanding cell wall or trigger cell to release toxic content that may lead to cell death or inhibit cell growth (Laura et al., 2012).

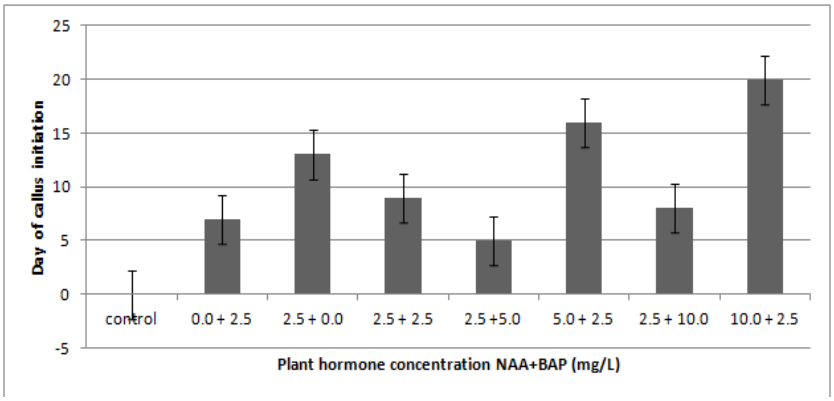

Figure 1. Days to callus initiation (*n*=20) with *C. frutescens* foliar explant due to different ratios of plant hormones (NAA and BAP), error bar indicates SEM (standard error of mean)

Table 1. Effects of NAA and BAP in different concentrations on foliar explant of *C. frutescens* at one month after culture

NAA:BAP (mg/L)	NAA:BAP ratio	Callus growth scale*	Callus morphological characteristic
Control	0:0	-	-
0.0:2.5	0:1	+++	Yellowish white with friable and soft texture
2.5:0.0	1:0	+	Brownish white with friable and soft texture
2.5:2.5	1:1	++	Pure white with friable and soft texture
2.5:5.0	1:2	++++	Pure white with friable and soft texture
5.0:2.5	2:1	+	Brownish white with non-friable and hard texture
2.5:10.0	1:4	++	Transparent white with friable and soft texture
10.0:2.5	4:1	+	Brownish white with non-friable and hard texture

* - = *none,* + = *poor,* ++ = *moderate,* +++ = *excessive,* ++++ = *highly excessive*

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

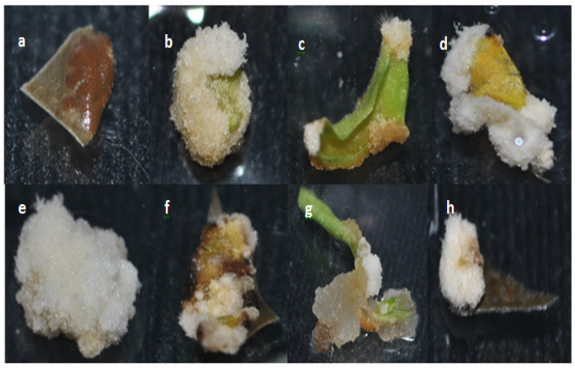

Figure 2. Callus culture of *C. frutescens* foliar explant with different treatments of NAA and BAP (mg/L) at one month; (a) control, (b) 0.0 + 2.5, (c) 2.5 + 0.0, (d) 2.5 + 2.5, (e) 2.5 +5.0, (f) 5.0 + 2.5, (g) 2.5 + 10.0, and (h) 10.0 + 2.5 NAA + BAP (mg/L)

Conclusions

The combination of NAA and BAP hormone at 1:2 was required to induce foliar explants of *C. frutescens* to reach the optimum level for callus induction in terms of best quality of callus morphology and induction and growth rate. The role of hormone combination between NAA and BAP showed tremendous positive effect in producing high and efficient amount of callus in the shortest period as compared to the use of single hormone of NAA or BAP. It was also able to produce white, friable, and soft callus that are more suitable for plant studies such as plant regeneration, organogenesis, somatic embryogenesis and other related studies in the future. It is also recommended that more further study on other relevant factors that may also contribute to increased callus induction such as pH, light and dark condition, media type and explant types should be carried out. The use of other type of plant hormone to replace NAA or BAP may also affect callus growth and morphology variation for callus induction of *C. frutescens*.

Acknowledgments

The authors would like to thank Faculty of Applied Science, University Teknologi MARA (UiTM), Shah Alam, for supporting this study. This study was funded by Research Management Institute, UiTM under the Excellent Fund (References No. 600 RMI/ST/Dana/5/3/DST 290/2009).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Improvement of *Arachis hypogaea* var. Margenta Seed Vigour by Hydropriming

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Introduction

Groundnut var. Margenta produced for seed purposes have poor germinability right after seed production and during early storage duration (Amyita et al., 2012). It shows that seed exhibits dormancy when it fails to germinate even though external conditions (e.g., temperature, moisture and air) are conducive or favourable. Therefore, seed treatment such as seed priming is a viable technology that may be an option to break dormancy and thus, improve germination rate, uniform seedling emergence, high vigour and better yields.

Seed priming is a technique of hydrating and redrying of the seed, which results in improved germination. Methods of seed priming can be divided into two groups depending on whether water uptake is uncontrolled (hydropriming) or controlled (osmotic priming and solid matrix priming) (Taylor et al., 1998). Common priming techniques include osmopriming (soaking seeds in osmotic solutions such as polyethylene glycol), halo priming (soaking seeds in salt solutions) and hydropriming (soaking seeds in water). In this study, the effects of hydropriming duration on Margenta seed vigour before and after various storage periods were evaluated.

Materials and Methods

All experiments were conducted in the Seed Quality Control Laboratory, Planting Material, Seed and Livestock Breed Production Unit, MARDI Headquarters, Serdang, Selangor. Groundnut seeds obtained from MARDI Station, Pontian, Johor, were dried under the sun to a moisture content of around 8%. Seeds were kept in air-tight polyethylene plastic bags and stored in a cold room at 10±2 °C for 2, 4 and 6 months before treatment. Seeds were soaked in distilled water for 0, 24, 48 and 72 hours at 25±2 ºC under dark conditions and dried back to its original seed weight (of around 8% moisture content). The seeds were then subjected to moisture content test, germination test and electrical conductivity test.

Two replicates of five seeds were used for moisture content determination for each treatment. Moisture content of all samples was determined on a fresh weight basis using the oven method at 103±2 ºC for 17±1 hours as recommended by the International Seed Testing Association (ISTA, 1985) for oily seeds. Four replicates of 25 seeds each per treatment were used for testing seed germination. Seeds were germinated in moist sterilized sand in germination boxes and incubated in a germination room at a temperature of 28±2 °C. The number of germinated seeds was recorded daily for 15 days. The mean germination time (MGT) was calculated based on the equation of Ellis and Roberts (1981) modified by Moradi Dezfuli et al. (2008). The time to 50% germination (T_{50}) was calculated according to the formula of Coolbear et al. (1984) modified by Farooq et al. (2005). A sample of 10 seeds was soaked in 100 ml deionized water at 25 ºC for 24 hours in an incubator. A conductivity meter, EUTECH Instruments CON 510, was used to measure electrical conductivity (EC) of seed leachates. Conductivity was expressed on a weight basis in $\mu Scm^{-1}g^{-1}$ of seed.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

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

Results and Discussion

Seed water uptake pattern

The water uptake pattern was determined by measuring the amount of water absorbed at 24 hours intervals related to the fresh weight of seed. Results (Figure 1) show that significant increase of water uptake occurred within 24 hours of soaking followed by a plateau phase. The rapid water uptake of dry seeds is mostly related to the low matrix potential and is associated with the interaction of water with seed surfaces and macromolecules (Bewley, 1997). The huge difference in water potential between the dry seed tissue and the ambient water potential presumably resulted in water movement from the higher to the lower water potential, which stopped when an equilibrium was reached (the plateau phase).

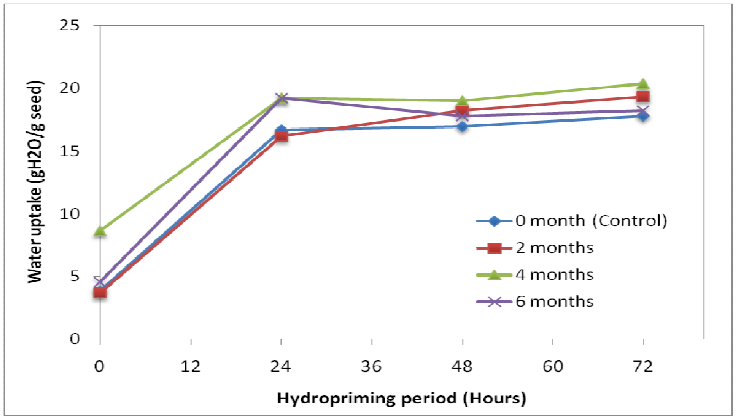

Figure 1. Effects of hydropriming duration on water uptake of seeds stored for 0, 2, 4 and 6 months

Seed germination

Results in Figure 2 show that germination percentage of non-stored seeds (Control) increased with prolonged storage period, indicating seed dormancy. The effect of priming duration was most obvious on the germination of non-stored seeds (Control). Germination of seeds stored for 2 and 4 months significantly increased upon hydropriming for 24 hours. A similar increase in seed germination of Bambara groundnut hydroprimed for 24 hours was also observed by others (Berchie et al., 2010; Ogbuehi et al., 2013). The increase in seed germination percentage is related to the resumption of metabolic activities during imbibition and the initiation of cellular events leading to radical emergence. However, hydropriming for 48 and 72 hours of seeds stored for 4 and 6 months did not have any significant advantage on germination. Hydropriming durations did not have any significant effect on germination of seeds stored for 4 and 6 months due to the dormancy broken during storage.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

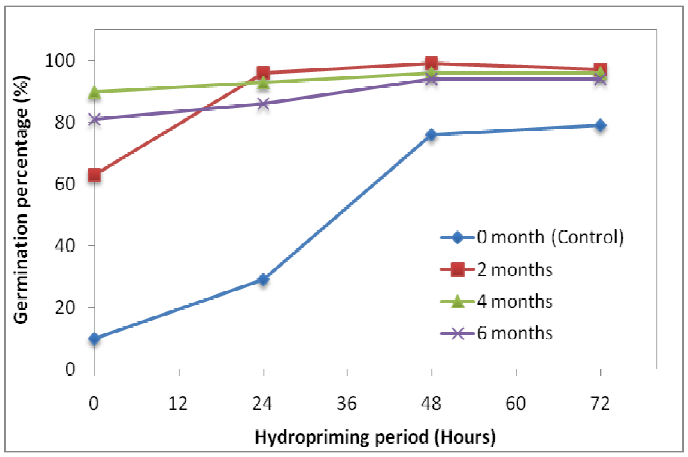

Figure 2. Effects of hydropriming duration on germination of seeds stored for 0, 2, 4 and 6 months

Speed of germination

Results (Table 1) show that hydropriming duration did not have any significant effect on germination speed of seeds stored for 0, 2 and 4 months. As for seeds stored for 6 months, there was a significant effect of hydropriming duration on germination speed. Earlier germination was recorded for hydroprimed seeds as indicated by lower value of MGT and T_{50}. The shortest MGT was obtained when seeds were hydroprimed for 72 hours as compared to those without hydropriming treatment and the seeds took 2.4 days, as compared to 3.6 days in seeds without hydropriming to achieve T_{50}. Therefore, the lower the MGT, the faster seeds germinated. Early seed germination was probably due to the completion of metabolic activities making the seeds ready for radical protrusion and the primed seeds germinated soon after planting as compared to non-treated seeds.

Table 1. MGT and T_{50} of seeds following hydropriming for 0, 24, 48 and 72 hours

Treatments	Storage Period (months)							
	0		2		4		6	
	MGT	T50	MGT	T50	MGT	T50	MGT	T50
Without hydropriming	9.3a	5.0a	7.1a	3.8a	7.4a	4.5a	9.2a	6.1a
Hydropriming for 24 hours	5.9a	3.1a	6.1a	3.7a	5.6a	3.3a	5.9b	2.9bc
Hydropriming for 48 hours	8.1a	4.7a	5.6a	3.6a	6.4a	4.1a	6.8b	5.3ab
Hydropriming for 72 hours	5.4a	3.6a	5.7a	3.6a	6.3a	4.3a	4.7c	2.4c

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

Seed vigour

Seed vigour was estimated by measuring the electrical conductivity of seed leachate for each soaking interval. Results (Figure 3) show that EC before hydropriming was high due to the rapid influx of water (from high to low water potential) which leads to leakage of solutes and metabolites into the surrounding solution (Bewley, 1997). Seed EC significantly decreased with hydropriming for 24 hours but increased thereafter. The decrease in EC was probably due membrane returning to their more stable configuration, in which solute leakage was minimal. The increase in EC after 24 hours of hydropriming was most probably due to the lost of cellular membrane integrity in seeds. Membrane integrity, as determined by electrical conductivity test, is closely related with seed vigor (Bruggink et al., 1991) and a study by Duke and Kakefuda (1987) reported that *A. hypogaea* L. seed testa prevented cellular membrane damage and it

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

can play a role in regulating the imbibition rate, thus affecting susceptibility to injury and subsequent germinability.

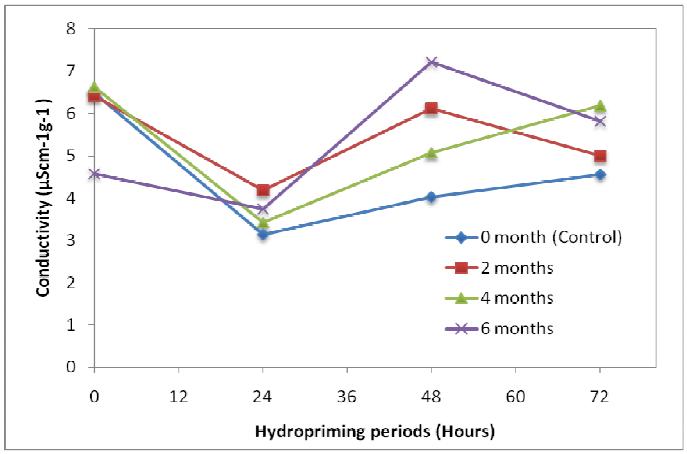

Figure 3. Effects of hydropriming durations on electrical conductivity of seeds stored for 0, 2, 4 and 6 months

Conclusions

Hydropriming for 24 and 48 hours increased germination and vigour of freshly processed seeds and seeds stored for 2 months. This study established the potential application of seed hydropriming for the improvement of groundnut var. Margenta seed vigour. However, optimization of the hydropriming treatments and other priming treatments needs to be further investigated.

Acknowledgements

The authors would like to thank MARDI for supporting this study via Research Grant JP-RC-0312. Thanks are also due to the Seed Quality Control Laboratory staff, especially Vigneshwaran s/o Poobalasingam and Shohime Ramli, for their technical assistance. A special appreciation also goes to the staff of MARDI Station, Pontian, Johor for their help throughout the study.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Phenolic Content of *Xanthostemon chrysanthus* after Treatment with Paclobutrazol and Potassium Nitrate

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Introduction

Xanthostemon chrysanthus (F. Muell.) Benth. or also known as golden penda is a medium-sized tree belonging to the family of Myrtaceace. Owing to its distinctive and bright yellow floral, this species becomes one of the popular ornamental trees in Malaysian cities. It is native to tropical northern Australia, New Guinea, Indonesia and the Philippines (Sosef et al., 1998). Its compound flowers also attract nectar feeding birds and insects which also add values into the landscapes.

It is well documented that phenolics play important roles in defense mechanism against various types of stresses caused by pathogens, pests or unfavourable growth conditions (Treutter, 2001; Pasqualini et al., 2003). In addition, an enhancement of phenolic compounds can be observed under different environmental factors and stress conditions (Diáz et al., 2001; Sakihama and Yamasaki, 2002). According to Davis and Sankhla (1988), triazoles were found to be highly effective in protecting plants from various environmental stresses that interfere with their normal physiological processes. Moreover, PBZ has other biochemical effect on plant, such as increase in levels of proline (Mackay et al., 1990), antioxidants (Bañón et al., 2003) and chlorophyll content (Watson and Himelick, 2004). It has been shown that water deficit led to an increase in the intensity of the blue fluorescence originating from plant phenolics, mainly from ferulic acid (Morales et al., 2005). Phenolics can neutralize light falling on the leaves through its transformation into blue fluorescence, which is no longer damaging, but can even be used for photosynthetic quantum conversion (Bilger et al., 2001). According to Kondo and Kawashima (2000), plants accumulate UV-absorbing flavonoids and other phenolic compounds to prevent the penetration of UV-B into the deeper tissues of the plant.

Therefore, this study aimed to determine the effects of PBZ and potassium nitrate (KNO_3) on total phenolic content (TPC) in the leaf of *X. chrysanthus* in three different growth stages i.e., before flowering, during flowering and after flowering. The outcome can be interpreted as the usefulness of such treatment for stress tolerance amelioration in this urban landscape tree.

Materials and Methods

Study location and plant materials

A study plot was established in Metropolitan Batu Recreational Park, Kuala Lumpur (3°12'49"N/101°40'43"E). Existing trees aged about six years grown in the recreational park were used in the study.

Experimental procedures

Cultar-250 formulation, 250 g a.i. PBZ per litre was used. Soil application of PBZ was carried out once at the commencement of the study at an application volume of 1 L per tree. Meanwhile, control plants were applied with 1 L of plain water. Quarterly, Krista-K Plus (13.7:0:38.4) as a source of potassium nitrate, KNO_3, was applied under the drip-line of the tree canopy using pocket system technique into the soil. The allocated amount of KNO_3 was equally applied in four holes for each tree. KNO_3 was applied in May, August and November 2011.

The experiment was arranged in a CRD with nine replicates, i.e. T1(0 g/L [1] PBZ + 0 g Krista-K Plus); T2(0 g/L PBZ + 100 g Krista-K Plus); T3(0 g/L PBZ + 200 g Krista-K Plus); T4(0.125 g/L PBZ + 0 g Krista-K Plus); T5(0.125 g/L PBZ + 100 g Krista-K Plus); T6(0.125 g/L PBZ + 200 g Krista-K Plus); T7(0.25 g/L PBZ + 0 g Krista-K Plus); T8(0.25 g/L PBZ + 100 g Krista-K Plus) dan T9(0.25 g/L PBZ + 200 g Krista-K Plus). A total of 81 trees were involved in this study.

Leaf sampling and preparation of sample

The first three fully expanded leaves were collected from each tree. The leaves were then washed under running water and fragmented into small pieces prior to extraction process. The samples were then soaked in 1:6 w/v of ethanol, in a sealed conical flask. It was then kept at room temperature for 72 hours on orbital shaker at 100 rpm of speed. After that, it was filtered by using Whitman no. 4 filter paper. The filtrate was then filled in an evaporating flask and subject to water bath (45 ^{o}C), followed by refrigeration (15 ^{o}C) and was lastly vacuum pumped (54 mBar) and rotated by using rotary evaporator at 100 rpm speed for 20 minutes. The crude extract was then weighed, labelled and stored in air tight amber bottle and placed in refrigerator at temperature of -20 to 4 ^{o}C until it was used for TPC analysis.

Analysis of TPC

Determination of TPC was performed using Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modifications. A 1.0 mg quantity of crude extract was extracted for 2 hours with 1.0 mL of solution containing 80% ethanol, concentrated hydrochloric acid and distilled water (8:1:1) at room temperature on a shaker set at 200 rpm. The mixture was centrifuged at 6,000 rpm for 15 minutes and the supernatant was decanted into vials. The supernatant was used for the determination of TPC. An amount of 200 µL supernatant extract was mixed with 400 µL Folin-Ciocalteu reagent (0.1 mL/0.9 mL) and allowed to stand at room temperature for 5 minutes. Then, 400 µL sodium bicarbonate (60 mg/mL) solution was added and the mixture was allowed to stand at room temperature for 90 minutes. Absorbance was measured at 725 nm by using a UV-spectrophotometer. A calibration curve was generated by using the gallic acid standard, and the levels of TPC in the samples were expressed as milligram equivalent to gallic acid per 1 g sample dry weight (mg GAE/g).

Data analysis

Data obtained were subjected to analysis of variance (ANOVA) and the treatment means were then compared ($p<0.05$) using Duncan's multiple range test (DMRT).

Results and Discussion

There was a significant difference found in TPC in the leaf of *X. chrysanthus* before flowering and during flowering stage, respectively (Table 1) Before flowering stage, T7 gave the highest TPC (20.33 mg GAE/g) as compared to the control (16.13 mg GAE/g), showing a difference of about 26% (Table 1). On the other hand, T4 resulted in higher TPC at flowering stage (24.74 mg GAE/g) while the control tree only had 16.82 mg GAE/g, giving 47% difference. At the flowering stage, both T4 and T7 had significantly higher TPC as compared to the control. However, there was no significant difference in TPC in the leaf amongst all the treatments after flowering stage (Table 1). This may be attributed to the prolonged growth and development or even towards aging stage in these leaves as there was generally minimum new shoot development during the energy consuming flowering stage. This study shows that PBZ was able to increase the TPC in *X. chrysanthus*. Increased phenolics content was also reported in leaf, stem and root of *Catharanthus roseus* following treatment with PBZ (Jaleel et al., 2006). Sankar et al. (2007) reported that water stress was minimized by the application of PBZ which then increased the antioxidant levels and activities of scavenging enzymes.

Table 1. TPC in *X. chrysanthus* after the application of PBZ and KNO_3

Treatments	TPC (mg GAE/g of dry weight)		
	Before flowering	During flowering	After flowering
T1	16.13 b	16.82 b	16.26 a
T2	17.37 ab	19.96 ab	18.77 a
T3	19.15 ab	19.71 ab	18.96 a
T4	19.07 ab	24.74 a	19.82 a
T5	18.78 ab	22.45 ab	19.92 a
T6	18.9 ab	21.44 ab	19.08 a
T7	20.33 a	23.75 a	20.45 a
T8	18.92 ab	21.03 ab	19.75 a
T9	18.45 ab	20.47 ab	18.84 a

Means followed by the same letter(s) within column do not differ ($p<0.05$) by Tukey's Honestly Significant Difference (HSD); T, treatments

Conclusions

PBZ (T4 and T7) increased the TPC in the leaf of *X. chrysanthus* before and during flowering. PBZ might be useful for improving growth and stress tolerance of ornamental trees which are usually grown under harsh urban environment.

Acknowledgements

Thanks are due to the Kuala Lumpur City Hall (DBKL) for site permission. This work was supported by the Ministry of Agriculture and Agro-based Industry Malaysia (05-03-10-SF1030).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Hexanal Treatment on the Main Phenolic and Volatile Compounds in Strawberry Fruit

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Introduction

Strawberry is one of the most consumed berries in the world and one of the important fruit crops cultivated worldwide. It is widely appreciated for its characteristic aroma, bright red color, juicy texture, and sweetness. Strawberry is rich in polyphenols and as it is consumed in high quantity, it can be a valuable source of phenolic compounds especially anthocyanins in the diet (Aaby et al., 2007; Aaby et al., 2012; Buendia et al., 2010). Strawberries are essentially fully ripe at harvest and highly perishable. Investigation on fruit ripening and senescence has shown that the softening process is accompanied with the initiation of membrane deterioration, possibly involving phospholipase D (PLD). An approach for delaying the progressive membrane degradation involved the use of hexanal (Paliyath et al., 1999; Paliyath et al., 2008). *In vitro* study has shown that hexanal can be a potent inhibitor of PLD activity, and enhance several beneficial qualities of fruit and vegetables, including colour, firmness and volatiles. Pre and postharvest applications of hexanal in several fruits, vegetables, fresh cut produce and flowers has shown promising results in enhancing their shelf-life (Paliyath and Subramanian, 2008). In this study, the main phenolic and volatile profile of the strawberry fruits was compared after subjecting the fruits to treatment with hexanal formulation.

Materials and Methods

Plant materials and hexanal treatment

Strawberry (*Fragaria x ananassa*) plants were planted in a matted row system under commercial conditions in an open field. Spacing between plants was 45 cm and 1.2 m between rows. Plants were allowed to runner freely to fill in the row. Row width was maintained at approximately 45 cm. Treatment or control applications were applied to each plot (1.5 m in length) with four replications. The treated plants were sprayed with 2% (v/v) hexanal formulation from a hand sprayer at intervals of 7 days beginning a week before harvest (white or pink fruit), whereas control plants were left untreated. The basic ingredients of the stock formulation included hexanal, 1% (v/v) geraniol, 1% (w/v) a-tocopherol, 1% (w/v) ascorbic acid, 0.1% (w/v) cinnamic acid, and 0.1% (v/v) Tween 80 dissolved in ethanol (10% v/v). Ascorbic acid (0.1% (w/v) was dissolved separately and added to the stock solution. Stock solution was mixed in the water to provide 2% final solution and sprayed at the top of the plants using a pressurized nozzle sprayer. One week after spraying, the strawberry fruits were hand-harvested at full red stage of maturity. Fruit samples were selected for uniform size, colour and absence of mechanical damage. Strawberries were frozen at -40 °C until analysis.

Extraction of polyphenols and LC-MS analysis of phenolic compounds

Ten strawberries were homogenized in 10 mL of HPLC-grade methanol (100%) using a Brinkman Homogenizer, fitted with a Polytron PTA 10 probe. The homogenate was centrifuged using a Sorvall RC-6 Plus centrifuge at 15000 x g for 20 min; supernatant was collected and aliquots were stored at -

20°C until further use. The extracts were dried using a stream of nitrogen to remove methanol. The dried residue was dissolved in distilled water and loaded onto a C18 Sep-Pak cartridge (1 mL volume, Waters Corporation, Massachusetts, USA). A total crude fraction was obtained by eluting the total loaded polyphenols directly with 100% methanol. Extracts of phenolic compounds filtered through a Millex HA 0.45 µm filter (Millipore Corp., Billerica, MA, USA), were analyzed on an Agilent 1100 series HPLC-MS (Agilent Technologies, Waldbarn, Germany) equipped with an autosampler, UV-visible detector and electrospray ionization was performed with an API-ES mass spectrometer. The phenolic compounds were separated using XTerra MS C-18 column (5 µm, 150 x 2.6 mm, Waters, Milford, MA) with water/formic acid (98:2, v/v) (A) and methanol (B) as mobile phases. For phenolic compound and anthocyanin analysis the gradient used was as follows: 0-2 min, 7% B; 2-30 min, 20%; 30-45 min, 30%; 45-50 min, 30%; 45-50 min, 35%, 50-60 min, 50%, 60-65 min, 80%; 65-67 min, 100%; 67-70 min, 100%; 70-73.50 min, 7%. The flow rate was 0.8 mL/min and the absorbance was recorded at 260 nm (phenolics) and 520 nm (anthocyanins). Nitrogen was used as the nebulizing and drying gas. ESI conditions were as follows: nitrogen pressure: 6 opsig; drying gas 12 L/min at 350°C; ion spray voltage, 4000 V and fragmentor voltage, 80 V. An appropriate aliquot of the samples, usually 20 µL (10 µg polyphenols) was injected for the analysis. The concentration of individual phenolic compounds was based on the % total area and the total polyphenols, as determined above. Phenolic compounds were identified by library (NIST 2008) search and comparison to the stored spectra of authentic compounds. All the analysis was carried out in four independent determinations.

HS-SPME/GCMS analysis of volatile compounds

Individual samples of strawberries were homogenized using a Brinkman Homogenizer, fitted with a Polytron PTA 10 probe to make a puree. Twenty grams of puree were then poured into a 250 mL flask and tightly closed. After 30 min, a solid-phase micro-extraction (SPME) injection unit Supelco® fiber (with the diameter 100 nm, coated with polydimethylsiloxane) was inserted in the head space and volatiles allowed to adsorb. GC-MS analysis was conducted with a Saturn 2000R (Varian Inc) GC-MS system. SPME fiber was inserted into the GC-MS inlet maintained at 250 °C and volatiles were desorbed for 5 min. The oven temperature was maintained at 40 °C and ramped up at the rate of 8 °C/min to 220 °C and held for 10 min. The flow rate of the carrier gas helium was held constant at 1 mL/min. The eluted compounds from the column were ionized by electron bombardment. Ions with m/z ratio from 40 to 450 were recorded for analysis. Volatile components were identified by library (NIST 2008) search and comparison to the stored spectra of authentic compounds. All the volatile analyses were carried out in four independent determinations.

Statistical analysis

Statistical analyses were conducted using a GraphPad Prism software (Prism 4 Statistics Guide, 2003). Treatment effects were analysed using a one way ANOVA. Means were separated using Tukey's test ($p < 0.05$). Means followed by the same letter are not statistically different.

Results and Discussion

The composition of anthocyanins in the control and treated strawberry are given in Table 1. A significant increase of pelargonidin-3-glucoside was discovered in treated strawberry. In this study, the hexanal formulation treatment had increased the pelargonidin-3-glucoside content while the other compounds from flavanols such as catechin and the derivatives of proanthocyanidins were decreased.

Table 1. Composition of phenolic compounds in control and treated fruits

Main phenolic compounds	Control	Treatment
Pelargonidin-3-glucoside	21.5 ± 1.0 a	26.5 ± 1.8 b
Pelargonidin-3-malonylglucoside	19.7 ± 1.0 a	15.1 ± 6.0 a
Proanthocyanindins	9.9 ± 0.3 a	5.0 ± 0.8 b
Catechin	2.6 ± 1.8 a	1.4 ± 0.8 b

Data are expressed as mg/100 g of fresh weight

The increase of pelargonidin-3-glucoside was suggested to be related to the phenylalanine ammonia lyase (PAL) activity. The activity of PAL has been suggested to increase concomitantly with the accumulation of anthocyanins during the ripening of strawberry (Cheng and Breen, 1991). The production of pelargonidin-3-glucoside through UDP-glucose:flavonoid-3-O-glucosyltransferase (3-GT) activity was shown to be parallel with the PAL activity (Given et al., 1988). Applying preharvest spraying of hexanal formulation 7 days before the harvest time could be the best time as the PAL activity starts to increase more than 8 fold after 23 days after anthesis, when anthocyanins accumulate at a remarkable rate (Cheng and Breen, 1991). By spraying hexanal formulation containing cinnamic acid, it might induce the synthesis of anthocyanins especially pelargonidin-3-glucoside. In sweet cherries, fruits treated with a hexanal formulation via a preharvest spray have better colour, brightness and firmness than unsprayed cherries even after 30 days of storage at 4□C (Sharma et al., 2010). In addition, the level of anthocyanins and some of phenolic compounds in sweet cherries were also maintained for up to 30 days in cold storage after spraying with hexanal formulation, up to 30 days in cold storage. Tiwari and Paliyath (2011) demonstrated that genes for ACC synthase, a component of ethylene signal transduction was down-regulated in hexanal-treated tomato. This suggests that the inhibition by hexanal might result from inhibition of ethylene action. The rate of decrease in hexanal treated fruit was more pronounced and may indicate that the decrease could be the result of parallel activation of downstream enzymes in anthocyanin biosynthesis. Thus parallel activation and extra supply of cinnamic acid during spraying may suppress the enzyme leucoanthocyanidin reductase that is involved in the catechin and proanthocyanidins production. The competition of the substrate also might happen between the enzymes that lead to the production of flavan-3-ols and anthocyanins.

Esters are an abundant class of aroma components and found to dominate in strawberries (Pérez et al., 1992). Esters encompass 25-90% of the total number of volatiles in ripe strawberry fruit. The change in ester volatiles in response with hexanal composition is given in Table 2. Some of the volatile esters in treated fruits such as butyl acetate, isoamyl acetate, ethyl hexanoate, 2-hexyl acetate, hexyl butanoate had higher content compared to control fruits. However ketone, lactone and acid compounds in treated fruits had lower content compared to control.

The production of the volatile esters has been suggested through the breakdown of the fatty acids (Guadagni et al., 1971). The enzymatic breakdown of unsaturated fatty acid into C6-aldehydes and alcohols are the major contributor to the flavor of immature ‘Cigaline’ and ‘Chandler’ strawberries. However, at full maturity C6 compound levels decrease drastically with increased furanone, acid, lactone, and ester production (Pérez et al., 1992; 1996). These results suggest the involvement of fatty acids in the production of strawberry volatiles. The treatments conducted at the immature stage resulted in a significant decrease in most of the volatile esters. This result suggests that the treatment successfully delayed membrane degradation due to the deficiency of substrate such as fatty acids derived from the membrane degradation for the volatile production. Hexanal formulation treatment increased the levels of caryophyllene oxide nearly 3 fold. The increase of caryophyllene oxide after hexanal treatment may contribute to the lowered degree of pathogen infections in strawberries (Paliyath, unpublished).

Table 2. Profile of volatile compounds in control and treated strawberry

Volatile compounds	Control	Treatment
Butyl acetate	0.3 ± 0.1 a	0.1 ± 0.1 b
Isoamyl acetate	0.4 ± 0.1 a	0.2 ± 0.2 b
Butyl butanoate	0.1 ± 0.02 a	0.1 ± 0.2 a
Ethyl hexanoate	0.3 ± 0.1 a	0.1 ± 0.04 b
(Z)-hexenyl acetate	0.3 ± 0.1 a	0.2 ± 0.2 a
2-Hexyl acetate	1.2 ± 0.7 a	0.5 ± 0.7 b
Hexyl butanoate	0.5 ± 0.2 a	0.2 ± 0.1 b
Hexyl butyrate	0.1 ± 0.04 a	0.3 ± 0.3 a
Methyl salicylate	0.2 ± 0.7 a	0.2 ± 0.1 a
Caryophyllene oxide	5.1 ± 2.0 a	14.7 ± 2.6 b
Ketone	3.9 ± 2.6 a	0.3 ± 0.4 b
Lactone	0.1 ± 0.1	nd
Acid	0.4 ± 0.3 a	0.1 ± 0.1 b

Means within a row having the same letter are not significantly different using Tukey's test ($p<0.05$).
The value given represents the % relative area and was obtained from four replicates.

Conclusions

When hexanal, a PLD inhibitor was applied to the strawberry fruits, the treatment greatly affected several phenolic compounds and volatile profile. Pelargonidin-3-glucoside, the most abundant anthocyanins in strawberry was increased by hexanal treatment. The lower productions in several ester volatiles in treated strawberry suggest that the degradation of membrane is minimized by the treatment and therefore it delays the production of volatile components. Hexanal treatment may thus provide increased nutritional quality as well as enhanced shelf life through limiting membrane deterioration in strawberries.

Acknowledgments

We wish to thank Valsala Kallidumbil, Ramany Paliyath, Renu Chandrasekaran and Sabri Pakdek for assistance in the field. This study was facilitated by the SLAB and Universiti Putra Malaysia.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Physico-Chemical Properties of Tomato Fruit (*Lycopersicum esculentum*) at Different Maturity Indices

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Introduction

The tomato (*Lycopersicum esculentum*) is one of the most widely consumed fruit vegetables in the world (Leonardi, 2000). Tomato can be consumed at any maturity index even when it is still immature. It is also widely used by the food industries as a raw material for the production of derived products such as purees or ketchup. A series of quantitative and qualitative changes in the chemical compositions take place during tomato fruit ripening (Petro-Turza, 1987). Thus, different indices of tomato may have different physico-chemical properties.

The fruit has three major parts i.e skin, outer pericarp, and locular cavity where the characteristics of each are different physically and chemically. During preparation and processing of tomato into products, some parts of tomato such as skin, outer pericarp, and locular cavity may be removed. Therefore the knowledge on the physico-chemical properties of tomato fruits at different maturity indices and at different parts (i.e skin, outer pericarp and locular cavity) will help to educate the consumers on the beneficial nutrients they obtained when they consumed the fruit and also make them aware of the benefits that they missed out from the tomato parts that had been removed. This will help the consumers to use tomato at its utmost benefit for better life.

Thus, this study was carried out to determine the physico-chemical properties of tomato at different maturity indices as well as at different parts of the fruit which are skin, outer pericarp, and locular cavity.

Materials and Methods

Tomato variety Holland 1039 from TKPM Lojing Kelantan was harvested at three different maturity indices (index 2, 4 and 6). The physico-chemical analyses were carried out at three different parts (skin, outer pericarp and locular cavity) for every maturity index.

Physico-chemical analyses

Color was determined using Minolta chromameter CIE Lab. Fruit firmness was measured using Texture Analyser TA. XTplus with P2N needle probe. The pH values for every part of the fruit were then determined using pH meter. Ascorbic acid contents of tomato fruits at different maturity indices and at different parts were determined based on colorimetric method by Jagota and Dani (1982). The pigments of lycopene, carotenoids, chlorophyll a and chlorophyll b were extracted using acetone-hexane (1:2) followed by absorbance reading at 503, 470, 662 and 645 nm respectively. The total soluble solids content was determined by using hand-held refractometer. Minerals (ash) content were also determined using muffle furnace at 550ºC for 6-8 h until white residues of constant weight were obtained.

Experimental design and statistical analysis

This study used Factorial Experimental (FE) in Complete Randomised Design (CRD). All the data obtained were analysed using 2-way analysis of variance (ANOVA). The differences between the means were determined at 95% confident level ($P < 0.05$) by Tukey's Test. The statistical program used was SAS version 9.3.

Results and Discussion

Color: Tomato at maturity index 2 showed the highest color L* values. Comparing among different parts, outer pericarp had the highest color L* values. Meanwhile, tomato index 6 and locular cavity part showed the highest a* values. However, for color b* values, locular cavity had the highest value compared to skin and outer pericarp. In tomato puree and tomato sauce production, the color and lightness of the product is one of the important factors that determine the product quality (United State Department of Agriculture, 1975).

Firmness: The firmness values at different maturity indices and at different parts of the fruit showed significant differences ($p<0.05$) statistically. Fruits from maturity index 2 showed the most firm fruit and the outer pericarp was the most firm among the fruit parts. Overall, outer pericarp from index 2 fruit had the highest firmness value. Texture is an important attribute in evaluating the quality of tomato fruit and it is determined by the fruit morphological and physiological characteristics such as epicarp firmness, amount of locule tissue and maturity stage (Sammi and Masud, 2007).

pH: Different maturity indices and different parts of the fruits had significant differences ($p<0.05$) pH values. Tomato index 2 showed the highest pH value compared to tomato index 4 and 6. The pH values were also the highest at skin part. Researchers of United State Department of Agriculture (2011) had found that most under-ripe to ripe, cooked tomatoes have a pH below and around 4.6. Unripe fruit will have lower pH than ripe fruit.

Ascorbic acid content: Tomato maturity index 4 showed the highest ascorbic acid content compared to others, meanwhile the locular cavity parts show the highest among parts of the fruit. Study done by Romero et al. (1992) had proven that ascorbic acid content is higher when it is still immature and decline as the fruit hits peak ripeness. Ascorbic acid levels in tomato are reported to range from 10-120 mg/100 g fresh weight (Hobson and Davies, 1971).

Lycopene content: Tomato maturity index 6 and at the skin part showed the highest lycopene content. The carotenoids in tomatoes, especially lycopene, increases significantly during the ripening process. Thus, its concentrations differ depending on the stage of maturity (Gross, 1996). The ripening of tomatoes is characterised by the softening of the fruit, the degradation of chlorophylls and an increase in the respiration rate and ethylene production, as well as the synthesis of acids, sugars and lycopene (Cano et al., 2003).

Carotenoids content: Among different maturity indices, tomato index 6 showed the highest carotenoids content where the skin part had the highest amount. The vibrant red color of tomatoes is due to the presence of the carotenoid, especially lycopene. Beta-carotene may also contribute to the color profile, particularly in the case of immature or orange pigmented tomatoes (Anthon and Barrett, 2008).

Chlorophyll a and b contents: Tomato maturity index 2 and skin part had the highest chlorophyll a values compared to other maturity indices and parts respectively. Meanwhile, tomato index 4 had the

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

highest chlorophyll b content compared to others which mainly found also in skin. The green color of unripe tomatoes is contributed by the chlorophylls a and b content in its fruit. Red and blue light were the most effective color in photosynthesis, which absorbed by chlorophyll a and b. The ratio of chlorophyll a to chlorophyll b in the chloroplast is 3:1 (Von Elbe and Schwartz, 1983).

Total soluble solids content: There was no significant difference ($p>0.05$) in the total soluble solid of tomatoes at different maturity indices and at different part of the fruit. Theoretically, total soluble solid content should be increasing with the increasing in maturity indices. However, the total soluble solids of tomato fruits in this study might be influenced by the low concentration of carbohydrates in its fruits. The changes in soluble solids content in tomato are correlated with hydrolytic changes in starch concentration into sugar (Sammi and Masud, 2007).

Ash content: Tomato index 4 had the highest ash content as compared to other indices and the skin part was the part where ash was found the most. The mineral composition in terms of ash, calcium, magnesium, iron, potassium and phosphorus revealed that traditional vegetables were sufficiently rich in mineral content. Results in the present study was in contrast with the study done by Motegaonkar and Salunke (2012) which revealed that tomato had low ash content at ripe index especially low in iron content.

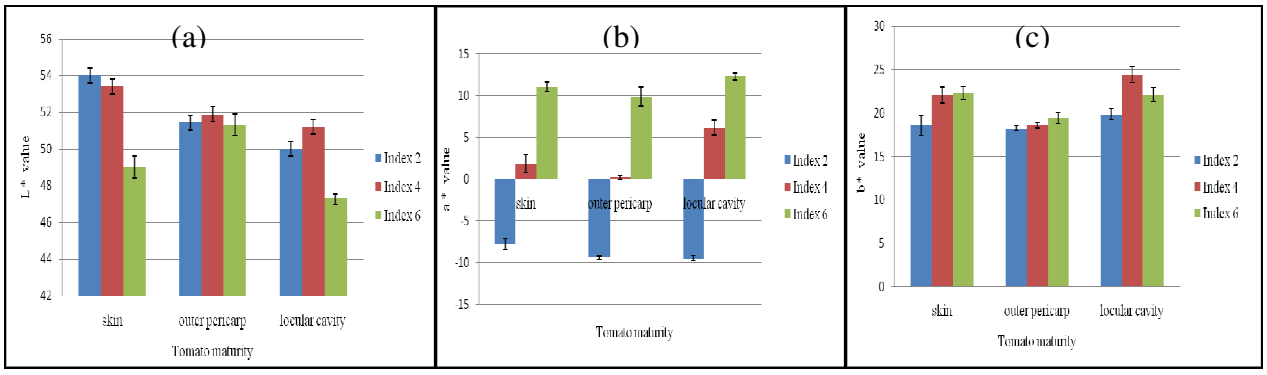

Figure 1: The colour of tomato at different maturity indices and at different parts of the fruits. (a) L* value; (b) a* value; (c) b value.

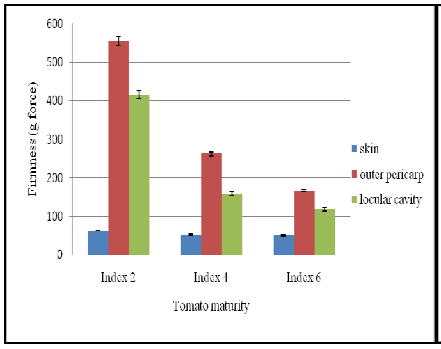

Figure 2: The firmness of tomato at different maturity indices and at different parts of the fruits

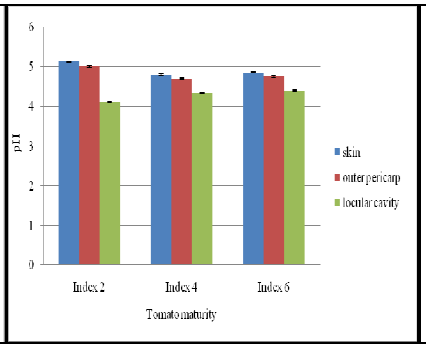

Figure 3: The pH values of tomato at different maturity indices and at different parts of the fruits

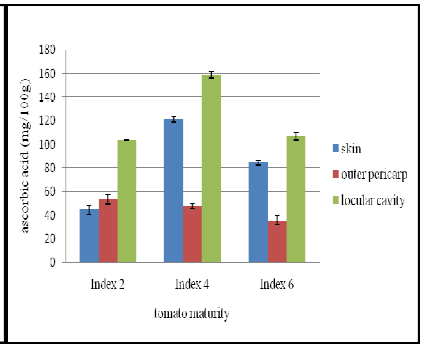

Figure 4: The ascorbic acid content of tomato at different maturity indices and at different parts of the fruits

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

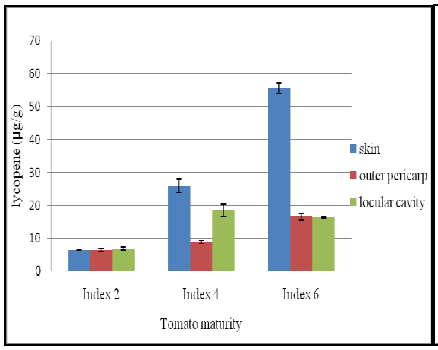

Figure 5: The lycopenes content of tomato at different maturity indices and at different parts of the fruits

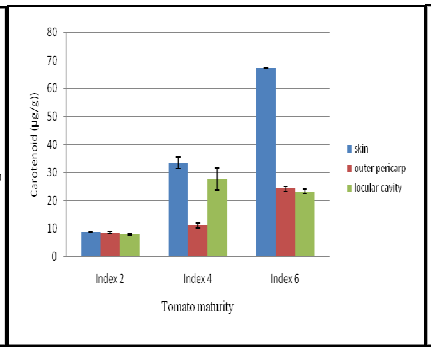

Figure 6: The carotenoids content of tomato at different maturity indices and at different parts of the fruits

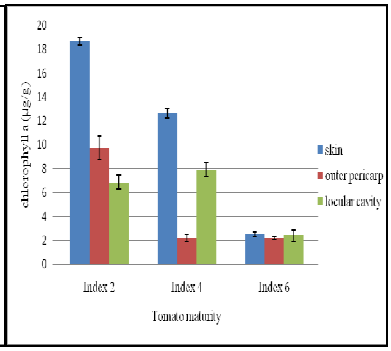

Figure 7: The chlorophyll a content of tomato at different maturity indices and at different parts of the fruits

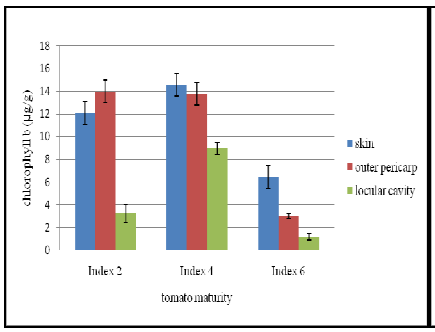

Figure 8: The chlorophyll a content of tomato at different maturity indices and at different parts of the fruits

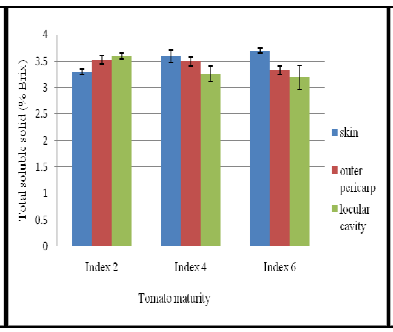

Figure 9: The total soluble solids of tomato at different maturity indices and at different parts of the fruits

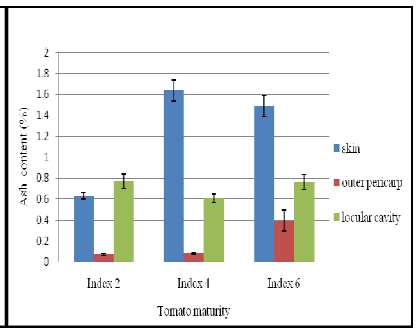

Figure 10: The ash content of tomato at different maturity indices and at different parts of the fruits

Conclusions

Tomato index 2 had the highest color L* value, firmness, chlorophyll a, and pH values. Meanwhile, tomato index 4 contains the highest ascorbic acid and ash contents and tomato index 6 had the highest values for color a*, carotenoids, and lycopene contents. Among different parts of the fruits, skin showed the highest values for color L*, chlorophyll a, and pH especially in tomato index 2. The skin of tomato index 4 contain the highest ash content while the skin of tomato index 6 had the highest color a* value and lycopene content. Outer pericarp part, showed was the most firm especially for tomato index 2. Meanwhile, the locular cavity had the highest in color b* value and ascorbic acid content mainly in tomato index 4.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Allelopathic Potential of Guinea Grass (*Panicum maximum*)

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Introduction

The desire to improve the year long production of high quality forage especially in the tropics and increasing concern about nitrogen fertilizer application to perennial pastures has increased the importance of intercropping grass with legume pastures. However, it is a known fact that successful establishment of these species as intercrops is beset with problems arising from competition for space, light, soil nutrients and possibly allelopathy.

Allelopathy is defined as biochemical interactions between one plant or microorganisms (algae, bacteria or virus) and another through the production of chemical compounds (secondary metabolites which influence, directly or indirectly, harm or benefit plant growth and development (Rice, 1984). Allelochemicals are present in almost all plants and in tissues like leaves, stems, flowers, fruit, seeds, roots and pollen and may be released from plants into the environment by volatilization, leaching, root exudation and decomposition of plant residues. Several forage grasses have been found to have allelopathic effects over other plants. Chou and Young (1975) found phenolic growth inhibitors and two unknown ninhydrin positive compounds from 12 subtropical grasses. Sunyata and Tosapin (2010) reported that water soluble extract from all parts of itch grass (*Rottboellia cochinchinensis*) had inhibitory effect on growth of some test plants, similarly, Chon et al. (2003) also found that aqueous extracts of lettuce leaf inhibited root growth of alfalfa and barnyard grass

The study was initiated with the objective of determining whether *Panicum maximum* had some allelophatic influence on the growth of some forage legumes based on the results of a competition experiment undertaken in 2010 involving *Panicum maximum* (guinea grass) and four legume forages; *Stylosanthes guinanensis* (stylo), *Centrosema pubescens* (centro) *Macropitilium bractaetum* (burgundy bean) and *Arachis pintoi* (*Arachis*). The dry matter yields of all legumes in mixtures irrespective of ratio combination were depressed by the presence of guinea grass (Baba et al., 2011)

The objective of the study was to determine whether water soluble root or leaf extract of guinea grass as well as soil incorporated root or leaf powder will affect growth and yield of the legumes in question

Materials and Methods

Experimental location

The experiment was conducted in the Nutrition Laboratory of Crop Science Department Universiti Putra Malaysia. The University is located at latitude of 3°02' N, longitude 101°42' E, and altitude of 31 m above sea level. Mean monthly rainfall from January to July 2012 was approximately 139.4 mm. Mean monthly minimum and maximum temperatures were 25.5 °C and 33.6 °C respectively while average monthly relative humidity was 93.4%.

Experiment 1 (Laboratory bioassay – effects on germination and seedling characteristics)

Root and leaf materials were obtained during a six weekly harvest from experimental plots used to study the performance of mixtures of guinea grass and each of the legumes earlier mentioned in year 2011. The harvested root and leaf materials were washed free of soil using tap water, and sun dried for a period of one week. Materials were later ground to pass through 2 mm sieve and then stored in air tight small plastic bottles. Ten g each of the ground root and leaf materials was then added to separate containers containing 100 ml of distilled water and left for a period of 24 hours to prepare 10% (w/v) root and leaf extracts solution. The contents were agitated with a magnetic stirrer for twenty minutes and filtered through two layers of white cheese cloth. Further solutions of lower concentrations (0.5, 1, 1.5 and 2% solution) were made from this stock solution. pH of the various extract concentrations was determined using a digital pH meter. Germination test was conducted using 8.5 cm petri dishes. Seeds of grass and legumes were placed in Petri dishes lined with 9cm Whiteman's filter papers (number 1) that were moistened with 5 ml of the appropriate concentrations of the water soluble root/leaf extracts. Control treatments were watered with distilled water. Number of germinated seeds was recorded on the second day of the experiment initially and every other day subsequently, until no further germination was observed. Radicle and shoot lengths were measured at the end of germination period for each species (usually 5 days). The experimental design was a 3 ×5 factorial in a completely randomized design.

Experiment 2 (Pot trial – effects of leaf powder and root powder)

The experiment was conducted at Faculty of Agriculture Farm (Field 2) University Putra Malaysia Malaysia. Leaf and root materials of *Panicum maximum* were obtained from the same location as in experiment 1. The materials were washed free of soil under a running tap water, sun dried for one week and then ground to pass through 2mm sieve. The resultant root and leaf powders were collected and stored in self sealed plastic until used. A soil mixture was prepared at the ratio of 3 top soil to 1 sand using a mechanical mixer. The soil mixture was filled into 36 plastic pots with the dimensions 19 cm width, 18cm length and 12 cm base width. Twenty g root powder each was incorporated into a total of 9 pots containing the soil mixture. Similarly, another 9 pots had leaf powder (20 g pot^{-1}) incorporated into the soils in them making a total of 18 pots. The soils in the remaining 18 pots had neither root nor leaf powder and thus were used for control plants. Water was sprinkled into the soils contained in the pots, thereafter, ten seeds each of stylo, burgundy and centro were sown at the soil surface and lightly buried (about 1 cm depth) . Each species was replicated 3 times, i.e. for both leaf and root powder incorporated. Controls were similarly replicated for both powders. The plants were subsequently watered using the sprinkler irrigation system. The seedlings in each pot were thinned to 5 plants per pot at exactly 3 weeks post planting. No fertilizer was applied. Harvesting was carried out at 6 weeks post planting, during harvest, height of the individual plants was taken from the soil surface to the tip of the shoot which represented shoot length. Root length was also measured. Total fresh weight of whole plant after harvest and dry matter yield were obtained after oven drying.

Results and Discussion

Experiment 1: Leaf extract

Different concentration of leaf extracts did not show significant effects on radicle length. In contrast, shoot length and germination percentage were significantly ($p<0.05$) depressed at 1.5% and 1% leaf extract concentration respectively. Interaction between species and concentration levels on shoot length revealed that shoot length was not affected by leaf extract concentration in burgundy and centro while the length was reduced ($p<0.05$) at 1% leaf extract concentration in stylo. Kato Naguchi (2004) observed a reduction in hypocotyl length of lettuce, timothy and ryegrass by leaf extracts of *Peuraria*

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

thumbergiana. The reduction in shoot length may be due to reduced rate of cell division and cell elongation as a result of the presence of allelochemicals (Javaid and Anjum, 2006).

Radicle length, shoot length and germination percentage differed significantly among legume species. The differences observed among species may be attributed to differences in seed size as large seed size meant greater carbohydrate reserve which may confer initial growth advantage in the growing seedling. The radicle length was greater ($p<0.01$) in centro compared to stylo and burgundy, the length in stylo was superior ($p<0.01$) to that of burgundy. In the case of shoot length, centro had the highest (8.7 cm) which was significantly greater ($p<0.01$) than burgundy and stylo. Germination percentage was observed to be highest in stylo (86.7%) and significantly higher ($p<0.01$) than those of burgundy and centro. Burgundy recorded the lowest germination percentage.

Experiment 1: Root extract

Different concentrations of root extracts did not significantly affect germination percentage or radicle length. However, shoot length was significantly depressed at 1.5% root extract concentration for Stylo (Figure 1) but not in other species.

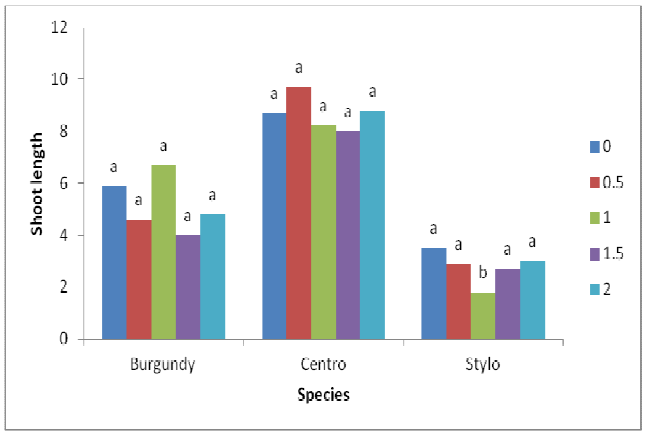

Figure 1. Interaction between legume species and leaf extract concentration on shoot length

Experiment 2: Leaf and root powder

Significant interactions were observed between species and powder type on dry matter yield, root and shoot lengths as shown in Figures 2, 3 and 4. Leaf powder significantly increased ($p<0.01$) dry matter yield in centro and stylo (Figure 2), shoot length in burgundy (Figure 4) and root length in stylo (Figure 2). In contrast the dry matter yield of burgundy was significantly ($p<0.01$) depressed by root powder (Figure 2). Root length in burgundy was also reduced significantly by root powder (Figure 3) while root length in centro was neither affected by root nor leaf powder. Xuan et al. (2005) reported reductions in root elongation, germination and growth of banyard grass following the incorporation of kava and alfalfa root powders.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

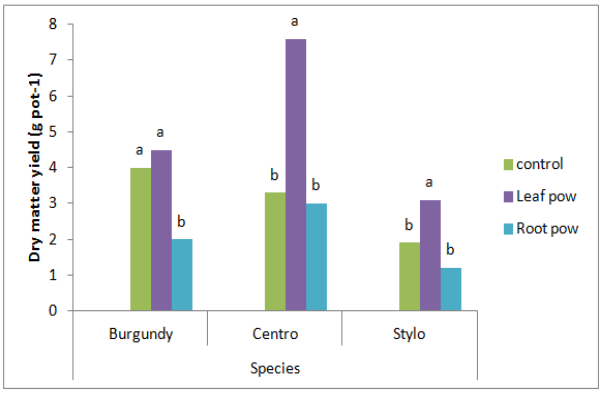

Figure 2. Interaction between legume species and root/leaf powder on dry matter yied.

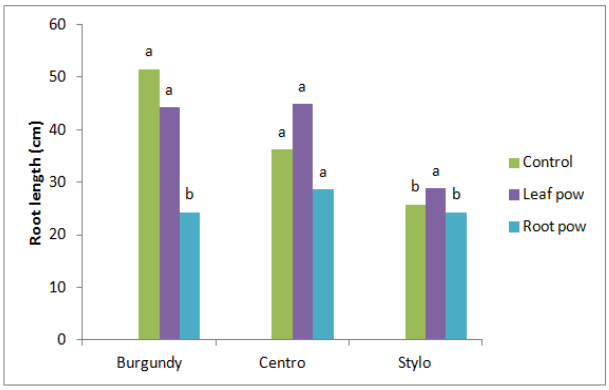

Figure 3. Interaction between legume species and root/leaf powder on root length

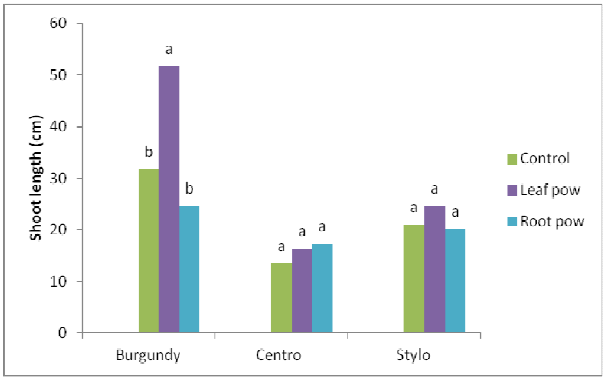

Figure 4. Interaction between legume species and root/leaf powder on shoot length

Conclusions

The results of the experiment indicate that *Panicum maximum* (root powder) showed allelopathic tendency towards burgundy, given the fact that, dry matter yield and root length were reduced significantly. The dry matter yields of centro and stylo were enhanced by leaf powder.

Acknowledgements

The Authors gratefully acknowledge the contributions of Universiti Putra Malaysia and Bayero University Kano, Nigeria for funds and permission to carry out the study respectively.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

An Alternative and Quicker Strategy to Improve Rice (*Oryza sativa)* Yield through Application of Phytohormones

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Introduction

Rice (*Oryza sativa* L.) is a staple food for more than two third of the world's population (Dowling et al., 1998; FAO, 2010). It has been estimated that more than 200 million tons of rice are lost every year due to environmental stresses, diseases and insects pests (Chen and Murata, 2002). In Malaysia, low yield productions form the potential yield is one of the factors that affect the total yield of rice that mainly at East Coast of Peninsular Malaysia such as Kelantan region which is one of the granary areas (second largest of granary area) in Malaysia with a commonly used variety, MR 219. However, the self-sufficiency level of rice for Malaysia is only 72% in 2010 and the Malaysian government has decided to self sufficiency by the year from 2015 to 2020 (MOA, 2011). Average yield for the country is about 4 t/ha (MARDI, 2009) and potential yield was 10 t/ha by using MR219 variety. An alternative and quicker strategy to increased yield production is through exogenous application, including phytohormones through foliar application to increase the grain filling of rice. Recently, this strategy has gained considerable attention because of its efficiency, feasibility, and cost and labor-effectiveness. These are either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yield, or facilitate harvesting. In this study, several phytohormones with different time of foliar application were used to evaluate the potential of the phytohormones in increasing the grain yield in glasshouse study.

Materials and Methods

Common widely cultivated rice variety, MR219 was used in this experiment as planting material. The seeds were sown overnight by using seed priming product from ZAPPA-PLUS (PeladangTech, Bangi, Selangor, Malaysia) and spread in wet tissue inside the tray for overnight. The seed were sown approximately after two days by direct seedling technique with 3 seed per pale with size 390 width × 390 Diameter × 350 mm height containing approximately 17 kg of soil obtained from a rice growing area Melor, Kelantan, Malaysia. The experiments were conducted under glasshouse facilities at Rice Research Centre, Field 10, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Four treatments of foliar applications [control (distilled water), commercial products (0.5% of Vita-Grow Plus), 0.1 mg/L of Epibrassinolide, 10µM of Spermine and 0.2% of pyroligneous acid, three time of foliar applications at 35 (single spray), 35+55 (double spray) and 35+55+85 (triple spray) DAS (Day after sowing) were evaluated in a randomized complete block design (RCBD) with 5 replicates. Data collections were taken after 5-10 days after foliar applications of phytohormones was applied. Foliar applications of each hormones were prepared by adding 0.01% surfactant of tween 20% and applied in the early morning around 8-10 am by using backpack sprayer with nozzles oriented vertical spraying until all the leaves were wet. In the flooding treatments, water level was maintained in between 5 to 10 cm in the pots. Compound fertilizers were applied accordingly to Department of Agriculture, Malaysia with four times application at different stages (subsidiaries fertilizer from government of Malaysia). Compound fertilizer with contains N:P:K (15:15:15 ratio) was applied at 15-20 day after sowing (DAS) with 260 kg/hectare),

urea in between 35-40 DAS (100 kg/hectare) and N:P:K blue (12:12:17:2TE) in-between 50-55 (260 kg/hectare) and at 70-75 DAS (125 kg/hectare). Crop growth, chlorophyll contents, photosynthesis rate and yield components including growth parameters were taken after 10 days application of treatments. The data were statistically analyzed using the analysis of variance (ANOVA) procedure in the SAS Statistical Software Version 9.0, using a CRBD. Duncan studentized range test was used to compare variation among the treatments ($p<0.05$).

Results and Discussion

Chlorophyll content was the higher in all treatments (42.23, 41.93, 41.80 and 41.30 SPAD units) at 45 DAS, spermine (39.73 SPAD units) and commercial product (38.07 SPAD units) at 65 DAS and commercial product (37.51 SPAD units), spermine (36.57 SPAD units) and pyroligneous acid (35.51 SPAD units) at 95 DAS (Table 1). One or two or three time of application of foliar application has no effect on chlorophyll content at 45, 65 and 95 DAS was observed. The higher photosynthesis rate in treatment epibrassinolide and spermine at 45 DAS, spermine at 65 DAS and pyroligneous acid at 95 DAS with 19.81, 19.77, 24.18 and 13.28 µmol CO_2 m^{-2} s^{-1} was observed, respectively. One and two times were adequate time of foliar application at 65 and 95 DAS with 22.41 and 13.17 µmol CO_2 m^{-2} s^{-1}, respectively. The highest leaf area at 45, 65 and 95 DAS was commercial product, spermine and epibrassinolide with 1339.04, 19.71.10 and 1766 cm^2, respectively. The time for foliar application was suitable with one and three times was obtained with 1923.36 and 1659 cm^2, respectively. All treatments shows greater chlorophyll content indicates that more pigments chlorophyll inside the leaf active for photosynthesis. Generally, chlorophyll contents were increased in all foliar application of phytohormones especially in commercial products and spermine during 45 to 95 DAS. Interestingly, spermine and epibrassinolide shows greater photosynthesis rate at 45, spermine only at 65 DAS and pyroligneous acid at 95 DAS. Similar results with chlorophyll contents as well. The photosynthesis rate was increased with 28, 34 and 26% at 45, 65 and 95 DAS. More chlorophyll contents will produce more assimilates from photosynthesis and carbon partitioning as well. Similar finding in leaf area was improved with 62, 11 and 31% larger in treatment with commercial products, spermine and epibrassinolide, respectively. Overall findings that foliar application of phytohormones improved chlorophyll content, photosynthesis rate and leaf area meter/hills as well compared than control. That indicates that contain more chlorophyll pigments in the leaf, more solar radiation absorbed by leaf for the conversion of light energy to be stored as chemical energy, photosynthetic potential and primary production as well (Fillela et al., 1995). In addition, leaf chlorophyll content is closely related to plant stress and senescence (Merzlyak et al., 1999).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Table 1. Chlorophyll content, photosynthesis rate and leaf area in different phytohormones and time of foliar application at 45, 65 and 95 DAS.

Treatments	Chlorophyll content (SPAD Units)			Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})			Leaf area (cm^2)/hills		
	45 DAS	65 DAS	95 DAS	45 DAS	65 DAS	95 DAS	45 DAS	65 DAS	95 DAS
Flooding conditions									
Control	37.63b	28.42d	22.02c	15.47d	18.05e	10.51e	828.42e	1772.66e	1344d
Commercial product	41.93a	38.07ab	37.51a	18.14b	21.38d	11.09b	1339.04a	1836.01d	1734b
Epibrassinolide	41.30ab	33.87c	30.60b	19.81a	24.13b	10.91c	1037.59d	1881.71b	1766a
Spermine	42.23a	39.73a	36.57a	19.77a	24.18a	10.63d	1319.07b	1971.10a	1311d
Pyroligneous acid	41.80a	36.40b	35.51a	16.08c	22.86c	13.28a	1146.48c	1864.70c	1474c
Time of foliar applications									
One	NA	34.66a	32.88a	NA	22.41a	10.84b	NA	1923.36a	1534b
Two	NA	35.93a	32.23a	NA	21.83b	13.17a	NA	1807.11b	1384c
Three	NA	NA	32.21a	NA	NA	9.84c	NA	NA	1659a

The values are the average of four replications. Means within column followed by the same alphabets are not significantly different at p<0.05 (Multiple Duncan Test). DAS= Days after sowing. NA=Did not analyze. One=45 DAS, Two=45+65 DAS and three=45+65+95 DAS time foliar applications.

Interestingly, all treatments shows greater grain number per panicle, panicle and grain weight per hill compared than control were obtained in Table 2. Three time foliar applications was most suitable in grain number per panicle but difference with panicle and grain weight per hill there are not significantly difference between time of foliar applications. However, treatments with epibrassinolide and spermine shown greater result in 1,000 grain weight with 25.74 and 25.61 g but not significantly difference between times of foliar applications were observed. Pyroligneous acid and spermine shows the highest grain filling at 95 with 92% was obtained. One or two time application of foliar was significant with 89 and 45.9%. Grain filling at 115 DAS was the highest percentage in epibrassinolide followed by commercial product and pyroligneous acid with 97.16, 96.56 and 96.07, respectively. Highest grain yield per meter squared was obtained in all treatments with 1288.7, 1229.4, 1145.3 and 1114.3 g per meter squared but no significantly different at time of foliar applications. Generally, all phytohormones were used in this experiment have positive result in enhanced grain number per panicle (12-18%), panicle per hills (23-46%), grain weight (7-35%) and directly to yield per meter squared. In additions, almost in all parameters were taken shows better results in all phytohormones compared than control. A foliar application was the fastest and directly strategy to enhance the plant growth and yields as well. The better and good contribution of photosynthetic, translocation of assimilates, source and sink concept was the factor that affecting in higher grain yield as well through greater plant height, tiller number, chlorophyll content, photosynthesis rate, dry biomass productions and leaf area, root length, surface and volume as shows in previous tables and figures. They are correlated to each other for better production of grain yields as compared than control. In spikelets, better superior and inferior spikelets also influence the production of grain filling as thus yield as well. This result might be correlated to this concept due to significantly increased main important rice yield components (tillers per unit area, spikelets per panicle, percentage of filled grains and grain weight) that influence the grain yield productions as described by Mohapatra et al. (2009) and Kato et al (2007). The yields per meter square were increased 30, 24, 15 and 12% in treatments commercial products, epibrassinolide, spermine and pyroligneous acid, respectively compared than control. The treatments have their own advantages such as commercial products and pyroligneous acid have rich nutrients components inside and growth regulators while epibrassinolide and

spermine have important roles in regulation of DNA replication and cell division, controlling of morphogenesis and senescence and resistance to environmental stresses (Galston and Sawhney, 1990; Martin-Tanguy, 2001; Kaur-Sawhney et al., 2003; Couée et al., 2004). As reported by Gifford and Evans (1981), a high yield in treatment with all treatments due to large amounts of assimilates partitioning and the associated high sinks strength by source sink relationship.

Table 2. Yield attributes in different phytohormones and time of foliar applications at 115 DAS.

Treatments	Grain number per panicle (no.)	Panicle per hill (no.)	Grain weight per hill (g)	1,000 grain weight (g)	Grain filling (%)	Grain weight (g) per m^2
Drought stress conditions						
Control	118b	13b	37.65b	24.17b	91.01d	994.6b
Commercial products	137a	19a	49.81ab	24.52b	93.13b	1288.7a
Epibrassinolide	136a	17ab	50.87a	25.74a	91.53c	1229.4a
Spermine	132ab	16ab	40.43ab	25.61a	94.41a	1145.3ab
Pyroligneous acid	139a	16ab	49.08ab	24.40b	93.73b	1114.3b
Time of foliar applications						
One	126b	16a	45.23a	24.6a	96.20a	1143.2a
Two	132ab	16a	46.52a	24.45a	96.22a	1159.7a
Three	139a	17a	44.95a	24.58a	95.94a	1160.5a

The values are the average of four replications. Means within column followed by the same alphabets are not significantly different at p<0.05 (Multiple Duncan Test). DAS= Days after sowing. NA=Did not analyze. One=45 DAS, Two=45+65 DAS and three=45+65+95 DAS time foliar applications.

Conclusions

In addition of foliar application of phytohormones, clearly indicated that increased the growth and yield of rice per meter square with one time of applications was sufficient. Rice yields component increased 12-30% with foliar applications of phytohormones as well.

Acknowledgements

The authors acknowledge the financial and technical support by Universiti Putra Malaysia and the Ministry Higher Education Malaysia for Long-Term Research Grant Scheme (LRGS)-Food Security UPM.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Influence of Seed Maturity Stages and Drying Methods on Seed Vigour of *Solanum melongena* L.

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Introduction

Several studies have showed that high quality seed is not only determined by seed germination performances but also seed vigour such as reported by Vidigal *et al*. (2011) in sweet pepper and Nautiyal et al. (2010) in groundnut. However, many factors could contribute to the variation in seed vigour such as the genetic information carried by the variety itself, harvesting time (seed maturity), mechanical damage during processing, and storage condition. In this study, two factors will be focused, harvesting time and mechanical damage during processing. In the case of brinjal, the most critical stage during processing is seed drying.

Seed maturity is one of main components affecting seed quality. It is a prerequisite for successful germination and emergence (Perry, 1982). According to Harringtons (1972), seeds attained maximum vigour at the end of the seed filling period. The stage was termed as physiological maturity (Sanhewe and Ellis, 1996). Suprakarn (2005) suggested harvesting fully ripe fruits (brownish colour) of purple variety of brinjal for seed production. Harvesting time for seed production may vary depending on cultivar, climate and growing conditions (Passam et al., 2010). Therefore, it is important for seed producers to determine the physical indicators of physiological maturity and harvesting time of each particular variety to ensure highest seed quality.

Besides that, different drying methods may also influence seed vigour. Studies done by Roopa (2006) on muskmelon, and Christinal and Tholkappian (2012) on chilli showed that different drying methods did influence seed vigour. A new technology for drying seed has been introduced by Rhino Research Group using Drying Beads®. It can reduce seed moisture content rapidly compared to conventional method. However, it is important to determine the effects of bead-drying on brinjal seed vigour as compared to the conventional method, sun-drying.

Therefore, this study was aimed at determining physiological maturity stage and its physical indicator, seed vigour of brinjal at different seed maturity stages (40 DAA to 60 DAA) and the effects of drying methods, namely sun-drying and bead-drying on seed vigour as indicated by the electrical conductivity test.

Materials and Methods

MARDI brinjal variety Mte1 was used in this study. Three hundred plants were grown on a 3 m x 100 m plot with a spacing of 1 m x 1 m. The crop was raised following the agronomic practices for seed production as recommended by Malaysian Agricultural Research & Development Institute (MARDI). Six hundred flowers at full anthesis were tagged from April 3 until April 22 in 2013. Fruits were harvested at 40, 45, 50, 55, and 60 days after anthesis (DAA). Fruit colour was recorded for the determination of physical indicator for harvesting by using colour chart Royal Horticultural Society (5th

EDITION, LONDON). For the determination of physiological maturity, fresh and dry weight of 100 seeds extracted from three fruits harvested at each harvesting time was determined by methods described by ISTA (2006).

Seed processing was done as recommended by MARDI. The balance of harvested fruits were sliced and fermented by soaking in water for 2 days at room temperature (30±2 °C). Good seeds that sank to the bottom were collected and cleaned using clean water while floated seeds and pulps were discarded. Extracted seeds were air-dried on mesh trays to remove seed surface moisture. A piece of tissue paper was used to check the existence of surface moisture and the process was stopped once no seed stuck to the tissue paper. The pre-dried seeds were divided into two batches. One batch of seeds was dried using sun-drying on mesh trays while another batch was dried using bead-drying (Drying-beads®) in an air-tight glass jar until moisture content of both seed batches reached 10%. Dried seeds were kept in air-tight glass bottles prior to quality testing. Electrical conductivity (EC) of seed was determined using a conductivity meter CON510 (EUTECH INSTRUMENTS, SINGAPORE). Seed vigour test was done following ISTA (2006). Four replicates of 50 seeds from each treatment were weighed and soaked in 50ml deionized water at 25 °C for 24 hours before the EC readings were taken. Results were expressed in $\mu S\ cm^{-1}\ g^{-1}$. Statistical procedure was carried out using the SAS software and data were analysed using ANOVA. Treatment means were compared using the Duncan's Multiple Range Test.

Results and Discussion

During ripening, fruit changes colour from purple to brown beginning from the tip of fruit towards the calyx. At 40, 45, 50, 55 and 60 DAA, the fruits have turned 20%, 60%, 85%, 95% and 100% yellowish brown, respectively (Figure 1).

Results (Figure 2) show that physiological maturity was achieved at 50 DAA, i.e when seed dry weight was at the maximum and seed fresh weight started to decrease. At this stage, fruits were observed with 85% yellowish brown and 15% light purple in colour. In contrasting reports, Demir et al. (2002) reported brinjal cultivar 'Pala' grown in Hatay, Southeastern part of Turkey in 1999 and 2000 showed that maximum seed dry weight was occurred at 40 and 42 DAA when fruits were observed with dull purple and bright purple in colour, respectively. In another study done by Yogeesha et al. (2008), results showed that maximum seed dry weight occurred at 53 DAA while fruits started turning to brown at 57 DAA. These differences might have occurred because of variation in type of cultivar used, climate and growing conditions (Passam et al., 2010).

Figure 1. Brinjal fruit harvested at different time (day after anthesis, DAA) showing colour change.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

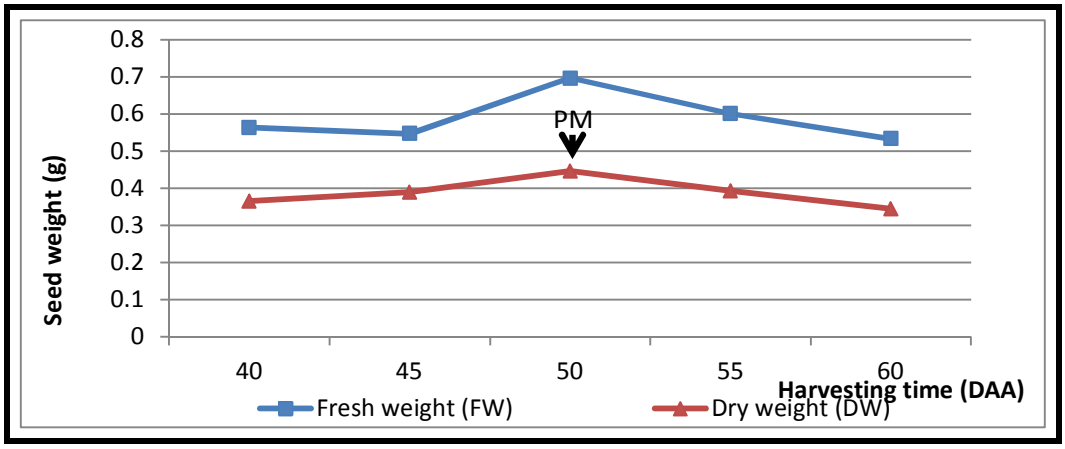

Figure 2. Physiological maturity (PM) of brinjal, *Solanum melongena* L.

After ANOVA, data have shown that different drying methods, both sun-drying and bead-drying do not have significant difference on seed vigour, however, seed maturity stages (DAA) did. It was proven by the similar pattern of electric conductivity values for both seeds batches dried using sun-drying and bead-drying at different maturity stages (Figure 3).

The highest electrical conductivity value was obtained at 40 DAA, and drastically dropped at 45 DAA, then increased at 50, 55 and 60 DAA. High electrical conductivity obtained at 40 DAA was probably because the seeds were still immature and their membranes were not well structured, thus causing more electrolytes leakages, therefore high electrical conductivity value was obtained. It indicates the seeds had poor seed vigour. Similar results were found by Martins et al. (2012) in brinjal and by Vidigal et al. (2009, 20 11) in sweet pepper.

At 45 and 50 DAA, low electrical conductivity values were observed. It was probably due to an increase of cell membranes integrity and subsequent reduction of electrolytes leakage along seed development. Similar results were reported by Vidigal et al. (2011) in sweet pepper and Demir and Ellis (1992) in tomato. According Bewley and Black (1994), during reserve deposition phase in final stage of seed maturation, there is also accumulation of potentially protective molecules especially *lea* (late embryogenesis accumulated) proteins and soluble sugars to prevent the membrane damage caused by water removal from seed tissues. Thus, seeds harvested at 45 and 50 DAA are considered as high vigour seed.

At 55 DAA and 60 DAA, the electrical conductivity increased more than 25 $\mu S\ cm^{-1}g^{-1}$ indicating decrease in seed vigour. Similar results were reported by Martins et al. (2012) in brinjal, in which electrical conductivity increased seven days later after seed reached physiological maturity at 70 day after pollination (DAP). By referring to classification of electrical conductivity values, these results suggested seed harvested at 55 DAA is not suitable for early sowing especially in unfavourable condition while seed harvested at 60 DAA has low vigour, thus not suitable for sowing (Milošević et al. 2010).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

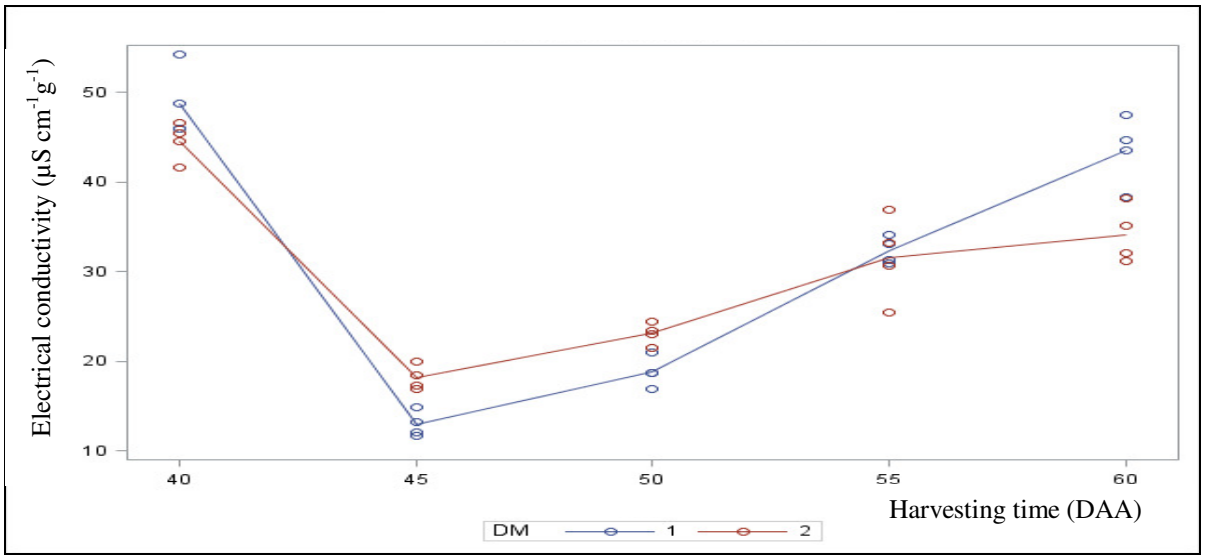

Figure 3. Changes of electrical conductivity values during seed maturation (µ*S* $cm^{-1}g^{-1}$) DM- Drying method, DAA- Day after anthesis, 1- Sun-drying, 2- Bead-drying

Conclusions

From the results of this study, it is suggested that seed vigour of Mte1 brinjal is not affected by both drying methods used in this study, however seed maturity stages did. Seeds were still immature at 40 DAA while seeds harvested at 60 DAA were over mature. Physiological maturity was determined to be at 50 DAA. Seeds harvested at 45 and 50 DAA had the highest seed vigour. Therefore, it is recommended to harvest the fruits as early as 45 DAA until 50 DAA when the fruits were observed with 60 to 85% yellowish brown in colour. Further tests on seed germination and seed emergence are being conducted.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Preliminary Study on Shoot Growth Subsequent to Grafting in Durian Clone D168

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Introduction

Durian is propagated vegetatively due to the advantages of preserved trait, smaller in stature and early fruiting (Brown, 1997) most often by the bud-grafting technique. Apart from 20 weeks of rootstock preparation, the technique takes around 20-22 weeks before the shoot reaches suitable size for transplanting in the field. Information on the dynamics of scion growth subsequent to grafting, however, is lacking and even though the entire union process is the same for all species, interaction between the various stages of the process and subsequent time for completion is plant specific (Dolgun et al., 2008). A study was conducted in order to improve the understanding on shoot growth rate in D168 durian (*Durio zibethinus*) as an attempt to shorten the production period of D168 durian planting material.

Materials and Methods

The experiment was conducted at the nursery of Planting Material, Seed and Livestock Breed Production Unit, MARDI. Rootstocks were produced by sowing seeds on a sand bed for 1 month and the seedlings were subsequently transplanted to 20 x 30cm perforated polyethylene bags. The plants were placed under 50% shade netting and watered twice a day. After 5 months, D168 scions from certified mother plants were collected and bud-grafted onto the rootstock. The shoot length, measured from the base to the tip of the shoot and number of fully expanded leaves that developed from scions were measured every week for the first 7 weeks and triweekly for the next 15 weeks after grafting.

Results and Discussion

Results shown that scion bud started growing between 2-3 weeks after grafting. Growth of shoots can be separated into three different stages with significant growth changes between them; lag phase (week 0-6), intermediate growth phase (week 6-16) and exponential growth phase (week 16-22 (Figure 1 and 2).

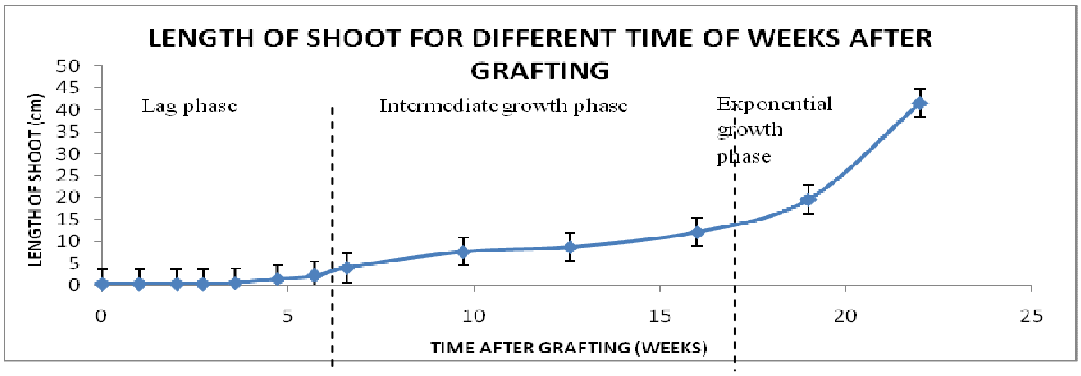

Figure 1. Length of shoot at different time (week) after grafting. *SE bars represent means from 10 samples

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

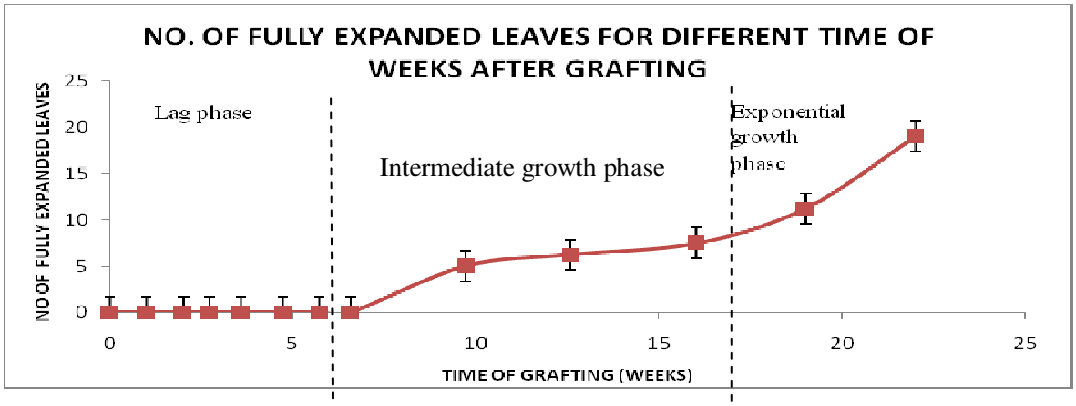

Figure 2. Number of fully expanded leaves at different time (week) after grafting. *SE bars represent means from 10 samples

During these phases, several major events occurred; cohesion of the rootstock and scion, proliferation of callus, cambial bridging and the healing of union (Hartmann et.al, 2010). From week 0 – 6, growth rate was slow at only 0.09 cm/week (Fig. 3). At this stage, proliferation of callus occurred during the first 6 weeks after grafting, thus explaining the slow growth of shoot.

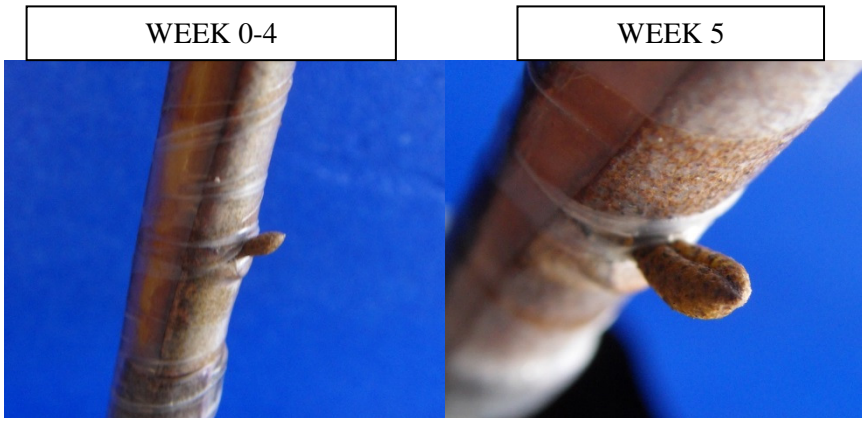

Figure 3. Shoot growth of D168 durian during the lag phase.

Growth slowly picked up from week 6 - 16 with a rate of 1.13 cm/week (Fig. 4). At the 10th week of grafting, several numbers of fully expanded leaves were recorded. During this phase, the callus that formed during previous phase initiated rootstock-scion interaction that create bridge or conducting tissues for water and nutrients transport to the scion, thus growth rate gradually picked up (Asante and Barnett, 1997).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

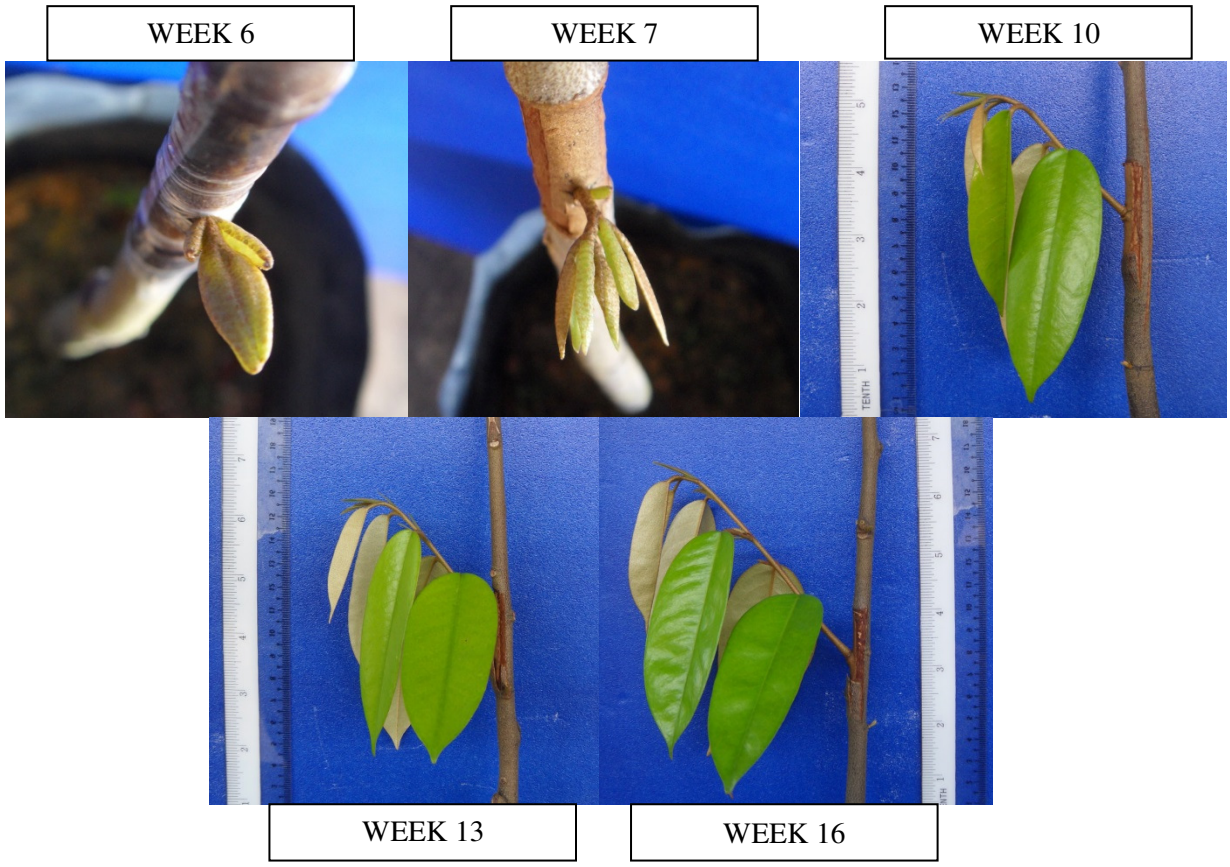

Figure 4. Shoot growth of D168 durian during the intermediate growth phase.

Growth rate was highest from week 16 - 22, at 7.33 cm/week (Fig. 5). Growth rate was highest during the exponential phase since numerous fully expanded leaves were formed and presumably the ability for photosynthesis increased.

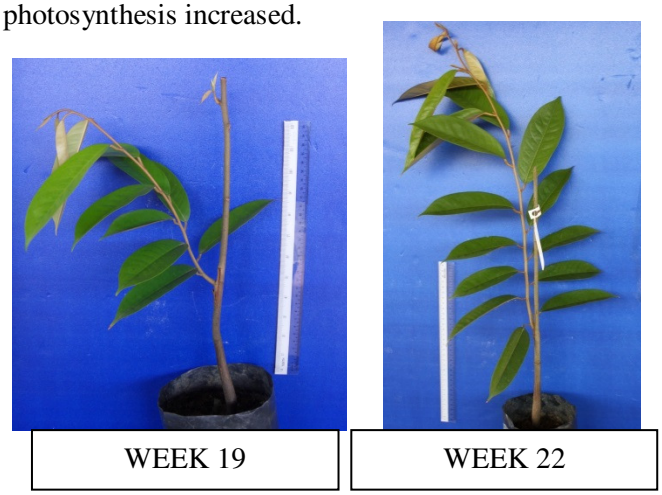

Figure 5. Shoot growth of D168 durian during the exponential growth phase

ISBN 978-967-10840-3-8
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Conclusions

From the results obtained in this study, it is quite obvious that cultural practices need to be tailored to the growth pattern, which signifies plant requirement especially in relation to water, nutrient and light availability.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

CHAPTER 5

BIOTECHNOLOGY

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The Effects of Different Concentrations of NAA and Phenylalanine on Oil Palm Embryoids Culture

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Introduction

Oil palm cannot be multiplied vegetatively since oil palm is a monocotyledonous species with a single growing apex (Rajesh et al., 2003). However, processes for the vegetative multiplication of oil palm through somatic embryogenesis have enabled the mass propagation of more than 1 million clonal plantlets to date (Aberlenc-Bertossi et al., 1999).

Somatic embryogenesis represents a sequence of process which is resulting in the formation of somatic embryos and has been acknowledged to possess many advantages for mass propagation (Nurul et al., 2013). MPOB has developed the basic protocol for liquid culture (Tarmizi, 2002a, b) which includes selection of a suitable callus (friable type), media formulation, aggregate sieving, maturation induction, embryoid regeneration and production of rooted plantlets in a dual phase system (solid and liquid media).
Appropriate composition and concentration of growth regulators are able to improve somatic embryo development (Gaspar et al., 1996; Tahardi et al., 2003). Various types of auxins – IAA, 2,4-D, NAA and 2,4,5-T were used to develop a tissue culture protocol for oil palm (Wooi, 1995). The switch from 2,4-D to NAA was encouraged as cultures on 2,4-D were particularly prone to genetic variation (Machakova et al., 2008; Sogeke, 1998; Sogeke et al., 1999). The previous findings have reported that L-phenylalanine as a nitrogen source can influence on the cells growth and can be used in the medium (Harding et al., 2009 and Palacio et al., 2011). In other way, phenylalanine also can increase the production of phenols which have influence on cell divisions and their expansion or even can leads to a cell death (Urmantseva et al., 2005 and Tamagnone et al., 1998). Thus, the objective of this study is to investigate the effects of different concentrations of NAA and phenylalanine on embryoids culture of oil palm PL213 cultivar.

Materials and Methods

Plant materials

This study was carried out using callus-derived somatic embryos (embryoids) of PL213 cultivar established from oil palm liquid culture system (Tarmizi, 2002a; Tarmizi, 2002b) to test the effects of NAA and phenylalanine on the growth and development of oil palm embryoids culture.

Embryoids multiplication

Embryoids of PL213 was first been multiplied on MS basal (MS0) medium for a month at 27±2 °C temperature and 16 hours light/day photoperiod.

NAA and media preparation

MS basal medium (Murashige and Skoog, 1962) was prepared with different concentrations of NAA (0, 0.5, 1.0 and 2.0 mg/L). The pH value was measured at 5.7-5.8 prior to autoclaving. Subculture was done by transplanting the cultures every two weeks for 8 weeks.

Phenylalanine and media preparation

MS basal medium (Murashige and Skoog, 1962) was prepared with different concentrations of phenylalanine (0, 75 and 100 mg/L). The pH value was measured at 5.7-5.8 prior to autoclaving. Phenylalanine stock solution of 1 mg/ml (w/v) was prepared separately and microfilter sterilized using 0.2 µm of filter membrane since it is heat sensitive. Subculture was done by transplanting the cultures every week for 28 days.

Embryoids culture experiment

2 g embryoids was inoculated to MS media supplemented with several concentrations of NAA (0, 0.5, 1.0 and 2.0 mg/L) and phenylalanine (0, 75 and 100 mg/L) for embryoids maturation and germination. Seven replicates for every treatment have been set and the cultures were incubated at 27 ± 2 °C temperature and 16 hours light/day photoperiod.

Data collection and statistical analysis

The growth rate of each experiment (NAA and phenylalanine) was evaluated and plotted by graph which comprising biomass fresh weight (g), number of shoots and number of roots. All data were presented as the average of seven replications for each treatment and evaluated statistically by one-way analysis of variance (ANOVA) using SAS software.

Results and Discussion

Effect of different concentration of NAA on oil palm embryoids culture

Data shown in Figure 1 illustrates the effect of the different concentration of NAA on the growth of PL213 embryoids culture within 8 weeks of culture. The growth rate of the culture was increased vigorously from week 6 to week 8 and the formation of shoots and roots started to appear at the second week of culture in all different treatments. The highest mean of biomass fresh weight (20.62±2.3 g), number of shoots (43±2) and number of roots (47±4) was achieved by MS0 (control), MS+2.0 mg/L NAA and MS+1.0 mg/L NAA, respectively. The results also indicated that there were significant differences ($p<0.0001$) between different concentration of NAA on fresh weight, number of shoots and number of roots.

These results show that embryoids maturation and germination of oil palm embryoids culture responded differently to various concentrations of NAA used in this study. In terms of fresh weight, additional 0.5 mg/L NAA gave the highest biomass of embryoids culture but high concentration of NAA (1.0-2.0 mg/L) produced cultures with slow growth rate. Besides, high concentration of NAA (2.0 mg/L) had stimulated tissue necrosis and excretion of phenolic compound into culture medium. A loss of embryogenic capability had been reviewed by Sarkar (2009) upon maintenance of cultures on high auxin media which is also being the cause of embryo development to be arrested.

Effect of different concentration of phenylalanine on oil palm embryoids culture

Figure 2 demonstrates the growth curves (fresh weight, number of shoots and number of roots) of PL213 embryoids culture in different concentration of phenylalanine within 28 days of culture. All treatments revealed that the growth rate of embryoids culture was gradually increased by time and the formation of shoots and roots started to develop rapidly after seven days of culture. MS0 (control) achieved the highest mean of biomass fresh weight (6.33±1.3 g) among the other treatments. Meanwhile, MS+100 mg/L phenylalanine gave the highest number of shoots (31±7) and the highest number of roots (37±5) was obtained from MS+75 mg/L phenylalanine (Figure 3). However, there was a significant different

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

(p=0.0001) between different concentration of phenylalanine on number of roots but no significant differences on fresh weight (p=0.1336) and number of shoots (p=0.0373).

Overall, it shows that appropriate concentration of NAA and phenylalanine are able to improve somatic embryo growth and development. However, the effects might vary from different types of growth regulators and amino acids. Therefore, an optimum concentration of each treatment should be used in order to get the highest growth rate of oil palm embryoids culture.

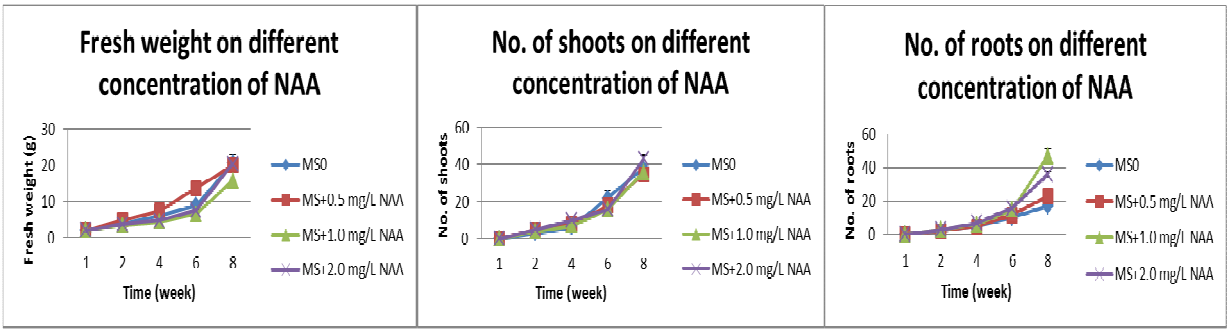

Figure 1. The growth of PL213 embryoids culture in different concentration of NAA within 8 weeks of culture.

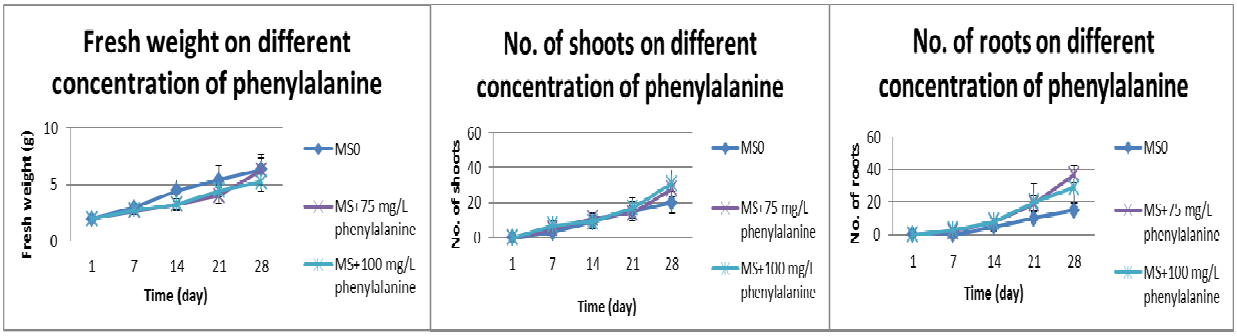

Figure 2. The growth of PL213 embryoids culture in different concentration of phenylalanine within 28 days of culture.

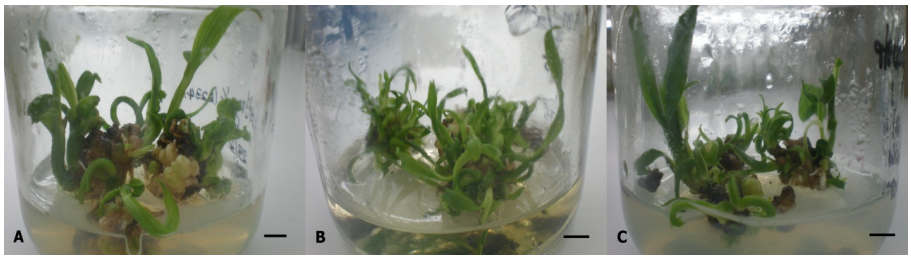

Figure 3. Embryoids culture of PL213 on (A) MS + 0.5 mg/L NAA (B) MS + 75 mg/L phenylalanine (C) MS + 100 mg/L phenylalanine after 28 days of culture (A to C bar: 1 cm).

Conclusions

In conclusion, this study showed that MS media supplemented with 0.5 mg/L NAA can increase the biomass of oil palm embryoids culture while MS media supplemented with 75-100 mg/L phenylalanine can produce high number of shoots and roots. The cultures responded differently to the different exogenous constituents added and it is suggested that an optimum concentration of NAA and phenylalanine can enhance the growth of oil palm embryoids culture in terms of biomass production.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

In vitro Direct Regeneration of *Hibiscus rosa-sinensis* in Modified Murashige & Skoog Medium Supplemented with Plant Growth Regulators

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Introduction

Hibiscus rosa-sinensis L. cultivar 'Brilliant Red', the national flower of Malaysia, is a five-petal, and brilliant red perennial herb. It belongs to the family Malvaceae and commands common names such as Chinese hibiscus, Red hibiscus, Shoe black plant or "*bunga raya*" in Malaysia. Some *Hibiscus* spp. could possess various bioactive activities such as lipid lowering activities (Gomathi et al., 2008), anti-tumoric (Lin et al., 2007), anti-diabetic (Venkatesh et al., 2008), as well as being used for paper-making (Ayadi et al., 2011). Conventional propagation means rely primarily on plant cuttings or grafting, which are problematic as they are time-consuming, difficult to mass propagate, require larger land size, or are season-dependable (in temperate region). Plant tissue culture technique provides an ideal alternative for propagating this plant cultivar, and to allow the establishment of a tissue culture system potentiating for molecular study to tap some potential commercial traits of this plant such as for its medicinal value and improved flower longevity or fragrance. In many cases, plant growth regulators (PGRs) were applied in plant tissue culture. Auxin is responsible for cell elongation (Ye, 2002) and tropistic response (Li et al., 2005), delays leaf senescence (Kim et al., 2011) but enhances root initiation on stem cuttings and lateral root development in tissue cultures (Pandey et al., 2011). Cytokinins play a vital role in promoting cell division (cytokinesis), *in vitro* morphogenesis, and division of procambial cells (Ye, 2002). Examples of cytokinins include 6-furfurylaminopurine (KIN), zeatin, and 6-benzylaminopurine (BAP). Despite the *in vitro* culture technique was established decades ago, progress in tissue culturing of *Hibiscus* spp. is still very much desired, and the study on the 'Brilliant Red' is still limited. In this study, we present the results on the effectiveness of PGRs supplemented with activated carbon (AC) in initiating shoots from *H. rosa-sinensis* nodal explants.

Materials and Methods

Explant surface sterilization

Nodal explants were harvested from young shoots of *H. rosa-sinensis* plants that grew beside the Sultan Abdul Samad Library, Universiti Putra Malaysia, Malaysia (Voucher Specimen Number: SK1560/08). Each harvested shoot contained a maximum number of three nodes counting from the shoot tip. The nodes were cut into 2 cm length before washing under running tap water for 30 min. A drop of detergent (Dynamo) was added into the water and the washing was continued for 1 min. The detergent was washed away under the running tap water, following which the explants were rinsed again three times with distilled water. Nodes were sterilised in approximately 500 ml of 40% (v/v) commercial Clorox solution [5.25% (w/v) NaOCl] for 20 min (Chew et al., 2012). A drop of Tween-20 was added to the Clorox solution prior to sterilisation. After each treatment, the explants were washed thoroughly with sterile deionised water three times (Misra and Chakrabarty, 2009), each wash comprised of 10 min. After the sterilisation steps above and prior to culturing the explants on the MS medium, each node was trimmed into 1 cm in length.

Culture medium

A modified full-strength Murashige & Skoog (MS) medium [40g/L sucrose + supplementations with myo-inositol, thiamine and nicotinic acid (concentration: standard MS vitamin)] was supplemented with PGRs, singly [BAP, zeatin, KIN, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene-3-acetic acid (NAA)]. The concentrations for IAA, IBA, NAA were 5-25 µM; those with BAP were 0.25-1.00 µM; with KIN were 1-10 µM and with zeatin were 0.5-2.5 µM. Meanwhile in another study, the above basal modified MS medium was supplemented with 0.3% (w/v) activated carbon (AC) (Chew et al., 2012) and PGRs in combinations (2 µM KIN: 2-10 µM IBA and 2 µM IBA: 2-10 µM KIN). The pH was adjusted to 5.7-5.8 using 1 M NaOH before adding 1.6 g/L Gelrite. The media were autoclaved at 121˚C for 15 min. The autoclaved media were dispensed as 10 ml into each 10 cm x 2.5 cm tube. A total of 45 replicates were prepared for each treatment. Observations were taken weekly for 6 weeks to record the percentages of survival and regeneration of explants, mean leaf length (cm), and mean number of leaves. Scoring of explant regeneration percentage was carried out when the number of explants regenerated was ≥ 5. All experiments were repeated once.

Statistical analysis

Quantitative and qualitative data were taken. The experiments were performed in a completely randomised design (CRD). The data were subjected to one-way analysis of variance (ANOVA) using SPSS version 16 software (SPSS, Chicago, IL.). Multiple comparisons among means were performed using Duncan's multiple range test (DMRT) with the level of significance at $p < 0.05$. Data were displayed as means ± standard deviations.

Results and Discussion

Supplementations of cytokinins (BAP, KIN, and zeatin) in the media were detrimental to the nodal explants, with a significant drop in the survival percentage starting from the 3rd week for zeatin and the 2nd week for BAP and KIN. Retardation of shoot elongation, early shoot tip necrosis (STN), and rapid aging of the explants (browning at 2nd or 3rd week) were also observed. These problems became more pronounced as the cytokinin concentration increased. A small amount of calli formed around the cut edges of the explants under the treatment with zeatin, especially at 1 µM, but no similar result was observed for the other two cytokinin treatments (result not shown).

Even though BAP has long been proven to be one of the most effective cytokinins for shoot regeneration, the result from Figure 1 contradicted the findings by Christensen et al. (2008) in which 2.2 µM BAP was reported optimal for shoot multiplication (2.3 ± 0.6 shoots per explant for cv. 'Cassiopeia Wind Yellow') using nodal explant of *H. rosa-sinensis*. This might be due to the different genotypes used in Christensen et al. (2008)'s research ('Cassiopeia Wind Yellow' and 'Caribbean Pink') and in this study ('Brilliant Red'). As a comparison to other species, Herath et al. (2004) reported that the optimum BAP concentration for shoot regeneration (76.5%) of *Hibiscus cannabinus* (cv. 'Tainung 2') was 8.8 µM and any further increment would negate the shoot number.

An earlier report on *H. cannabinus* (cv. 'Everglades 71', 'Tainung 1', and 'Tainung 2') direct shoot regeneration study revealed that shoot growth was suppressed with a BAP concentration of more than 4.4 µM (Zapata et al., 1999). Both reports above contradicted the latest findings from Ayadi et al. (2011) (*H. cannabinus* cv. 'Guangdong 743-2'), who concluded that the optimum medium for direct shoot regeneration of *H. cannabinus* was actually hormone-free (shoot induction: 90.5%). Again, a plausible explanation for such varied findings could be the differences in the genetic background of the cultivars

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

used. Ccomparing with *Hibiscus sabdariffa*, the conclusion from Gomez-Leyva et al. (2008) contradicted the report from Govinden-Soulange et al. (2009), with the former research group concluded that 17.74 µM BAP was optimum for direct shoot regeneration (100%, cv. Colima) while the later team showed that hormone-free MS medium was the best (81.0 ± 5.32%, cv. 'Local'). In *H. sabdariffa*, KIN was reported as a major contributor to shoot regeneration (Govinden-Soulange et al., 2009).

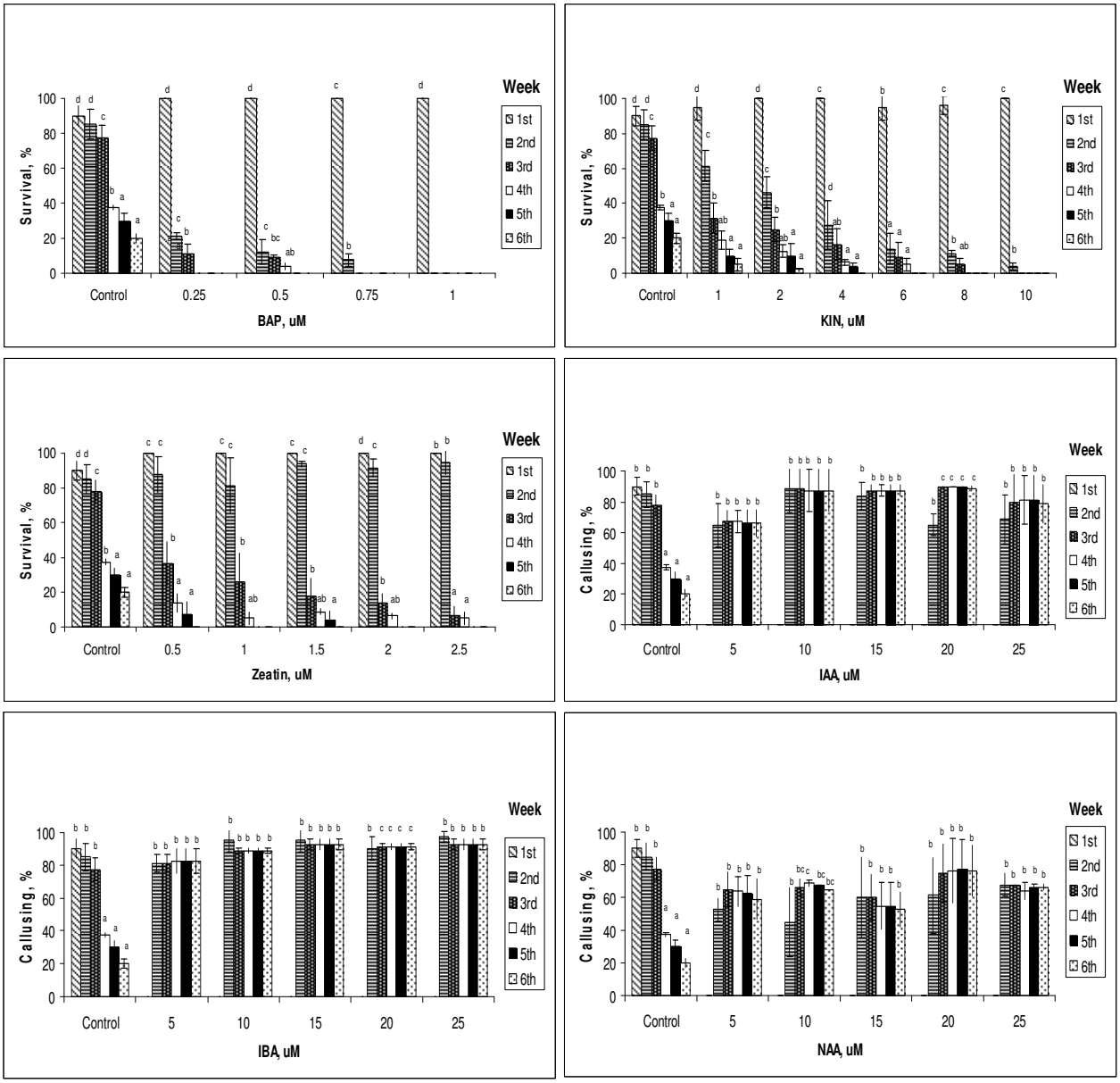

Figure 1. Survival and callusing of nodal *H. rosa-sinensis* explants treated with cytokinins and auxins. The nodal explants were cultured on modified basal MS media supplemented with different PGRs. Data were taken weekly for 6 weeks. Different letters (a, b, c, d, e) indicate values that are significantly different at $p<0.05$ by DMRT. Data indicates means ± standard deviations.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The effects of auxins, namely IAA, IBA, and NAA, on the growth of the nodal explants were summarised in Figure 1. As shown in the charts, all auxins under study promoted callusing, and in many cases, halted the emergence of shoots (or if the shoots appeared, elongation of the shoots was inhibited). Generally, calli emerged at the 2nd week and continued to enlarge in diameter thereafter. The calli formed were friable and whitish. However, IAA-induced calli were morphologically observed to be slower in size increment; instead, root formation from the explants was enhanced (result not shown).

In this study, IBA at 20 µM was found to be the best PGR for inducing callusing rapidly (91.3 ± 1.8%), starting at 2nd week. The diameter of the callus clump reached 1 cm after 4 weeks of culture (treatments at ≥ 10 µM of NAA or ≥ 15 µM of IBA). Consistent with the significant role that auxin plays in callus formation, in this study, the concentrations of auxins applied were at least 10 times more than that of the cytokinins used (except KIN). The callus size induced by auxin at concentrations < 5 µM was insignificant (result not shown).

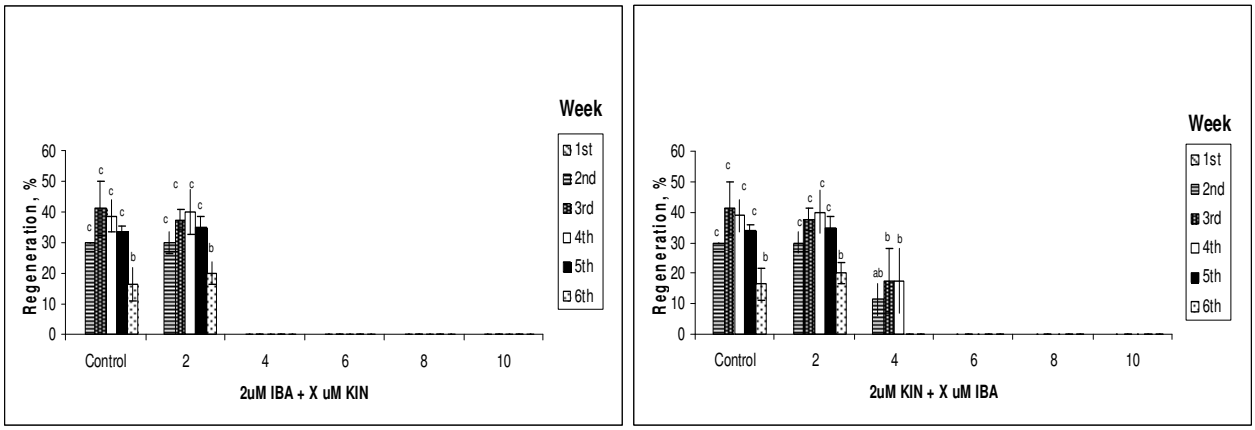

Figure 2. *In vitro* regeneration of nodal *H. rosa-sinensis* explants. The nodal explants were cultured on modified basal MS media supplemented with 2µM KIN+IBA. Data were taken weekly for 6. Different letters (a, b, c, d, e) indicate values that are significantly different at $p<0.05$ by DMRT. Data indicates means ± standard deviations.

Govinden-Soulange et al. (2009) showed that PGR combinations could generate optimal growth result in terms of shoot multiplication (*H. sabdariffa*). They suggested that cytokinin-auxin synergistic effect should be further investigated. Chen et al. (2010) had demonstrated how to maximise the number of shoot per explant (12.97 ± 0.20) and frequency of shoot regeneration (98.33 ± 0.15%) on *H. cannabinus* cv. P_3B by using BAP, IAA, and F-68. Figure 2 summarised the results for IBA and KIN combination in the ratios of 1:1, 1:2, 1:3, 1:4, or 1:5, and *vice-versa*. The concentration of KIN was set at 2 µM because a lower concentration seemed to be less toxic to the nodal explant, as shown in Figure 1. The shoot regeneration rate at '2µM KIN + 2µM IBA' was comparable to that of the control (35-40%, no PGR) from the 3rd to the 5th weeks (Figure 2).

Treatment of '2µM KIN + 4µM IBA' had a much lower regeneration rate than that of lower treatment strength (about half of the control and '2µM KIN + 2µM IBA') (Figure 2) while the rest of the combinations were unsuitable for shoot regeneration. It is still ambiguous whether the result from '2µM KIN + 2µM IBA' was actually due to the synergistic effect of KIN and IBA because AC in the media could act as a nutrient or organic substance absorber (Pullman and Johnson, 2002) to scavenge the KIN and IBA, resulting in a medium which is seemingly somewhat like "PGR-free", especially when the

PGR concentration is low. Such trend could be observed for all the hormones as their concentrations increased. In short, despite PGR was found to give a regeneration percentage of 37.5 ± 3.5 (3rd week), overall PGR-free medium is, however, recommended for the *Hibiscus* cultivar used in this study.

Conclusion

Basal modified MS medium supplemented with 0.3% (w/v) activated carbon is suitable for direct shoot regeneration of *H. rosa-sinensis* L. cv. 'Brilliant Red' nodal explants. Any cytokinin added would be harmful to the explants *in vitro* survival.

Acknowledgements

Chew Tiong Dar is a recipient of a Graduate Research Fellowship from the Universiti Putra Malaysia. Authors would like to thank Associate Professor Dr. Rusea Go, Department of Biology, Faculty of Science, Universiti Putra Malaysia, for her invaluable assistance in identifying the plant species.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Comparison in Multiplication Rate of MD2 Pineapple Micropropagation with Liquid MS, Solid MS and Temporary Immersion System

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Introduction

A new pineapple hybrid, MD2 pineapple, bred by Hawaii Pineapple Research Institute and later developed by Del Monte Fresh Produce Hawaii Inc. was introduced into the fresh fruit market in 1996. The demand for MD2 pineapple for fresh fruit market has increased since its release in 1997 due to the taste and fruit characteristics. Wardy et al. (2009) concluded that MD2 pineapple is more preferable by consumers due to the taste, colour, and high sugar content. High concentration of ascorbic acid in MD2 pineapple provides an environment which constrains microbial growth making it suitable for export market due to longer post-harvest shelf life. Chan et al. (2003) described MD2 pineapple as golden ripe pineapple with a more cylindrical shape of fruit with an average weight of 1.3 to 2.5 kg. MD2 pineapple also is more resistant to internal browning compared to other pineapples.

In Malaysia, the current market price of MD2 pineapple is RM5.90 per kg. As one of the biggest pineapple producing country with strategic geographical location for MD2 pineapple plantation and global exportation, the demand on MD2 pineapple has increased in the past few years. Based on the characteristic and potential of MD2 pineapple to penetrate global fresh fruit market, MD2 pineapple has been listed as one of the core crops in Entry Point Project 7 (EPP-7) under the National Economic Key Area (NKEA) with RM1.4 billion funds allocated for the improvement and development of MD2 pineapple together with other important fruits and vegetables including J32 jackfruit, *Eksotika* papaya, Cavendish banana, B10 starfruit, and KR_1 rock melon, tomato, capsicum, and lettuce (PEMANDU, 2011).

However, shortage of planting materials is one of the major problems in MD2 pineapple plantation in Malaysia. Conventional method of obtaining the planting materials from the sucker, crown, and slips of the pineapple may consume up to 16 to 18 months which is after the fruit was harvested (Hepton, 2003). Imported planting materials for MD2 pineapple are costly as the value could reach up to RM1.80 each. Based on this situation, tissue culture technique could be seen as the most effective solution as it can provide numerous number of planting materials which are identical to its mother plant in a shortest time (Hamad and Taha, 2008a). In this study, three tissue culture systems for MD2 pineapple were studied and compared for the highest rate of micropropagation in order to select a suitable tissue culture system that could be implemented to overcome the shortage of MD2 pineapple planting materials in Malaysia.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Materials and Methods

In vitro shoots initiation

MD2 pineapple's suckers were obtained from the MD2 pineapple plantation by Koperasi Serbaguna Anak-anak Selangor (KOSAS) at Kuala Kundang, Selangor. The leaves were removed and the meristem was cut up to the size of 6 to 8 cm in diameter and 9 to 12 cm in height. The meristem was washed thoroughly under tap water to remove excessive dirt before transferred into the laminar flowhood. The meristem was further sterilized with alternating chlorox (50%, 30% and 10%) and sterilized distilled water for 30 minutes and 10 minutes, respectively. The meristem was trimmed in laminar flowhood by cutting the sides of the meristem up to the size of 2 cm in diameter and 4 cm in height. The meristem was further cut vertically into four sections and each section was placed onto suitable shoot initiating medium containing full strength solid Murashige & Skoog (MS) medium (Murashige & Skoog, 1962) supplemented with 2.5 mg/L BAP, 0.1 mg/L NAA, 1.0 mg/L TDZ, and 30% glucose. The explants were incubated in the absence of light for 10 days before being transferred onto fresh solid medium without TDZ for shoot proliferation up to M_1V_2 for further use.

Solid incubation system

Shoots produced from the shoot initiation process with an average size of 1.5 to 2.0 cm with 4 to 5 leaves were singularly trimmed and sub-cultured on solid MS medium supplemented with 2.5 mg/L BAP, 0.1 mg/L NAA, and 3% glucose with three shoots per jam jar. Shoots were incubated under 16 hours photoperiod at room temperature. The number of new shoots produced was recorded after 60 days of incubation. All procedures were handled with aseptic technique in sterile environment to avoid contamination.

Liquid shake system

Shoots produced from shoot initiation process with an average size 1.5 to 2.0 cm with 4 to 5 leaves were singularly trimmed and sub-cultured into liquid MS medium supplemented with 2.5 mg/L BAP, 0.1 mg/L NAA, and 3% glucose with three shoots per flask. Shoots were incubated under 16 hours photoperiod at room temperature on a shaker at 100 rpm. Number of new shoots produced was recorded after 60 days of incubation. All procedures were handled with aseptic technique in sterile environment to avoid contamination.

Temporary immersion system

Shoots produced from shoot initiation process with an average size of 1.5 to 2.0 cm with 4 to 5 leaves were singularly trimmed and sub-cultured into an *in vitro* bioreactor device called Récipient à Temporaire Automatique (RITA®) with three shoots per bioreactor. Liquid MS medium supplemented with 2.5 mg/L BAP, 0.1 mg/L NAA and 3% glucose were added into the device. Sterilized air was pumped into the device every two hours for 20 minutes and the shoots were incubated under 16 hours photoperiod at room temperature. The number of new shoots produced was recorded after 60 days of incubation. All procedures were handled with aseptic technique in a sterile environment.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Statistical analysis

The experiment was handled in completely randomized design (CRD) with 10 replications. The data collected were analyzed with Microsoft® Excel and SAS for least significance difference (LSD) at $p<0.05$. The experiment was repeated twice.

Results and Discussions

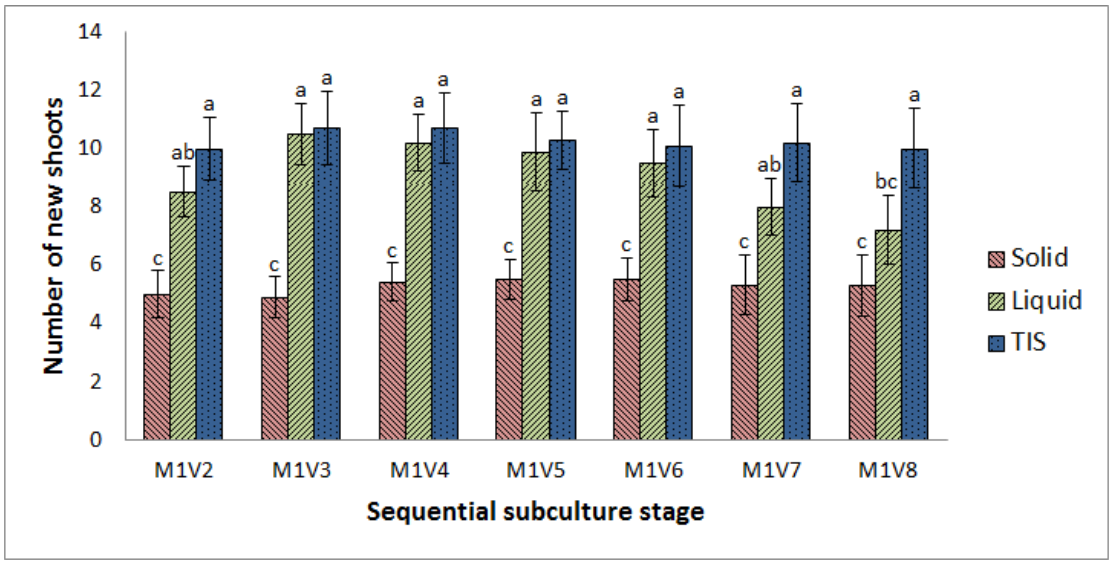

Figure 1. Comparison of the difference in numbers of new shoots produced in solid incubation system, liquid shake flask system, and temporary immersion system (TIS) of MD2 pineapple throughout second subculture (M_1V_2) up to the eighth subculture (M_1V_8) after 60 days of incubation. Different letters (a, b, c) indicate values that are significantly different at $p<0.05$.

In solid incubation system, the highest number of new MD2 pineapple shoots produced was observed at M_1V_5 and M_1V_6 with 5.5 ± 0.7 new shoots per explant. There was no significant difference in the increase or decrease of number of new shoots produced by using this system as seen throughout M_1V_2 to M_1V_8 which indicates that subsequent subculture does not affect the number of shoots produced. Advantages of solid incubation system could be seen as cost effective in terms of the use of electricity for automated system such as the use of high powered automatic shaker in liquid flask system and automated sterilized air pump used in temporary immersion system. However, the number of shoots produced was significantly low compared to liquid shake flask and temporary immersion system.

The highest number of shoots produced by using liquid shake flask system was observed at M_1V_3 with 10.5 ± 1.0 shoots produced per explant. There was a significant increase in the number of new shoots produced between M_1V_2 and M_1V_3. This may be due to the change of the type of medium used previously on M_1V_1 during shoot initiation which uses solid MS medium as shoot multiplication medium. The change of medium used in M_1V_2 which uses liquid MS medium may not provide suitable growth environment for the explant. Prior to adaptation, the number of new shoots produced increased significantly in M_1V_3. However, there was also a significant decrease in number of new shoots produced using liquid shake flask system in M_1V_7 and M_1V_8. This indicates that the increase number of subculture affects the number of new shoots produced which may be due to the loss of competency of the meristem

of the explants. The reduction in the competencies of a meristem may be due to the subculturing effect that prevents pre-axillaries and new axillaries to complete its maturation (Hamad and Taha, 2008b). Voyiatzi et al. (1995) and Harris and Mantell (1991) reported that destruction of shoot tips chemically and manually, respectively, caused a reduction in the rate of shoot production in tissue culture of hybrid tea rose and Paeony.

In temporary immersion system, the highest number of new shoots produced per explant was observed at M_1V_3 and M_1V_4, respectively. There was no significant difference in the number of new shoots produced throughout the subculture up to M_1V_8 which indicates that the number of subculture does not affect the number of new shoots produced using this system. It was also noted that the height of new shoots produced in temporary immersion system is more uniform with an average shoot height of 3.2 ± 1.3 cm as compared to height of new shoots produced in liquid shake flask and solid incubation system with an average shoot height of 3.0 ± 1.8 cm and 2.8 ± 1.6 cm, respectively.

Significant difference in comparison of the number of new shoots produced between solid incubation system and liquid shake flask and temporary immersion system may be due to the effectiveness of the nutrient intake from the media provided by the nature of a liquid medium. The TIS was utilized based on the principle that provides temporary contact between the explant and liquid medium rather than permanent contact which was not provided by the liquid shake flask system (Etienne and Berthouly, 2002). This prevents anomaly in liquid shake flask tissue culture especially by avoiding hyperhydricity while providing better aeration and gas exchange. This superior quality of temporary immersion system over liquid shake flask may indicate the higher number of new shoots produced as well as the better stability achieved throughout subcultures of MD2 pineapple by using the TIS.

Conclusions

Based on the comparison with solid incubation system and liquid shake flask system, TIS produced the highest multiplication rate with 10.7 ± 1.3 new shoots per explant while producing shoots with greater height and better uniformity. Thus, temporary immersion system may appear to be a better solution in producing high number of MD2 pineapple planting materials in a short period of time in order to fulfil the increasing demand by the MD2 pineapple plantation in Malaysia.

Acknowledgements

The authors would like to thank Koperasi Serbaguna Anak-anak Selangor (KOSAS) for providing fresh MD2 pineapple planting materials and special appreciations to Agensi Nuklear Malaysia (ANM) for providing the facilities needed for this study.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Evaluation of Sucrose Concentration and Gelling Agent on Pitaya Callus Morphology and Betalains Production in the Culture

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Introduction

Red pitaya, *Hylocereus polyrhizus* is a member in Cactaceae family and commonly known as red dragon fruit. Its exotic characteristics with delicious flavour, attractive deep red coloured and juicy fruit flesh has made it become popular in a fruit market. The deep red coloured fruit flesh attributed with betalains pigment that compose of red-violet betacyanins and yellow betaxanthin contribute to the antioxidant property. Hence, red pitaya is identified as a significant source of antioxidants (Rebecca et al., 2010) and also an alternative source of natural food colourant (Woo et al., 2011a, Woo et al., 2011b, Phebe et al., 2009 and Azerado, 2009).

Production of fruit crop through field cultivation will not meet the growing demands of betalains pigment as an alternative source of antioxidants and natural food colourant. In vitro technology has been widely exploited to enhance the production of valuable secondary metabolites. Biosynthesis of betalains using different plant in vitro system of *Beta vulgaris* including cell suspension (Leathers et al., 1992), hairy root (Pavlov and Bley, 2006) and callus cultures (Girod and Zryd, 1991) were reported. Betalains pigment was also successfully produced from stem callus culture of *Zaleya decandra* (Radfar et al., 2012) and cell suspension culture of *Portulaca sp.* (Noda and Adachi, 2000).

However, obtaining the comparative yields of betalains from plant in vitro system is crucial. Optimization of the pigment production can be achieved by selection of highly productive cell lines (Akita et al., 2000) or manipulation of culture media (Girod and Zryd, 1991 and Akita et al., 2002). In this study, we evaluated the effect of sucrose concentration and gelling agent on red pitaya callus and betalains production in the culture.

Materials and Methods

Establishment of explants

Red pitaya fruit was washed with running tap water, followed by surface sterilized using ethanol in laminar flow. The fruit was halved and fruit flesh was excised to be used as explant. All seeds were removed and the flesh was cultured onto culture media in the size of 1 cm^2.

Effects of sucrose concentrations

Three basal media (MSO, MS media supplemented with 1 mg/L NAA and MS media supplemented with 1 mg/L TDZ) were used to examine the effect of different concentrations of sucrose. All 3 basal media were supplemented with 0.3% (w/v) phytagel and various sucrose concentrations (30, 60 and 90 g/L). The pH of the media was adjusted to 5.8 prior to autoclaved at 121 °C for 30 minutes.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effects of gelling agent

Three basal media, MSO, MS media supplemented with 1 mg/L NAA and MS media supplemented with 1 mg/L TDZ were used to examine the effect of gelling agent. All 3 basal media were supplemented with 30 g/L sucrose and solidified with 0.3% (w/v) phytagel, phyto agar or gelrite. The pH of the media was adjusted to 5.8 prior to autoclaved at 121 °C for 30 minutes.

Culture condition and maintenance of callus culture

All the experiments were performed with 20 replications and 6 explants in each replicate. The cultures were kept in dark condition at 25 °C and the subculture was done at 1 month interval onto the same treatment media. Data were recorded every month before subculture.

Analysis of betalains content

One month callus culture was harvested for pigment content analysis. Betalains content in the callus culture was analysed by using a spectrophotometer. Two pigments, betacyanin indicated by red-purple colour pigment and betaxanthin indicated by yellow-orange pigment were detected at absobances at 537 nm and 480 nm, respectively.

Results and Discussion

Different sucrose concentrations and gelling agents were examined in 3 different types of basal media. In general, red coloured pigmented callus was produced in all media, but in different intensity. Callus produced in culture media supplemented with 1 mg/L NAA or TDZ was found darker red in colour compared to callus grew in MSO media. However, only soft and watery callus was observed in all treatment media.

Sucrose is one of the components added into plant tissue culture media as carbon source and osmotic regulator (Shahnewaz and Bari, 2004). The result obtained from this study showed significant effect of different sucrose concentrations on callus growth. Optimal callus growth was observed in media supplemented with 30 g/L sucrose and callus growth was found to be stunted in media added with 60 and 90 g/L sucrose. As stated by Gerdakaneh et al. (2009), fresh weight of strawberry callus reduced by the increase of sucrose concentration in culture media. This may be due to the osmotic effect occurred leading to the inhibition of nutrient uptake by the callus cultures.

Concentration of carbon source was reported to give a great effect on betalains biosynthesis in *Beta vulgaris* root and cell suspension culture, respectively (Bhagyalakshmi et al., 2004 and Akita et al., 2002). Higher concentration of sucrose increased betalains production as shown in Figure 1(a). The highest betalains content was observed in 90 g/L sucrose and 1 mg/L NAA callus culture. Betalains content produced from the callus culture had direct relation to the intensity of the callus colour formed. However, betalains production increased with a reduction of callus biomass when higher sucrose added.

On the other hand, pigmented callus with different colours were obtained when different gelling agents tested. Culture media solidified with phytagel and phyto agar formed dark red coloured pigmented callus while gelrite resulted in the formation of yellow-orange coloured callus. Different type of callus developed may influenced by different impurities of the gelling agents tested that affect water and element uptake from the culture media.

This study also revealed that betalains biosynthesis in callus culture was influenced by gelling agents used to solidify culture media. Figure 1(b) shown Phytagel and Phyto agar enhanced betalains production among the gelling agents tested. Betalains content was found significantly higher in Phytagel and Phyto agar solidified media compared to Gelrite solidified media, especially when 1 mg/L NAA added. The data also indicated that pigmentation was closely related to the betalains content of the callus culture.

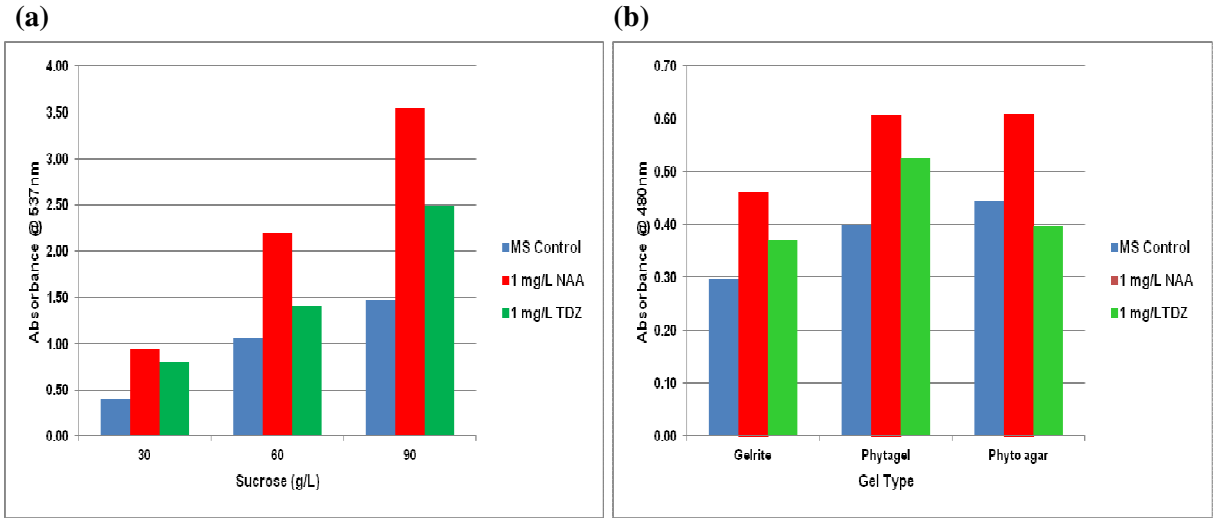

Figure 1. Influence of (a) sucrose concentration and (b) gelling agent on betalains biosynthesis in red pitaya callus culture.

Conclusions

Sucrose concentration in culture media was found greatly influenced the callus growth, colour intensity of the pigmented callus and betalains production of the culture. On the other hand, the three gelling agents evaluated did not affect callus growth but giving impact on callus pigment synthesis and betalains production of the culture.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Effect of Acute Gamma Irradiation on *in vitro* Growth of *Stevia rebaudiana* Bertoni

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Introduction

Currently, herbal based products are highly demanded in Malaysia and also around the world because of their values in medicinal, healthcare and beauty products. *Stevia* is also showing great demands and currently being used as medicinal products. *Stevia rebaudiana* Bertoni is a branch bushy shrub of the Asteraceae family, native to the Amambay region in the north east of Paraguay (Soejarto, 2002). It grows up to 1 m tall (Mishra et al*.,* 2010). The leaves of stevia have both functional and sensory properties superior to those of many other high-potency sweeteners, and is likely to become a major source of high-potency sweetener for the growing natural food market in the future (Goyal et al*.,* 2010). The leaves contain a large amount of stevioside (Geuns, 2004), which is formed by three molecules of glucose and one molecule of steviol, a diterpenic carboxylic alcohol. Pure extract stevioside is non caloric and 300 times sweeter than sugar (Bhosie, 2004). Because of the herbal industry's great demand and its effect on Malaysia economic, *S. rebaudiana* as herb, would be more valuable in the near future. Hence cultivation of stevia under controlled environment could be proposed in order to meet the growing requirement for natural medicine. Radiation treatment of plants is one of the most common techniques for induction of plant mutations. These mutants are useful for developing new plant varieties as well as for functional studies of genes (Hase et al*.,* 2000, Shikazono et al*.,* 2003, Tanaka et al*.,* 1997, 2002). Exposure of *S. rebaudiana* to radiation shows the potential of gamma rays for the stimulation and enhancement of steviol glycosides which can be utilized further as a natural sweetener. This study was carried out to study the effect of acute gamma irradiation on growth and multiplication rate of *in vitro* shoots of *S. rebaudiana* Bertoni.

Materials and Methods

Sterilized shoot tips and nodal segment (with a single axillary bud) were cultured onto semi-solid MS Medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/l 6-furfurylaminopurine (kinetin) together with 3% (w/v) sucrose and 2.4 g/l gelrite. The pH of the medium was adjusted at 5.8 with 0.1 M NaOH before autoclaving at 121°C for 15 min. After 7 days of culture, the explants were irradiated with acute gamma radiation at 0, 10, 20, 40, 60, and 80 Gy using our Gamma Cell facility and the irradiated explants were incubated in the incubation room at 24±2 °C with 16 h photoperiod. The number of explants used in this study was six for each dose with five replications and this experiment was repeated twice. After 4 weeks of culture, percentage of survival, length of plants and number of new shoots formed were measured. The data were analyzed by SPSS statistical analysis version 19 using one way ANOVA with Duncan's Multiple Range Test.

Results and Discussion

This radio-sensitivity test was conducted as to identify the LD50 for *in vitro* stevia shoots and to select effective doses to be used for the *in vitro* mutagenesis. Results from Table 1 showed significant difference for the percentage of the survival amongst shoots irradiated with lower and higher doses of acute gamma radiation. All non- irradiated shoots (control/0Gy) and shoots irradiated at 10.0 Gy showed

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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100% survival rate with the highest number of new shoots formed, 5.00±1.98 and 5.06±1.98 respectively. It was observed that, the survival rate of the shoot tips declined with the increasing dose of gamma radiation. At 60 Gy and 80 Gy, the shoot tips demonstrated 0% survival, all were killed.

Table 1. Effect of different doses of acute gamma irradiation on *S. rebaudiana* Bertoni growth after 4th week's culture

Dose	Survival rate (%±SD)	Length of plant (cm±SD)	No of new shoot form (mean±SD)	No of leaves (mean±SD)
0	100a	5.74±2.43a	5.00±1.98a	26.96±7.92a
10.0	100a	4.96±1.49b	5.06±1.98a	24.13±9.84a
20.0	73.33±44.98b	1.35±1.12c	1.40±1.10b	8.37±5.16b
30.0	46.47±50.74c	1.20±1.49c	0.93±1.23b	4.77±5.78c
40.0	40.00±49.83c	0.74±1.35cd	0.90±1.63b	4.40±6.99c
60.0	0	0	0	0
80.0	0	0	0	0

**Data was taken from 5 replicates with twenty explants. Significance p<0.05 using Duncan's Multiple Range Test. SD= Standard Deviation.*

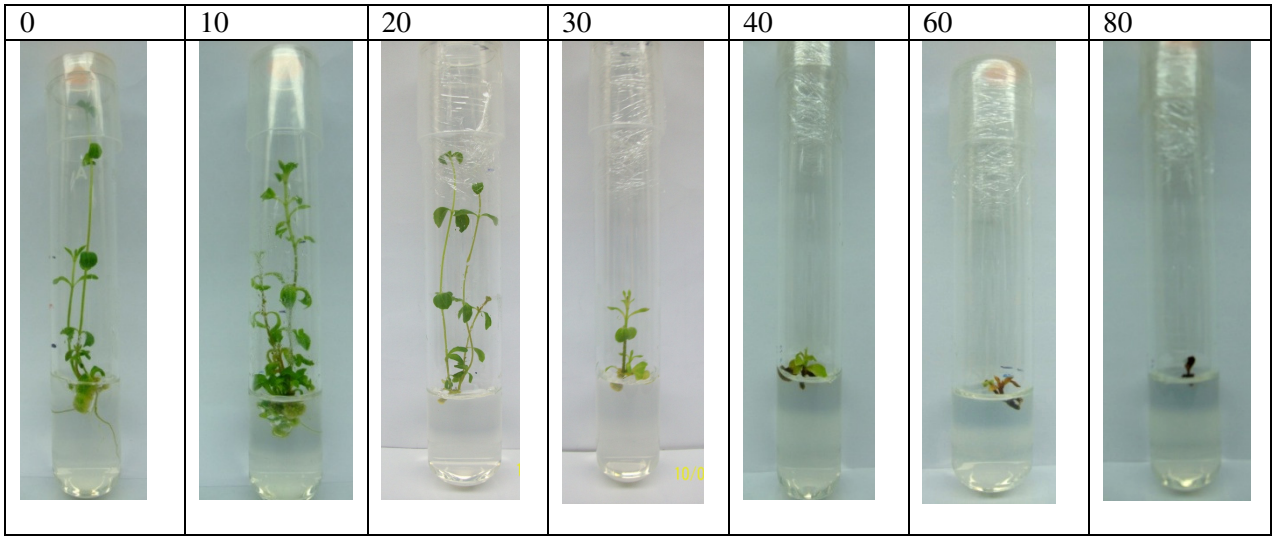

Figure 1. Effect of gamma radiation on plantlets growth after 4 weeks of culture in MS + 0.5 mg/L kinetin

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

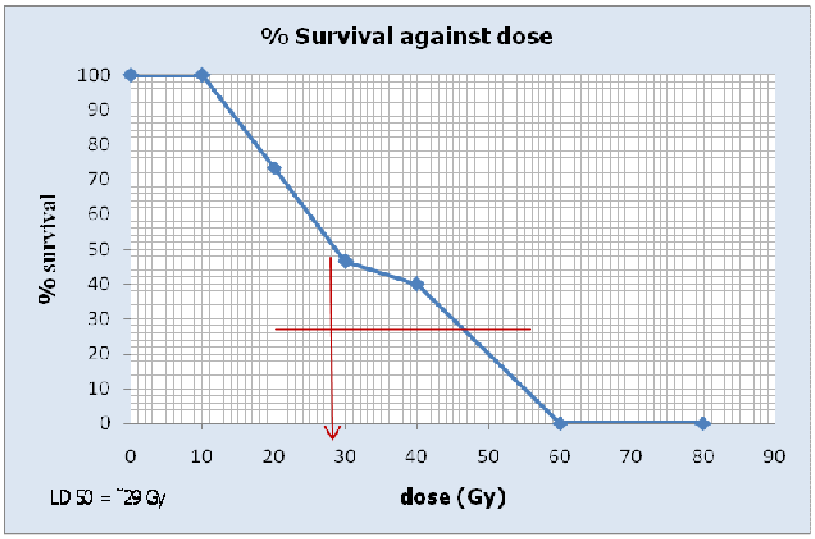

Figure 2. LD_{50} of *S. rebaudiana* after 4 weeks of culture

From the LD50 results, it was clearly demonstrated that with the increasing gamma dose, the survival rate of stevia shoots showed significant declining. Based on the figure above, LD50 for the stevia (the dose that killed 50% of the irradiated explants) was at 29 Gy. From this study, the effective doses were selected at 10, 20 and 30 Gy. These three selected doses were applied for the *in vitro* mutagenesis of the stevia shoots. To date, shoot tip explants were irradiated with these doses and regenerated shoots were sub-cultured up to M1V4 generation to reduce the effect of chimeras.

Conclusions

The radiosensivity test for *S. rebaudiana* Bertoni has been successfully optimized and established. The LD50 for stevia shoots was successfully determined and the effective doses were selected at 10, 20 and 30 Gy. These three selected doses were applied for the *in vitro* mutagenesis of the stevia shoots.

Acknowledgements

The authors would like to thank the management of Malaysian Nuclear Agency for their continuous assistance, advices and supports on this project. Special thanks is also extended to the Ministry of Science, Technology and Innovation (MOSTI) for providing fund for this project under the Science Fund grant 02-03-01-SF0163: Effects of chronic irradiation on growth and multiplication rate with reference to enhanced production of steviol glycoside in *S. rebaudiana* Bertoni.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Ethephon Induces Changes in the Expression of *Hevea* B Serum Proteins

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Introduction

Hevea brasiliensis latex is the superior source of commercial natural rubber. It is a living cytoplasmic content of the laticifer in a polydispersed system. It has a variety of molecules such as rubber particles, proteins, carbohydrates and other cell contents. In order to increase the latex yield, ethephon, an ethylene releasing compound has been used as a stimulant. Treatment of ethephon on rubber tree promotes prolongation of latex flow. One of the main inherent limitations of latex flow is the occurrence of plugging event inside the laticifer. Previous findings showed that plugging in the latex vessels was related to the composition of B serum and its proteins.

B serum is derived from the membrane-bound organelle in the bottom fraction of centrifuged latex. Lutoids as the major organelle in the bottom fraction of centrifuged latex are known to have both lysosomal and vacuolar characteristics. The protein content of B serum can be classified into three groups namely acid hydrolases, defense and miscellaneous proteins (d'Auzac et al., 1995). This indicates the important role of this organelle in defense mechanism, plant growth and development, as well as stress response. Meanwhile, ethylene is known to regulate multiple gene expression especially genes related to wounding, plant defense and resistance (Zhong and Burns, 2003). This paper described the effect of ethephon treatment on B serum proteins using Western blot analysis. Four proteins selected for this study were beta-1,3-glucanase, microhelix complex protein, hevein and early nodule specific protein (ENSP). All the four proteins were categorized as defense-related proteins.

Materials and Methods

Ethephon treatment and latex collection

Ethephon (2.5%) was applied to the tapping cut and on the renewing bark 2 cm above the cut of RRIM 600 trees that were tapped on a half spiral, third daily system. Latex samples from stimulated and control tress were collected before stimulation and 2 days after stimulation. Three trees were stimulated and three trees were untreated, as the control. Latex from stimulated and control trees were collected and analyzed for protein changes in B serum.

Sample preparation

Fresh latex was centrifuged at 44 000g for 1 h at 4 ^{o}C and three distinct fractions were obtained namely the rubber cream, C serum and bottom fractions (Moir, 1959). The bottom fraction which contained lutoids was further washed with 0.4 M mannitol to remove excessive C serum from the lutoids. Centrifugation was carried out prior to the freeze-thaw method to rupture lutoid membrane and released the fluid content which was B serum. The ruptured lutoids were subjected to centrifugation at 5 000 rpm for 30 min at 4 ^{o}C to separate the supernatant (the B serum).

Dialysis of B serum

Two millilitres of B serum proteins were dialyzed using SnakeSkin™ (Pierce, IL, US) tubing molecular weight cut-off 3000 Da against water overnight at 4 ^{o}C. Centrifugation was carried out to fractionate the dialyzed B serum into supernatant and precipitate.

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein separation was then performed in a 15% SDS-PAGE and the gels were stained using Coomassie Brilliant Blue.

Immunoblotting

Prior to this, protein in all samples was quantified using Quick Start™ Bradford Protein Assay (BioRad, USA). For this experiment, the concentration of total B serum proteins was identical at 200µg/mL while the concentration of protein standards was set at 50 to 150 µg/mL. Separated proteins from the SDS-PAGE were blotted onto a nitrocellulose membrane using electrophoresis method for 16 h. Subsequently, the nitrocellulose membrane was blocked with 5% non-fat milk in PBS and then incubated for 90 min with monoclonal or polyclonal antibodies as the primary antibody. After three washes with PBS-milk, the nitrocellulose membrane was incubated for 1 h with the secondary antibody, anti-mouse IgG conjugated or rabbit anti-goat IgG conjugated to alkaline phosphatase. After three washes with PBS-milk, the nitrocellulose membrane was incubated for 10 min in Tris-buffered saline (TBS) before being immersed in 5-bromo-4-chloro-3-indolyl phosphatase for visualization.

Results and Discussions

Four proteins were subjected to Western blot analysis as the semi-quantitative analysis to detect protein expression changes between control and ethephon-treated samples. Proteins were probed with rabbit polyclonal anti-hev b6, anti-hev b4 and anti-hev b13 for detecting hevein, microhelix complex protein and ENSP respectively (Figure 1). Meanwhile, for beta-1,3-glucanase detection, protein was probed with mouse monoclonal anti-hev b2. Protein standards used in this experiment were the purified protein for each of respective proteins.

When B serum proteins from control and ethephon-treated were detected with mouse monoclonal anti-hev b2, a strong band was obtained at approximately 34 kDa and a faint band on top of it for both protein samples. However, the intensity of protein band detected in ethephon-treated sample was 2 fold stronger than the control. Beta-1,3-glucanase which belongs to PR group 2 is identified as an inducible protein especially with the presence of elicitors such as ethylene and also wounding activity. This protein seems constitutively expressed in rubber tree because of regular wounding by tapping and then intensified with ethephon stimulation.

Similar to microhelix complex protein, apparently two major bands were more discernible with stronger intensity in the treated protein sample although several bands were also detected in both B serum proteins. Seemingly, observation through ultra-cytology method also showed that microhelices were also increased as a result of yield stimulation (Gomez and Moir, 1979). Hence, it supports that microhelix complex protein is affected with ethephon stimulation. Microhelix complex protein is deemed to have significant contribution in *Hevea* defense mechanism as it carries a component of cyanogenicglucosidase and a lecithinase-homologue.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Meanwhile, five bands were obtained from control and ethephon-treated from B serum proteins when detected with rabbit polycclonal anti-hev b6. Three out of five bands for ethephon-treated samples showed stronger signal compared to the control. Two of them were found at higher molecular weight and one was obtained at low molecular weight. Hevein is a major soluble protein in latex and has vacuolar targeting sequence. It is an acidic protein appears to be a lectin, it can fix chitin and has antifungal properties (Van Parijs et al., 1991). Several studies have demonstrated the presence of variants of hevein, prohevein and pseudohevein as well at the protein, mRNA and gene level (Beintema, 2010). However, Arokiaraj andYeang (2006) findings showed that ethephon stimulation on rubber tree does not increase latex hevein protein at mRNA level. Thus, the intention of using polyclonal antibody in this experiment is to amplify signal from target protein with low expression level, as the target protein will bind more than one antibody molecule on the multiple epitopes.

On the contrary, B serum proteins probed with polyclonal anti-hev b13 showed comparable signal intensity in both control and treated samples. Early nodule specific protein is homologue to patatin and showed lipase and esterase activity (Arif et al., 2004). It appears that yet it is classified under defense-related proteins, ethephon stimulation does not affect much on ENSP.

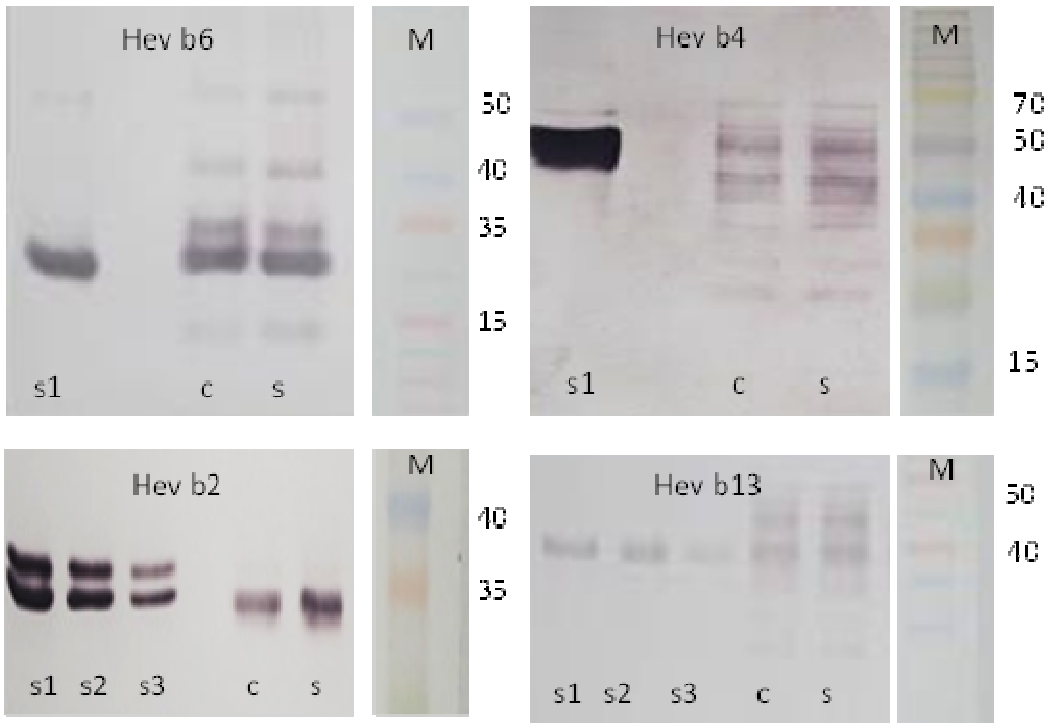

Figure 1. Western-immunoblot of SDS-PAGE separated B serum proteins probed with rabbit polyclonal anti-hev b6, anti-hev b4 and anti-hev b13 each for hevein, microhelix complex protein and ENSP respectively. Meanwhile, for beta-1,3-glucanase detection, protein was probed with mouse monoclonal anti-hev b2.
Top left: hevein Top right: microhelix complex protein Bottom left: beta-1,3-glucanase Bottom right: ENSP
M: Protein marker, C: B serum control E: B serum ethephon-treated S1: Protein standard (150µg/ml), S2: protein standard (100µg/ml), S3: protein standard (50µg/ml).

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Conclusions

These semi-quantitative results showed that three out of four B serum proteins selected were affected during ethephon stimulation application. All these proteins are located inside the lutoids; vacuolar and lysosomal-like organelle. These observations indicated that organelle bound proteins were involved in the ethylene signaling mechanism.

Acknowledgements

The authors wish to thank the Directorate of the Malaysian Rubber Board for the fund received under SEAC grant (S2010/BTP/2011(33)/213 to carry out this research.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Purification of Polyclonal Antibody against Recombinant RTBV and RTSV Coat Protein

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Introduction

Rice Tungro disease, a major rice viral disease in Southeast Asia, is caused by the simultaneous infection in paddy plants of Rice tungro bacilliform virus (RTBV), a double-stranded DNA virus and Rice tungro spherical virus (RTSV), a single-stranded RNA virus. The ''Tungro virus complex'' of RTBV and RTSV is transmitted solely by the Green leafhopper (GLH), *Nephotettix virescens* (Hibino and Cabauatan, 1987). Since mid-1960, rice production in South and Southeast Asia has seriously been affected by the viral disease causing 5 to 10% losses of rice yield annually with an estimated annual loss of about US$1.5 X 10^9 worldwide (Hossain and Pingali, 1998). Areas where rice tungro virus disease is epidemic are small in relation to the total rice production of a region or country. Nonetheless, affected fields may suffer a total yield loss causing a significant impact on the livelihood of farmers in Asia, who generally depend on the crops produced on relatively small farms (Azzam and Chancellor, 2002). The tungro disease symptoms include stunting, yellow or yellow-orange leaf discoloration and reduce tillering which are often confused with non-pathogenic disorders such as nutritional deficiencies, excess water after drought or insect injury which causes similar symptoms.

Therefore, it is important to identify or develop methods which are suitably reliable and accurate for detecting the rice tungro pathogens. Moreover, regular monitoring of viruses in the fields by economical diagnostic tools would facilitate the eradication and escape procedures for virus diseases, which is important in preventing further spread of the virus disease. Immunodiagnostic techniques to detect viruses are relatively more specific, sensitive and reliable than symptom identification or by insect transmission of the viruses to assay plants. However, low titre of virus in the vascular tissue of infected rice plants has caused the purification of virus relatively difficult for production of polyclonal antibodies. Alternatively, recombinant coat protein of the virus, which may be produced in large volume whenever necessary, can be prepared as the antigen for the production of antibody (Abu Bakar et al., 2010). This paper describes the purification of polyclonal antibody against recombinant RTBV and RTSV coat protein from rabbit serum and evaluation of the sensitivity of the purified antibodies with real samples.

Materials and Methods

Rabbit antiserum against RTBV and RTSV were diluted with saturated ammonium sulphate with continuous slow stirring for 30 min at room temperature. The serum mixture was then centrifuged at 5000 rpm for 30 min at 4 ^{o}C. The pellet was then resuspended in PBS and dialyzed three times in 0.01M buffer to remove the ammonium salt. The partially purified antiserum was then run through a protein A affinity column (Protein A Sepharose^{TM}) using AKTA Prime Protein Purifier instrument. Phosphate buffer (0.1 M, pH 7) and glycine buffer (0.1 M, pH 3) were used as the binding and elution buffer, respectively. Fraction tubes giving the highest absorbance reading at 280 nm were collected, pulled together and added with 1 M of Tris-HCl as the neutralized buffer. The collected fraction containing the purified antibodies was then dialyzed, freeze-dried and kept at -20 ^{o}C. To determine the amount of protein in the freeze-dried purified antibodies, BCA protein assay was conducted.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

A small amount of the purified antibodies at a concentration of 1 mg/mL was treated with 50 mM β-mercaptoethanol at 95 °C for 5 min and the light and heavy chains were resolved by denaturing gel electrophoresis (SDS-PAGE) on a 12% gradient gel. Partially purified antibodies before being run through the chromatography column were also placed on the same gel. The gel was then stained with Coomasive Brilliant Blue R-250 Staining Solution (Bio-Rad, Hercules, CA).

In performing the ELISA to test the sensitivity of the purified antibodies, wells of a 96 well microtiter plate was coated with 100 µL of 0.01 mg/mL antibody against RTBV or RTSV accordingly. The plate was incubated for 4 h at 37 °C before being washed three times with PBS-Tween at 200 µL/well. Unoccupied sites on the polystyrene well surface were blocked by treating with 0.5 % (w/v) of powdered milk in PBS: 250 µL/well for 1 h at 37 °C. Then, extracted samples of healthy and infected rice plants were added at 100 µL/well. The plate was then incubated overnight at 4 °C and washed three times with PBS-Tween. The infected samples were artificially infected with RTBV and RTSV through its vector, the green leafhopper in a control environment at MARDI Seberang Prai. The antibody of interest was then added again to the wells accordingly and incubated for 2 h at 37 °C. For visualization of the immunological reaction, anti rabbit IgG-alkaline phosphatase conjugate was used and the presence of the enzyme was revealed by the color reaction with p-nitrophenyl phosphate (PNPP).

Results and Discussion

Among the many candidates for the salting out method, ammonium sulphate is the most often used salt in the 'salting out' fraction method as it is a convenient purification step due to several reasons such as at its saturated point (760 g/L), it is of sufficiently high molarity where most proteins are precipitated and thus most proteins can be fractionated and it is also able to accommodate large amount of sample (Stocheck, 1990). As for purification using protein A chromatography column, it has been reported that high yields of pure IgG antibody could be effectively obtained using Protein A as its molar dissociation constant is at about 10^{-7} which reflects its high affinity for the Fc region of immunoglobulin (Huse et al., 2002) . Figure 1 shows the chromatogram of antibodies fraction elution from Protein A affinity column with two peaks where the first peak signaled the unbound protein from the antiserum being eluted and peak 2 signaled the bound proteins which included the antibody of interest being eluted. This also suggested that the binding and elution buffer used in this purification step were suitable in purifying the antibodies. Based on the protein assay, for RTBV antiserum and RTSV antiserum, 1 mg of the freeze-dried product is equivalent to 0.545 mg and 0.048 mg of protein respectively.

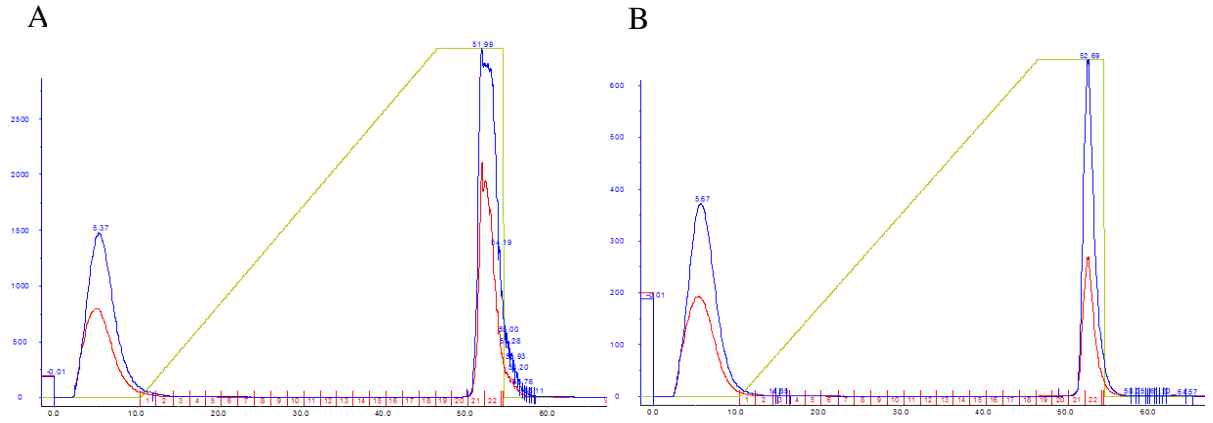

Figure 1. Chromatogram of antibody elution from Protein A affinity column using AKTA prime protein purifier. A: IgG against RTBV and B: IgG against RTBV. The green line represents the addition of elution buffer in percentage into the column.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Rabbit IgG is composed of two identical heavy chains, each containing ~52,000 Da total mass of amino acids and two identical light chains, each approximately 24,000 Da (Miller et al., 2003). SDS-PAGE showed that, while the partially purified antibodies had a few bands, both purified antibodies against RTBV and RTSV revealed only two bands in between 50,000-75,000 Da and in between 15,000-35,000 Da. As there were only two bands present in the purified antibodies that are within the range of rabbit IgG heavy chain and light chain relative molecular mass, the purification of antibodies were deemed successful.

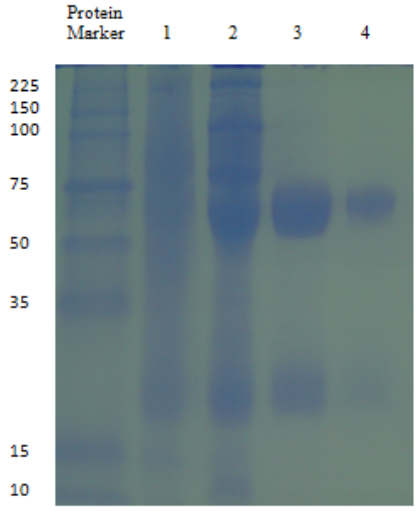

Figure 3. Coomassie Brilliant Blue stained gel after denaturing gel electrophoresis (SDS-PAGE) of partially purified and freeze-dried purified antibodies against RTBV (lane 1,3) and RTSV (lane 2,4)

The sensitivity of both antibodies was then determined by ELISA where artificially infected rice plants were used as samples and healthy rice plants as control. From the ELISA results, the purified antibody against RTBV produced very high 'healthy' background. Therefore, as shown in Figure 2, antibodies against RTBV at a concentration of 0.01 mg/mL antibody, there was no significant difference in the ELISA absorbance reading between healthy and infected sample whereas for antibodies against RTSV, the absorbance reading for infected samples was two times higher than for healthy samples.

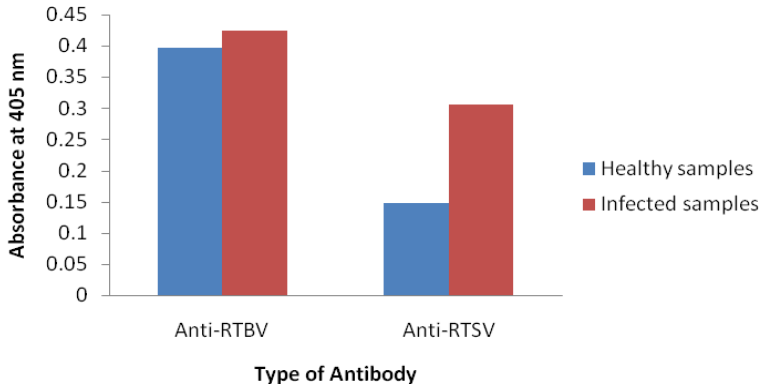

Figure 2. Sensitivity test of the purified antibodies using ELISA.

Conclusions

Antibodies against RTBV and RTSV produced using recombinant RTBV and RTSV coat proteins as the antigens, were successfully purified using ammonium sulphate and protein A affinity column. The antibody against RTSV has the potential to be further applied in the development of screening methods for tungro disease.

Acknowledgements

We are grateful to Dr Norliza Tendot Abu Bakar (Biotechnology Research Centre, MARDI Serdang) for supplying the antisera at the beginning of this project. This work was supported by the Ministry of Science, Technology and Innovation (MOSTI) under the Top Down Nano Directorate Fund (RB5023NF10).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Enhancement of Betalain Production in Dragon Fruit Callus Culture Using Plant Growth Regulators

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Introduction

Dragon fruit or commonly known as pitaya (*Hylocereus polyrhizus)* is a member of the *Cactaceae* family. The fruit flesh is red, yellow or white when ripened. Dragon fruit have been reported as rich in betalains especially in red dragon fruit which has the similar array of colour pigments found in beetroot. Thus, it now becomes a new focus source of natural red dye. Betalains is a secondary metabolite pigment, which is water soluble and containing cluster of nitrogen that play a role in producing red-purple and yellow-orange pigments. Currently, betalains is one of the five most widely used colourant in food industries (Jackman and Smith, 1996). The use of natural pigment as food colourant has now become more important because synthetic food colourant causing side effect on public health.

Betalains is one of the major categories of natural pigment in plant besides anthocyanins and other flavonoids. Betalains is found more stable, not influenced by pH, three times stronger colouring strength and suitable for cold temperature foods colouring without changing the food taste compared to anthocyanins (Nottingham, 2004). Its stability at pH 3 to 7 allows betalains to be used as food colourant at neutral and low acidity level (Stintzing and Carle, 2007). However, obtaining a strong and stable colour from fruit is problematic during processing and storage.

Besides, betalains were reported to have anti-aging, anti-inflammatory, anti-toxin, reducing risk of blood clots and anti-cancer properties. Clinical studies also showed that betalains can increase energy level, clean dead cells from our body as to prevent infection or diseases. In recent years, biotechnology approach has been explored to produce pigments using *in vitro* system. A few systems have been reported to successfully produce secondary metabolite including hairy root culture, suspension culture and callus. Callus culture was reported as a better system for producing secondary metabolite pigments compared to cell suspension culture. This mainly because of the morphology appearance and colour characteristic can be observed easily to facilitate the choice of colour pigment (Stafford and Warren, 1991). In MARDI, a research project has been carried out to identify pigments from dragon fruit. However research on production of betalain pigments using callus culture of dragon fruit has not been exploited. Therefore, this study aims to develop callus culture system for high production of betalains. Effect of different concentration of various type of plant growth regulators, dichlorophenoxy acetic acid (2,4-D), naphthaleneacetic acid (NAA), and thidiazuron (TDZ) on callus growth and pigment produced were examined. The best treatment which give high betalain content will be used for mass-propagate callus for subsequent experiment.

Materials and Methods

Callus induction and pigment production

The pigmented callus was induced using flesh of red dragon fruit that were cultured on the callus induction medium consisting of full-strength Murashige and Skoog, (Murashige and Skoog, 1962) supplemented with 50 mg/L myo-inositol, full-strength MS vitamin, 3% (w/v) sucrose, plant growth

regulator and 0.3% (w/v) phytagel. The cultures were grown at 25 ± 2°C in the dark for one month. The combination of plant growth regulators used consists of thidiazuron (TDZ), naphthaleneacetic acid (NAA) and dichlorophenoxy acetic acid (2,4-D) range from 0 to 5 mg/L. A total of 108 treatments have been investigated. There were 6 explants in each treatment, and each treatment was replicated twenty times.

Multiplication of callus

One month callus derived from each treatment was excised and subcultured onto fresh callus induction medium for multiplication. Subculturing was done for three months at monthly interval to determine the best medium for callus multiplication.

Betalain content in the callus culture was then analysed by using spectrophotometer. Two pigments, betacyanin indicated by red-purple colour pigment and betaxanthin indicated by yellow-orange pigment were detected at absorbance at 537 nm and 480 nm, respectively.

Results and Discussions

Effects of different plant growth regulator combinations on callus induction and pigment production

Previously, the investigation using single plant growth regulator to induce pigmented callus has been carried out. The cultures were kept in light and dark condition at 25±2ºC. It was observed that all the fruit flesh cultured in light condition gradually turns to colourless and none of them produced pigmented callus after 4 weeks. As reported by many researchers, betalains stability was influence greatly by light. Expose to light condition caused degradation of betalains by 15 to 16% respectively. Therefore for subsequent experiment, only dark conditions were applied.

Various concentrations and combinations of dichlorophenoxy acetic acid (2,4-D), naphthaleneacetic acid (NAA), 6-benzylaminopurine (BAP) and thidiazuron (TDZ) were evaluated. Figure 1 shows that the effect of plant growth regulators on pigmented callus and callus morphology were varies when different combination and concentration of plant growth regulator used. In general, combination of BAP or TDZ with NAA produced red pigmented callus while combination of BAP or TDZ with 2,4-D produced yellow-orange pigmented callus. The callus produced in treatments supplemented with 2,4-D produced are small and soft. This callus was not maintaining the colour after second sub-cultured. Its colour changed from yellow-orange to brown which indicated that degradation of betalain pigments occurred. Therefore, 2,4-D was found not suitable for enhancement of betalains in dragon fruit callus culture.

Combination of BAP and NAA was found to be the best in inducing high intensity of pigmented callus as compared to TDZ. Most of the pigmented callus produced in BAP and NAA combination is dark red. The highest intensity of pigmented callus with the maximum betalains content was observed in the callus cultured on MS medium supplemented with 3 mg/L NAA and 3 mg/L BAP. The average callus size is more than 2 cm. However, the callus is less friable as compared to callus produced in treatment combination of TDZ and NAA. The dark red pigmented callus started to turn brown after third sub-cultured.

On the other hand, treatments combination of TDZ and NAA produced red pigmented and more friable callus. The fast growing callus with bigger size which more than 2 cm without much browning was observed on MS medium supplemented with 4 mg/L NAA and 2 mg/L TDZ. The red and friable callus was maintained even after third round sub-cultured. However, the betalains content was lower than the

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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combination of BAP and NAA (Figure 1). The pigmented callus produced from four selected treatments were further analysed using LC-MS/MS.

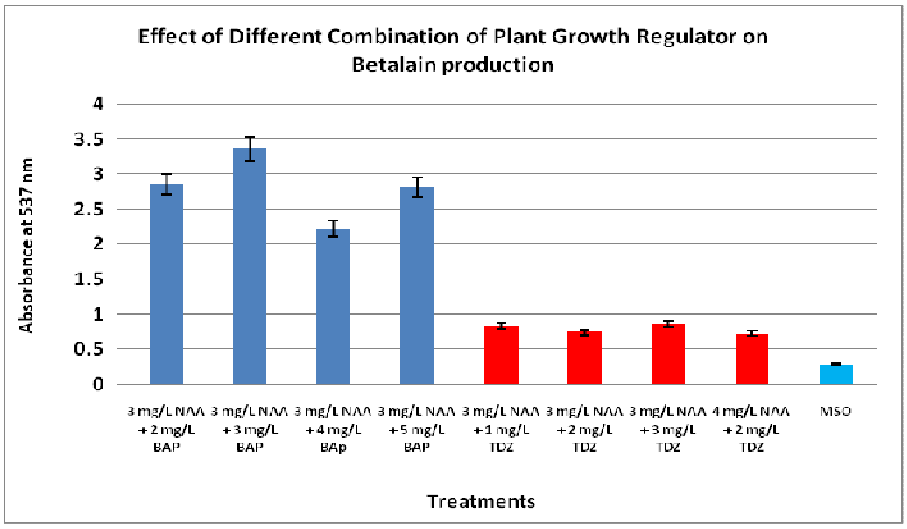

Figure 1. Effect of different plant growth regulator on betalain production

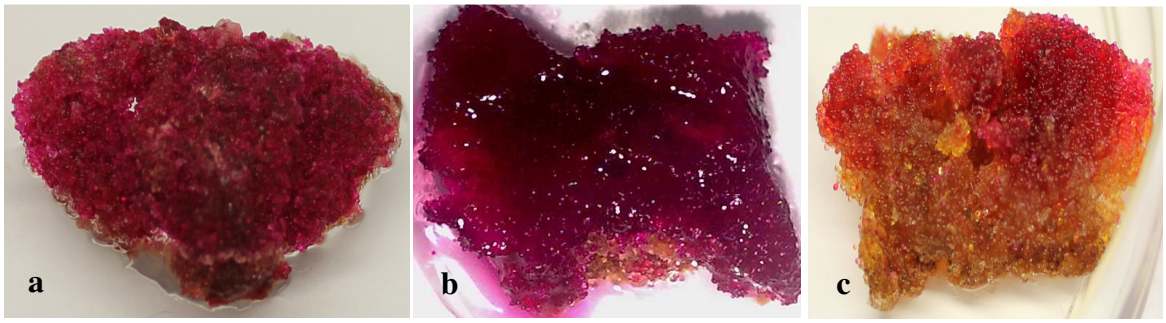

Figure 2. Morphology of pitaya callus cultured on MS media supplemented with different hormone combination. a; Combination of TDZ and NAA, b; Combination of BAP and NAA and c; Combination of 2,4-D and NAA. Callus cultured in MS media supplemented with TDZ and NAA showed darker red compared to callus cultured in MS media supplemented with 2,4-D.

Conclusions

Callus produced in treatment combination of BAP and TDZ with NAA was dark red in colour while with 2,4-D produced yellow-orange colour callus. Among the different type of plant growth regulators examined at different concentrations, the highest intensity of pigmented callus were observed on MS supplemented with 3 mg/L BAP and 3 mg/L NAA. Thus, BAP, TDZ and NAA were used to mass-propagate callus for subsequent experiment.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Expression of *Polygonum minus* Farnesol Dehydrogenase Recombinant Protein

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Introduction

Polygonum minus or kesum from Polygonaceae family is an aromatic plant which has been recognised by the Malaysian government in the Herbal Product Blueprint as an essential oil-producing crop (Wan Hassan, 2007). *P. minus* is shown to have antioxidant (Huda-Faujan et al. 2007), antimicrobial (Suhaila et al., 1996) and anticancer properties (Murakami et al., 2000). *P. minus* produced a broad range of secondary metabolites such as sesquiterpenes. Many plants defend themselves against insect herbivory through the production of secondary metabolites which interfere with insect physiological functions. Recently, farnesol and farnesal have received considerable attention as another valuable intracellular metabolite of sesquiterpenoid metabolism. Farnesol dehydrogenase (FDH) (EC 1.1.1.216), is an enzyme involved in insect juvenile hormone biosynthesis and plays important roles in insect morphogenesis (Hammock, 1985; Riddiford, 1994; Roe and Venkatesh, 1990) and reproduction (Venkatesh et al., 1987; Wyatt and Davey, 1996). For this reason, interfering with its biosynthesis has long been considered a promising strategy for the development of target-specific insecticides. Farnesol dehydrogenase activity was found in sweet potato (Inoue et al., 1983), *Manduca sexta* (Baker et al., 1983), mosquito (Mayoral et al., 2009) and *Arabidopsis thaliana* (Bhandari et al., 2010). Herein we report the isolation of cDNA clone, which encodes farnesol dehydrogenase from *P. minus* and the expression of recombinant protein in bacterial expression system, *Escherichia coli*.

Materials and Methods

Plant Materials

P. minus plants were harvested from the plot in Universiti Kebangsaan Malaysia (UKM). The sample was washed thoroughly with distilled water, ground in liquid nitrogen and directly used for RNA isolation.

Isolation of FDH Full-Length cDNA Clone

Total RNA from *P. minus* was extracted using the modified method described by Lopez-Gomez and Gomez-Lim (1992). To amplify the full length FDH cDNA clone, RACE technique was used. The first strand cDNA was obtained and amplified using the SMART RACE cDNA Amplification Kit (Clontech). cDNA was sythesized from 1 µg total RNA isolated from *P. minus* according to the manufacturer's protocol. Full-length cDNA clones were constructed based on long-distance PCR (LD-PCR) strategy. A pair of new primers was designed against the 5' and 3' untranslated region (UTR) of FDH cDNA. These primers and the first strand cDNA were utilized to obtain the full-length sequence by LD-PCR, using Advantage 2 polymerase mix (Clontech). Alignment of deduced amino acid sequences of *P. minus* FDH with other farnesol dehydrogenase encoding sequences were obtained using the ClustalW program.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Recombinant protein expression

ORF of FDH cDNA clone was subcloned into pQE-2 expression vector (Qiagen, USA). Recombinant expression of FDH protein was performed in bacterial expression system, *E. coli* M15. The proteins were expressed in Luria Bertani (LB) medium containing 100 µg/ml ampicilin and 25 µg/ml kanamycin overnight at 16 °C after induction with 1mM isopropyl-β-D thiogalactoside (IPTG).

Results and Discussion

FDH full-length cDNA clone was obtained by RACE and LD-PCR approaches. The full-length FDH cDNA sequence was found to consist of 1023 bp ORF. BLASTx analysis showed that the deduced amino acid sequence of the cDNA was highly similar to NAD(P)-binding Rossmann-fold superfamily protein from *Theobroma cacao* (76%), Rossmann-fold NAD(P)-binding domain-containing protein from *A. thaliana* (71%) and (72%), dihydroflavonol-4-reductase from *Solanum lycopersicum* (73%), *vitis vinifera* (74%), *Cucumis sativus* (78%) and *Fragaria vesca* (76%) (Table 1). In addition, the FDH amino acid sequence was found to contain the conserved NAD(P)-binding domain which is commonly found with a core Rossmann-type fold (Figure 1). SDS-PAGE analysis showed that a ~40.6 kDa soluble protein was successfully expressed (Figure 2).

Table 1. Similarity between amino acid sequence of farnesol dehydrogenase from *P. minus* with amino acid sequence from other plants.

Organism	Enzyme	E-value	Identity (%)	Score
T. cacao	NAD(P)-binding Rossman-fold superfamily protein	1e-170	76	491
S. lycopersicum	dihydroflavonol-4-reductase	8e-170	73	489
V. vinifera	dihydroflavonol-4-reductase	1e-165	74	478
C. sativus	dihydroflavonol-4-reductase	1e-164	78	475
F. vesca	dihydroflavonol-4-reductase	2e-164	75	475
A. thaliana	Rossmann-fold NAD(P)-binding domain-containing protein	4e-164	70	474
A. thaliana	Rossmann-fold NAD(P)-binding domain-containing protein	2e-159	72	462

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

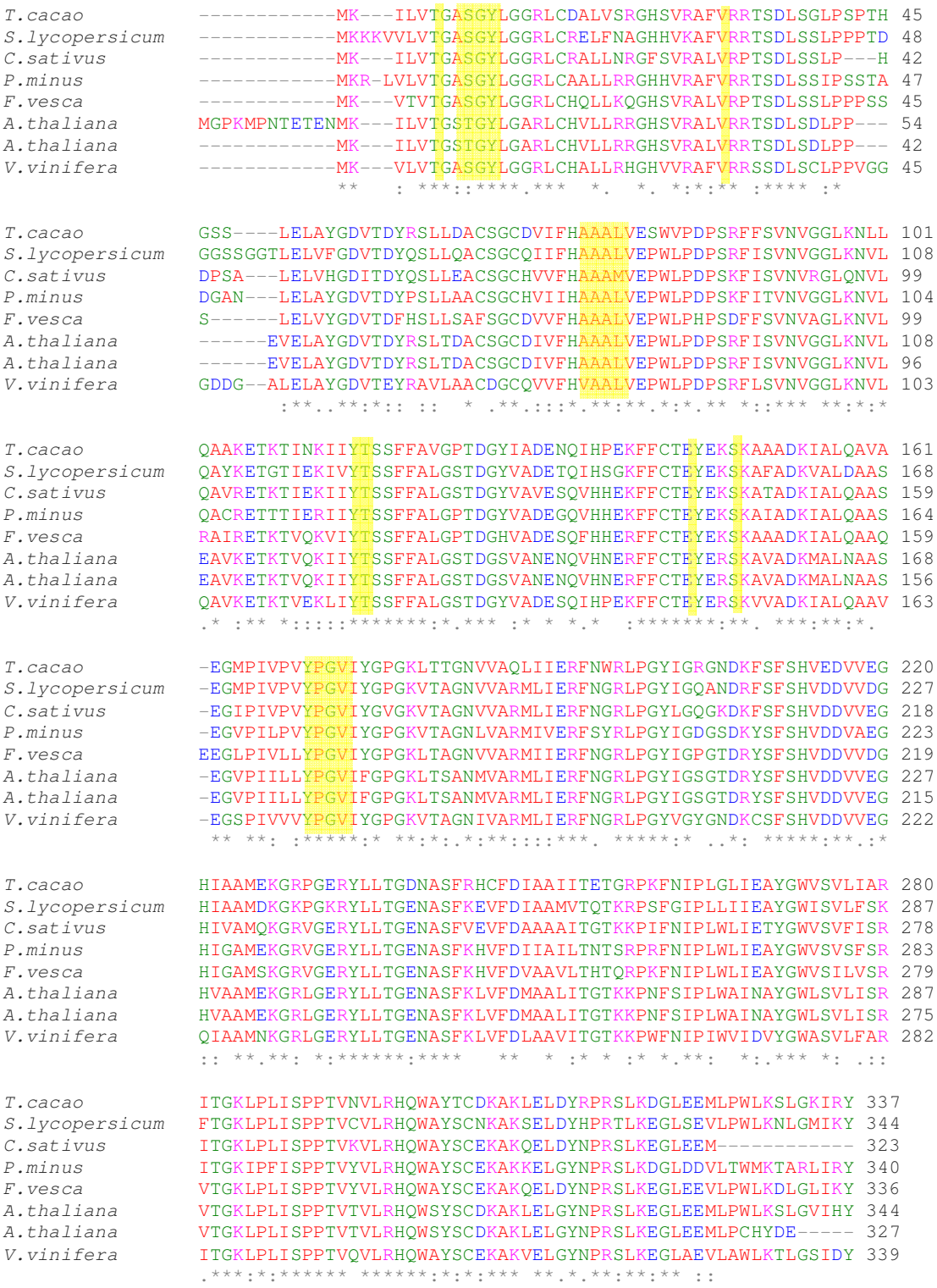

Figure 1. Multiple sequence alignment between amino acid sequence of FDH *P. minus* and these from *Theobroma cacao*, *Solanum lycopersicum*, *Vitis vinifera*, *Cucumis sativus*, *Fragaria vesca* and *Arabidopsis thaliana* using ClustalW. indicated the conserved NAD(P)-binding domain.

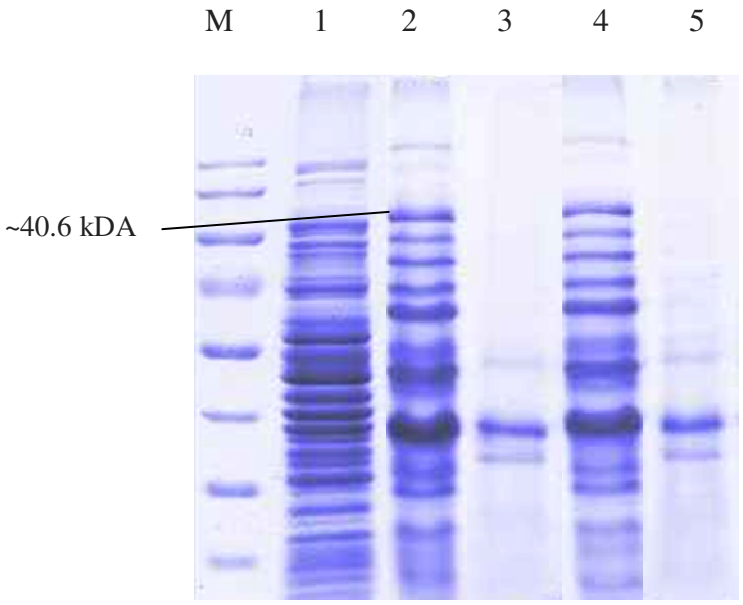

Figure 2. SDS-PAGE analysis of FDH recombinant protein. Lane M: Protein marker; lane 1: uninduced; lane 2 & 4: soluble protein; lane 3 & 5: insoluble protein.

Conclusion

A full-length cDNA clone encoding FDH with the size of 1.1 kb was successfully isolated. Recombinant protein of FDH was successfully expressed.

Acknowledgement

This work was supported by AP2011 grant.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Response of *Kappaphycus alvarezii* Upon Different $CdCl_2$ Concentration Treatment

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Introduction

Heavy metal is a naturally occurring element in nature but extremely toxic for living organism if present at high level. Release of the heavy metal to the environment was contributed by heating systems, metal industries, incinerations, traffics, cements production and phosphate fertilizer (di Toppi and Gabbrielli, 1999; Benavides et al., 2005; Gratão et al., 2005). Almost 30 000 tons of Cadmium (Cd) were released to the atmosphere annually with an estimation of around 4 000 to 13 000 tons contributed by industrial activities (ATSDR, 2005). Cd is classified as Class 1 Carcinogen by International Agency for Research on Cancer (IARC, 1993). Cd enters human and animal body systems via the contaminated plants they consumed. Even though Cd^{2+} is non-essential nutrient for plants, it may enter the plant system through transmembrane carriers engaged in the uptake of essential macronutrients such as Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} (Clemens, 2006; Roth et al., 2006). Thus, plants have developed several mechanisms to cope with heavy metal presence in order to preserve their metabolic activities. Metal exclusion, active excretion, restricted distribution of the metal in sensitive tissues, metal binding on the cell wall, chelation by organic molecules and compartmentalization in vacuoles are some of these mechanisms (Benavides et al., 2005; Gratão et al., 2005). *Kappaphycus alvarezii* is a red marine alga that is commercially cultivated along the coastal region of Sabah, Malaysia for its valuable carrageenan. This study was focused on the determination of lethal Cd concentration on *K. alvarezii* and the time needed by the latter to absorb Cd in the toxic environment to the safe level.

Materials and Methods

Sample preparation

Fresh *K. alvarezii* obtained from Semporna, Sabah was cleaned and kept in a custom-made tank filled with artificial seawater which consisted of sterile water and sea salt. Salinity and temperature were maintained at 28 ppt and 26 ^{o}C, respectively. Guillard's F/2 nutritional medium was added into the tank and water was pumped and filtered constantly to ensure adequate aeration while maintaining the cleanliness. The sample was kept under 12:12 hours of dark and light cycle with intensity of 20 µmol photon/m^2/s. The samples were acclimatized for at least 24 hours before the toxicity tests were carried out.

Viability Test

Fresh *K. alvarezii* sample was cut into algal disc shapes with a diameter of 2 and 3 mm using scalpel. All the algal discs were soaked in the Cd free seawater with salinity at 28 ppt at 26 ^{o}C for a day to stabilize it before the experiment was carried out. Thirty algal discs were placed in each petri dish filled with 20 mL sterile seawater enriched with Guillard's F/2 nutrient. Cd concentrations used in the experiment were 0, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µM with three replications for each concentration.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Petri dishes with the sample were incubated at 26 ^{o}C with 100 rpm shaking for 16 days. The seawater medium and nutrient were changed every two days to avoid nutrient depletion. Numbers of living algal discs were recorded every two days until day 16.

Chlorophyll content analysis

One hundred mg fresh *K. alvarezii* discs from all treatments were soaked in 7 mL of preheated dimethyl sulfoxide at 65 ^{o}C for 45 minutes until the samples become colorless. The samples were then removed from the test tube before another 3 mL DMSO was added. Three mL of the extracts was aliquoted into clean cuvette. Absorbance readings at 645 and 663 nm were recorded using UV-Vis Spectrophotometer.

Total protein content analysis

Fresh sample (0.5 g) from each treatment was ground using mortar and pestle before it was homogenized in 1 mL of phosphate buffer (pH 7.5). The mixtures were then centrifuged at 5000X g for 10 minutes. Pellet was suspended in 500 µL trichloroacetic acid before centrifuged again for another 15 minutes at 8000X g. The pellet was then diluted in 1 mL 0.1 M sodium hydroxide (NaOH). The protein sample absorbance readings at 595 nm were recorded.

Statistical analysis

Means and standard deviations of the recorded data from each analysis were calculated. Analysis of variance, ANOVA were also carried out to validate the significant difference of varied $CdCl_2$ concentrations for each analysis.

Results and Discussion

Viability test

The viability test showed that the number of living algal discs at the last day of experiment increased as the concentration of the $CdCl_2$ increased (Figure 1). Algal discs without $CdCl_2$ treatment were dead at day-8. Generally, algae exposed to high concentrations of heavy metal tend to die faster than the control, but the reverse was observed for the response shown by *K. alvarezii.* High concentrations of $CdCl_2$ seemed to promote the survival of the sample. This might be due to the unique ability of *K. alvarezii* to absorb and chelate heavy metal. It also showed that 500 µM of $CdCl_2$ was still tolerated by the plant.

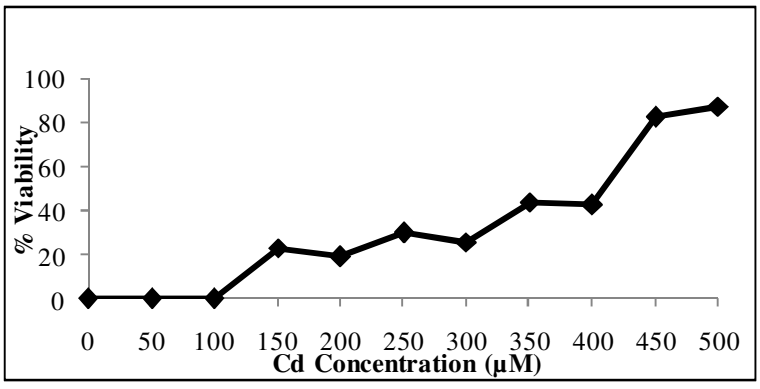

Figure 1. Percentage of living algal discs for every $CdCl_2$ treatments after 16 days.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Chlorophyll content analysis

The total chlorophyll content continued to decrease until day 10 before starting to increase until day 15 (Figure 2). Chlorophyll content is often measured in plants to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Parekh, 1990). The decline in the chlorophyll content of *K. alvarezii* exposed to Cd stress is expected as it must has triggered the inhibition of several proteins involved in chlorophyll biosynthesis such as protochlorophyllide reductase (Van Assche and Clijsters, 1990) and δ- aminolevulinic acid dehydratase (ALA- dehydratase) (Padmaja et al., 1990). Reactive oxygen species (ROS) that are highly produced during stress were also reported as the factor contributing to chlorophyll damage. This indicates that the decrease in *K. alvarezii* chlorophyll content was due to Cd stress. Researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals such as in two wheat varieties to which Cd and Pb were applied, total chlorophyll decreased 50% (*Triticum aestivum* cv. Gerek 79) and 70% (Bolal, 2973) (Oncel et al., 2000). The decrease in chlorophyll content due to heavy metal stress was also reported in almond (Elloumi et al., 2007) and sunflower (Zengin and Munzuroglu, 2006).

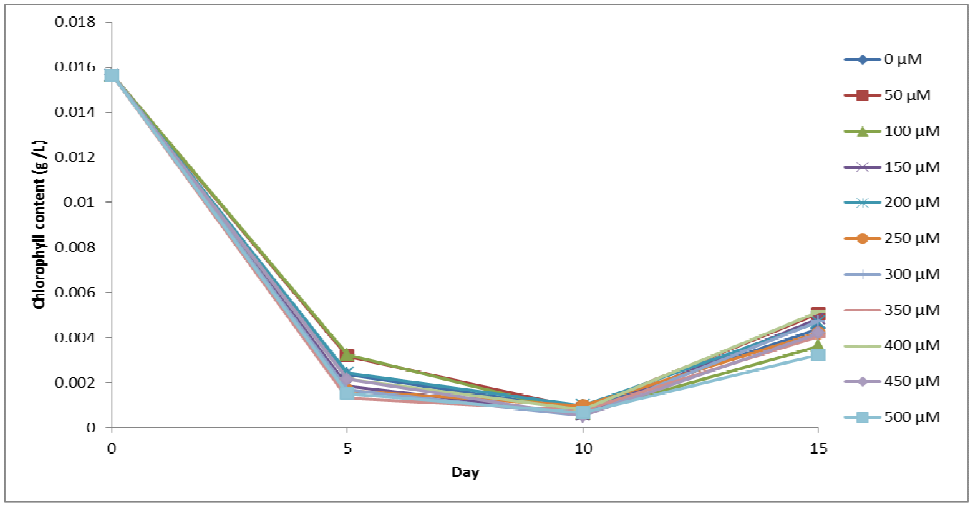

Figure 2. Total chlorophyll content for treatments using $CdCl_2$ treatments at day 0, day 5, day 10 and day 15.

Total protein content analysis

The total protein content analysis showed that there was no specific pattern on protein content in the sample treated with different concentrations of $CdCl_2$ (Figure 3) . Most of the treatments showed that protein content decreased at day-15 but there was an increment of protein content at day-5 for some of the samples. Level of protein content for the controlled sample decreased the most at day-15 even though it showed the highest protein content at day-5 compared to others. Heavy metal stress has been shown to induce a variety of proteins resulting in overall increase in protein content (Shah and Dubey, 1997). The decrease in protein content at higher $CdCl_2$ concentrations may be due to protein degradation process as a result of increased protease activity (Palma et al., 2002). Plants that are tolerant to heavy metal will have an increment in the soluble protein content due to the rapid protein production involved in primary and secondary response to metal toxicity. Proteins involved in the plant defense systems especially in metal chelating process must have been highly synthesized to cope with the stress introduced. A research

done by Ahmad and Jhon (2005) has shown that protein content increased with the presence of heavy metal stress in *Pisum sativum*.

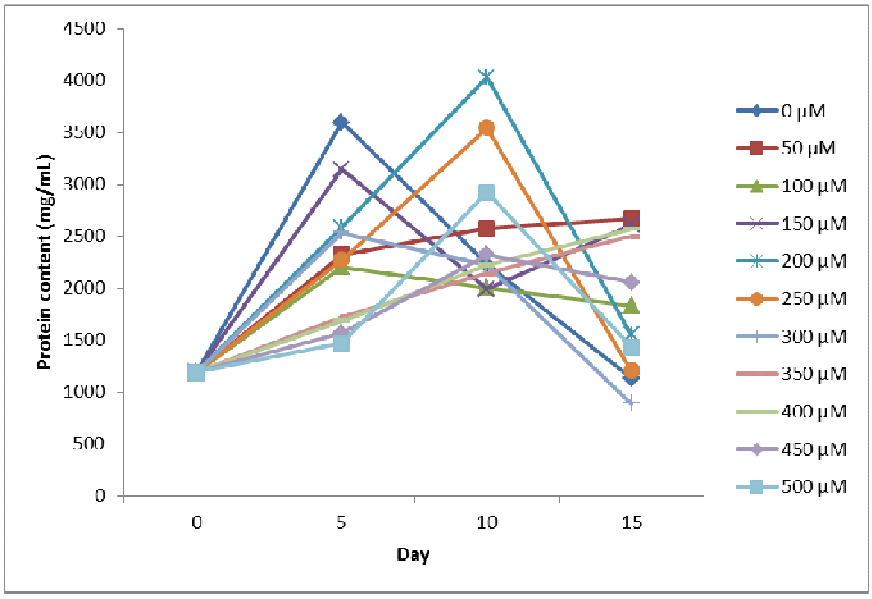

Figure 3. Total protein content for every $CdCl_2$ treatments at day 0, day 5, day 10, and day 15.

Conclusions

In conclusion, our result indicates that the exposure of *K. alvarezii* results in decrease in chlorophyll content but increase in protein content. Viability percentage is higher with higher Cd concentrations. *K. alvarezii* is highly tolerant towards Cd stress which makes it suitable to be used as a heavy metal accumulator in heavy metal affected sea.

Acknowledgement

This research was funded by ERGS/1/2012/stg08/UKM/02/10 grant.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

A Quick Protocol to Facilitate the Selection of Potential Transgenic Papaya Lines for Field Evaluation.

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Introduction

Genetic engineering has become an important tool for improvement of crop quality. Although the process of generating transgenic papaya plant appears simple and straight forward but interpretation of the results could be complicated due to large number of putative transgenic plants need to be screened. Polymerase chain reaction (PCR) results can determine the presence of transgene but is not good enough to assist in determining the potential line that should be processed for field evaluation. Thus, the selection of PCR positive transgenic plants for further evaluation needs careful consideration. Previously, transgene expression in transgenic plant can be determined using Northern analysis. Currently, with the advanced technology, the expression profile of transgene in PCR positive plants can be estimated using real-time PCR and the gene expression results can be used to assist in selecting the transgenic lines needed for further field evaluation.

In this study, the embryogenic Eksotika papaya calli were transformed with 1-*aminocyclopropane-1-carboxylic oxidase* 2 *(ACO*2) gene. This gene is closely associated with fruit ripening in Eksotika (Abu Bakar et al., 2001). In this study, *ACO*2 gene was transformed into Eksotika papaya to prolong the shelf life of the papaya fruit. In our analysis of hundreds of transformed plants, we found that by assessing the gene expression profile helped to significantly reduce the task of having to screen through a large number of transformants for field evaluation. Hence, in this study we report the results of gene expression profile of transgenic papaya lines produced from transformation using *ACO*2 gene compared to non-transformed seedling-derived control plants. The results obtained were able to better facilitate the selection of potential transgenic papaya lines for field evaluation.

Materials and Methods

Agrobacterium-mediated transformation and plant regeneration

One-month-old embryogenic cultures of Eksotika papaya were transformed with the antisense *ACC Oxidase* 2 (pASACO2E1) construct using a previously established *Agrobacterium*-mediated transformation method for Eksotika (Vilasini et al. 2000).

Analysis of transgenic plants

The putative transgenic plants were further analysed using PCR to examine the presence of the transgene(s) in the plant genome. PCR analyses were carried out using the primer pair flanking the 35S promoter and the *ACO*2 gene (ACO2 forward 5' ACTGACGTAAGGGATGA-3' and ACO2 reverse 5-TACATTGCCGTAGATGA-3') and also a primer pair for the *npt*II gene (nptII forward 5'-CCTTATCCGCAACTTCTTTACC-3' and nptII reverse 5'-CACCATGATATTCGGCAAGCAG-3'). The following thermal cycling conditions were used: 2 min at 94°C; 35 cycles of 30 sec at 94°C, 45 sec

at 62 °C and 1 min 30 sec at 72 °C; and finally at 72 °C for 10 min. Twenty microliters of each PCR amplified products were subjected to 1.0% (w/v) agarose gel electrophoresis.

Planting and shelf life analysis of planted transgenic lines

24 independently selected transgenic antisense plants were transferred into soil, with a planting distance of 2.2 m between rows and 2 m within each row, in a netted field measuring approximately 24 m x 18 m x 5.2 m. The transgenic and control fruits were harvested at index 2 and placed on working bench at ambient temperature (25± 2 ºC) for visual examination of the duration to achieved full yellow colour index (index 6).

Analysis of relative gene expression

The housekeeping genes used in this study were 18S ribosomal RNA, 40S ribosomal protein and actin genes. The real-time PCR analysis was carried out using the ABI PRISM 7700 system (Applied Biosystem, California, USA). Real-time PCR was performed with a final volume of 20 µL. The reaction consists of 2 µL of diluted cDNA, 10 µL (1 X) SYBR green master mix, 200 nM each sense and antisense primers, and the final volume was adjusted to 20 µL using sterile distilled water. The PCR cycling conditions were a pre-denaturation step at 94ºC for 2 min, followed by 40 cycles repetition of the following steps: 94ºC denaturation for 15 sec, 55ºC annealing for 30 sec, 72ºC extension for 30 sec and ending at 95ºC for 1 min.

Gene expression analysis was carried out on leaf samples of *in vitro* and field grown putative transgenic plantlets. The C_t value was determined using the real-time PCR instrument's software (ABI PRISM 7700, Applied Biosystem, California, USA). Each DNA template was analysed three times and the experiment was repeated twice. The average of two biological replicates was used for correlation analysis.

Table 1: List of primer pairs used for real-time PCR

Name	Sequence (5'-3')	Length (bp)	Amplicon Size (bp)
*ACO2*F	GCTGGGTTTTACTCTTTTATGTG	23	
*ACO2*R	ACTTCCAAACACCATGATTAGGG	23	140
ActinF	TTCCACTATGTTCCCTGGTATT	22	
ActinR	TCCTATCCAGACGCTGTATTTC	22	119
18_SRF	TTGTTTGATGGTATTTGCTACTCGG	25	
18_SRR	TGAATCATCAGAGCAACGGGCAGAG	25	136
40sRPF	TGGCAAAGCCTACAAAGACTATCA	24	
40sRPR	AGGAATGGGAAGGGAGGGAGAT	22	77

Results and Discussions

Generating transgenic antisense Eksotika papaya plants

Transformation of the *ACO*2 gene into 6,000 embryogenic calli successfully generated 60 putative transformed papaya lines after 4-month selection on kanamycin medium. Out of 60 putative calli transformed with pASACO2E1, 46 were positives for the integration of *npt*II and *ACO*2 genes. The selected putative transgenic lines were successfully transferred into soil with high survival rate.

Gene expression analysis of transgenic papaya plants

Expression study of each putative transgenic papaya lines produced was analysed using mRNA extracted from young leaves samples. Gene expression levels of the *ACO*2 gene in all 46 putative transgenic lines were compared to the wild type seed-derived control plants. The analysis of gene expression data was carried out following Pfaffl's method (2001) which was based on relative expressions of the target gene expressed in the sample and a control in comparison to an internal control gene expression. Of these, 42 independent transgenic lines showed reduction in expression levels of *ACO*2 transgene. The highest reduction expressions of targeted gene were shown in Line 3-1 and Line 27-3 with 5-fold reduction. Other transgenic lines showed reduction of expression level of *ACO*2 between 1- to 3-fold reduction. The expression level of *ACO*2 in non-transformed tissue culture and non-transformed wild type seed-derived control plants are similar. This indicated that non-transformed tissue culture without gene of interest had no significantly effect on the expression of *ACO*2 gene.

Gene expression analyses were also carried out on 24 field grown transgenic papaya lines in the nethouse. Based on the gene expression results, it showed similar pattern of expression whereby the antisense putative transgenic lines L3-1 and L27-3 exhibited higher reduction of *ACO*2 expression levels. Based on these gene expression findings, it proved that the antisense of *ACO2* gene worked in reducing the expression of *ACO*2 gene in the transgenic papaya plants.

The determination of gene expression using real-time PCR is much more convenient as compared to Northern analysis and also can speed up the molecular analysis process. The gene expression level was in qualitative form which can be used to distinquish the level of expression in each putative transgenic line produced. The results are very useful in assist for selecting of putative transformed lines for further field evaluation.

Effect on shelf life on reduction of ACO2 expression in transgenic plants

Further field trial of the transgenic papaya was done to evaluate the effectiveness of antisense technology in knocking down the *ACO*2 gene expression. To facilitate this field evaluation, selection of potential independent transgenic lines was done based on the results of gene expression study. Since the main aim of the transformation process was to reduce the gene expression of *ACO*2, therefore the selected transgenic line for further field evaluation was focused on positive lines that produced low levels of *ACO*2 expression. Twenty four independent transgenic papaya plants were transferred into soil in nethouse to evaluate the effectiveness of this antisense technology in knocking down the expression of *ACO*2 gene.

Eleven transgenic lines showed delayed in skin colour development as compared to non-transformed wild type seed-derived control fruit. Based on the results obtained, it showed that the shelf of the fruits was approximately associated with the level of *ACO*2 expression produced in each transgenic lines. Non-transformed wild type seed-derived control fruit required 4 days to developed full yellow colour index (Index 6). The lowest levels of *ACO*2 gene expression produced by transgenic Line 27-3 resulted the longest shelf life of fruit which required 14 days to achieve full yellow colour. These shelf life analysis results suggest that, the levels of *ACO*2 gene expression in transgenic lines is somehow associated with the shelf life of the transgenic produced. Although the shelf life results is not hundred percent aligned with the gene expression data, but expected results can be predicted. Therefore, the expression level of transgene of *in vitro* putative transgenic plants can be used to select a potential transgenic plant to be planted for further evaluation.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Conclusions

Gene expression study using real-time PCR is useful for precise and fast track selection of potential transgenic Eksotika papaya lines targeting for field evaluation. It minimizes the number of plants to be analysed and allows tracking of the transgene expression in real time.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

CHAPTER 6

PEST AND DISEASE MANAGEMENT

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Determining Critical Period of Crop-Weed Competition for Aerobic Rice

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Introduction

Critical period of weed control (CPWC) is that period of crop life cycle during which crop must be kept weed-free to avoid undesirable economic yield loss (Evans et al., 2003)**.** Theoretically, weed infestation before or after CPWC is not a threat, and should not cause considerable yield loss (Knezevic et al., 2002). Thus, yield obtained by weeding during critical period only is almost similar to that obtained by keeping weed-free throughout. Studying CPWC helps identify residual action required for pre-emergence herbicide, improve timing and decrease the amount of post-emergence herbicide applications, and thus may reduce potential environmental and ecological degradation (Zimdahl, 1980). Since the introduction of CPWC concept in the 1960's countless studies have been conducted around the globe to determine the CPWC in rice (Azmi et al., 2007; Juraimi et al., 2009). Aerobic rice, growing rice in non-puddle soil like an upland crop, is gaining popularity day by day as a water wise technology (Tuong and Bouman, 2003; Anwar et al., 2010). But, this technology is impeded by high weed pressure because of dry tillage and aerobic soil conditions (Zhao et al., 2006; Anwar et al., 2011; Juraimi et al., 2013), and hence weed management has been a challenge for this promising technology (Anwar et al., 2012). It is not unlikely that a switch from flood irrigated to aerobic system will certainly bring a change in species composition, pressure and emergence pattern of weeds resulting in a new dimension of weed-crop competition. Studies on single and multispecies weed interference effects on rice have been extensively conducted in different rice ecosystems, but very little effort has been made so far to determine CPWC in aerobic rice systems. Therefore, the objective of the present study was to determine the critical period of weed control for aerobic rice.

Materials and Methods

The field trial was conducted during the off season 2010 (May-July) and main season 2010/2011 (November – January) under the field conditions at Field 2, Universiti Putra Malaysia, Malaysia. An aerobic rice line AERON 1, sourced from International Rice Research Institute (IRRI), was used as the plant material in this study. The experiment was conducted following a randomized complete block design with three replications. For determining critical period of crop-weed competition, a quantitative series of treatments consists of two components a) increasing duration of weed interference (weed competition for 2, 4, 6 or 8 weeks after seeding (WAS) followed by weed free condition till harvest) and b) increasing length of weed-free period (weed-free for 2, 4, 6 or 8 WAS followed by weed competition till harvest) were imposed. In addition, season long weedy check and weed-free check were included as controls. No herbicide was used and all weed control was accomplished by hand weeding. The experiment was conducted under naturally occurring population of mixed weed species. The experimental field was dry-ploughed and harrowed, but not puddle during land preparation. Rice seeds were directly dry-seeded in rows with 15 cm intra-row spacing. The field was maintained under non-saturated aerobic conditions throughout the growing season. In both seasons, the trial was primarily rain-fed, but supplemental sprinkler irrigation was applied when needed. Overflow canals were maintained to facilitate drainage whenever heavy rainfall resulted in water log. Data were collected on weed biomass, rice biomass and rice yield. Statistical Analysis System (SAS 9.1) software was used to analyze the data, including analysis of variance (ANOVA) and comparison of means based on a protected LSD procedure

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

at 5% level of probability. The CPWC was determined based on Logistic equation and Gompertz model with the help of sigma-plot software for yield loss levels of 5 and 10%.

Results and Discussion

The naturally occurring weed community in the experimental site was comprised of 23 species in off season and 18 species in main season. However, the weed population was mostly dominated by broadleaf weeds with little contribution from grasses and sedges. Due to aerobic soil conditions very high weed biomass were found in both seasons (Table 1). Higher weed pressure was observed in the main season than in the off season. Weed biomass increased with the increasing duration of weed interference period up to 6 WAS and thereafter declined in both seasons. In contrast, weed biomass decreased with increasing duration of weed-free period. The high weed pressure observed in this study confirms the findings by several researchers (Mahajan et al., 2009; Jaya-Suria et al., 2011; Anwar et al., 2012) who reported that weed pressure in aerobic rice is highest among all rice ecosystems. Biomass production and grain yield of AERON 1 were significantly influenced by weed interference period in both seasons; rice biomass and grain yield were increased with increasing length of weed- free period for up to 6 WAS after which no significant improvement was observed. In contrast, both biomass and grain yield were significantly decreased with increasing span of weed interference period up to 8 WAS, and thereafter remained unchanged. Apparently, the grain yield recorded was slightly higher in the main season than in the off season. Season long weed-free conditions produced a yield advantage of 115 and 122% over season long weedy conditions in the off and main season, respectively (Table 1). Weed competition throughout reduced rice yield by approximately 55% in both seasons as compared with season long weed-free period. These values are very close to those previously reported, where season long weed competition reduced yields by 50% (Johnson et al., 2004). Juraimi et al. (2009) recorded 79 and 66% yield reduction in rice due to weed competition till harvest under flooded and saturated conditions, respectively. Chauhan and Johnson (2011), on the contrary, reported as high as 95% yield reduction in aerobic rice due to weed competition throughout the crop growing season.

Table 1. Effect of duration of weed competition on weed biomass, rice biomass and rice yield in off season 2010 and main season 2010/2011.

Weed competition period	Weed biomass (g/m^2)		Rice biomass (g/m^2)		Rice yield (t/ha)	
	Off season	Main season	Off season	Main season	Off season	Main season
Weedy until 2 WAS	155.33 de	188.33 e	660.71 ab	669.60 b	3.37 ab	3.46 ab
Weedy until 4 WAS	215.00 cd	239.0 de	631.00 b	652.00 b	2.99 a-c	3.07 bc
Weedy until 6 WAS	520.33 a	548.33 a	443.53 d	431.30 d	2.5 c-e	2.59 d
Weedy until 8 WAS	503.00 a	532.33 a	235.42 e	205.55 e	1.98 e-f	2.01 e
Weedy check	390.00 b	431.67 b	169.00 f	130.49 f	1.68 f	1.73 e
Weed-free until 2 WAS	342.00 b	364.67 c	247.48 e	253.67 e	2.22 df	2.43 d
Weed-free until 4 WAS	256.67 c	286.00 d	553.00 c	589.07 c	2.79 b-d	3.01 c
Weed-free until 6 WAS	164.33 de	181.67 e	664.36 ab	654.23 b	3.22 ab	3.51 a
Weed-free until 8 WAS	107.33 e	101.33 f	673.00 ab	687.58 ab	3.49 a	3.76 a
Weed-free check	-	-	679.45 a	729.33 a	3.61 a	3.84 a

Within a column for each factor, means sharing same alphabets are not significantly different at P=0.05 probability level according to least significant difference test. WAS= weeks after seeding

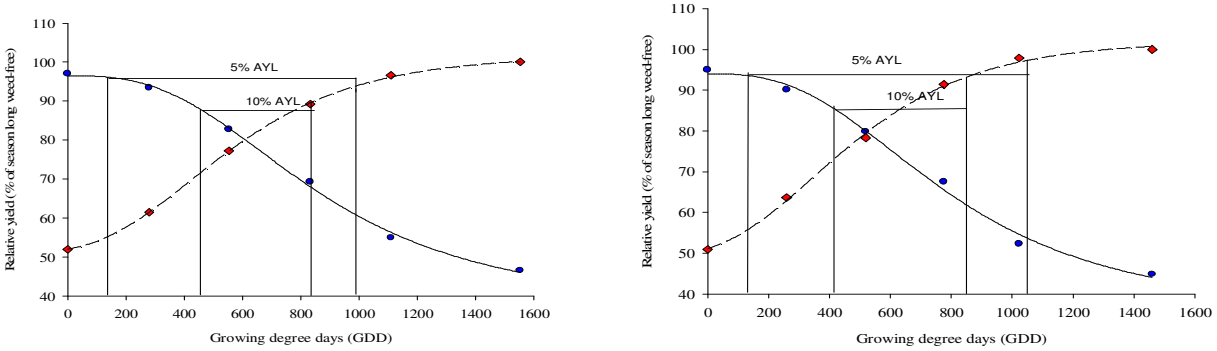
Critical period of weed control (CPWC) was determined using relative yield (% of season long weed-free yield) and growing degree days (GDD) as quantitative variables in the regression analysis. Predicted and observed relative rice yields as affected by weed interference and weed–free periods in the off and main seasons are shown in Figure 1. Responses were highly significant as indicated by high R^2 values. In

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

the off season, the beginning of CPWC based on 10% accepted yield loss (AYL) occurred with 456 GDD corresponding to 23 days after seeding (DAS). In contrast, for the same AYL in the main season, weeds were required to be removed at 412 GDD, corresponding to 21 DAS. The end of the CPWC at 10% AYL occurred at 832 GDD or 40 DAS in the off season and 847 GDD or 43 DAS in the main season. At 5% AYL, the onset of CPWC occurred at 137 GDD or 7 DAS in the off season and 131 GDD or 7 DAS in the main season. Weeds had to be controlled until 987 GDD or 49 DAS in the off season at a 5% AYL. In contrast, for the same AYL in the main season, rice fields should be kept weed-free until 1044 GDD, or 53 DAS (Table 2).

Table 2. Estimated critical periods of weed control for two acceptable levels of crop losses in off season 2010 and main season 2010/2011.

Yield loss levels (%)	Critical period							
	Off season 2010				Main season 2010/2011			
	Onset		End		Onset		End	
	Growing degree days	Days after seeding	Growing degree days	Days after seeding	Growing degree days	Days after seeding	Growing degree days	Days after seeding
5	137	7	987	49	131	7	1044	53
10	456	23	832	40	412	21	847	43



Off season 2010

♦ RY= 50.7278+50.8116*exp(-exp(-(x-418.5238)/315.6532))
R^2=0.999

● RY=38.5194+56.3398/(1+abs(x/859.9014)^3.1213)
R^2=0.998

Main season 2010/2011

♦ RY= RY=61.1348+39.4928*exp(-exp(-(x-479.7600)/217.4354))
R^2= 0.999

● RY= 38.5968+52.2125/(1+abs(x/794.2458)^3.4376)
R^2= 0.994

Figure 1. Influence of weed interference on relative yield of aerobic rice variety AERON 1 in the off season of 2010 and main season of 2010/2011. Increasing duration of weed interference (●) data fitted to the logistic equation; increasing weed-free period (♦) data fitted to the Gompertz equation. The dots and the lines represent observed relative yield and fitted models, respectively. AYL= accepted yield loss; RY= relative yield.

It was evident from our study that CPWC of AERON 1 was variable in length between seasons, and was a bit longer in the main season than in the off season. The beginning of the critical period was relatively stable between seasons, while the end, on the other hand, was more variable. The onset of CPWC was delayed and ended earlier as the predetermined AYL was increased from 5% to 10%. Our findings closely resembles to those reported by many researchers. Juraimi et al. (2009) suggested that direct seeded rice should be kept weed-free for 2-71 DAS in saturated condition and 15-73 DAS in flooded condition. In the Philippines, Chauhan and Johnson (2011) estimated CPWC of aerobic rice as between 18 and 52 DAS to obtain 95% of weed-free yield. Juraimi et al. (2009) also estimated a longer CPWC in the main season and a shorter one in the off season in both flooded and saturated conditions. Johnson et al. (2004) also observed differences in CPWC between seasons in rice.

Conclusions

The study portrays the significance of CPWC determination on sustainable weed management in aerobic rice. The practical implication of this study is that under similar field conditions aerobic rice fields should be kept weed-free during 7-49 DAS in the off season and 7-53 DAS in the main season to achieve 95% of weed- free yield, and 23-40 DAS in the off season and 21-43 DAS in the main season to achieve 90% weed-free yield. Since 5% yield loss level would not be practical from economic view point, a 10% yield loss may be considered sufficient in terms of economic returns, and this level can be achieved by early post-emergence application of herbicide or weeding between 10-15 DAS followed by a post emergence application or weeding between 30-35 DAS. Since weeds emerging after this period causes no substantial yield looses, the need for applying additional herbicides or weeding more than 2 times as practiced by many farmers is not warranted, and this would result in significant cost savings.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Preliminary Study: Enzyme Inhibition Biosensor for Dithiocarbamate Fungicides Detection

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Introduction

Pesticides play an important role in order to get high achievement in agricultural product through the control of pests (Van Dyk and Pletschke, 2011). Dithiocarbamates (DTCs) are a group of organosulfur compounds that have extensively been used as fungicides in agriculture for more many years. It forms the most important class of fungicides for broad spectrum control not only for vegetables but also for a variety of diseases on seeds and fruits (Noguer et al., 2001). Commonly used fungicides in this group are such as zineb, maneb, mancozeb, ziram and thiram. The widespread use of pesticides without control can have a detrimental effect on human health. Although acute toxicity of these compounds is low, these fungicides constitute a pesticide family of environmental concern since many reports suspected them of inducing neurological troubles resembling Parkinson disease (Soleo et al., 1996), carcinogenesis, teratogenesis, mutagenesis and goitrogenesis. Other than that, dithiocarbamate was also easily transported in soils due to its solubility in water. According to Malaysian Food Act 1983 (2009 edition), maximum residue limits (MRLs) for dithiocarbamate in vegetables and fruits is 10 ppm.

There are many methods available for pesticide detection: chromatographic methods such as gas chromatography (GC) and high performance liquid chromatography (HPLC) coupled with mass spectrometry (MS). These high technology methods are very sensitive and reliable but they have major drawbacks such as complex and time consuming treatments of the samples which need extraction of pesticides, extract cleaning, solvent substitution and etc. Furthermore, they can only be performed by highly trained technicians and are not convenient for on-site or on-field detection.

There is a need for rapid detection and on-site use for monitoring the MRLs of dithiocarbamate pesticide in vegetables and fruits. Biosensors are potentially useful as they detected pesticides quickly and have been active in the research area for some years (Gamal, 2010). Enzymatic determination of pesticides is most often based on inhibition of the activity of selected enzyme. Enzyme inhibition by pesticides was used for measuring purpose using the electrochemical sensor (Trojanowicz 2002, Silvia and Arben, 2003). Wiegand-Rosinus et al. (1990) have proof that aldehyde dehydrogenase was strongly inhibited by dithiocarbamates fungicides compounds. Based on this fact, we try to study the inhibition of aldehyde dehydrogenase activity using biosensor approach for dithiocarbamate detection.

Materials and Methods

Reagents

Aldehyde dehydrogenase from *Saccharomyces cerevisae*, β-nicotinamide adenine dinucleotide (NAD^+) 99% purity, propionaldehyde, zineb (zinc-(ethylenebis)-dithiocarbamate) and Phosphate buffer.

Enzyme assay

About 10 µL of enzyme, 10 µL of 0.1 mM NAD^+, 20 µL of 50 µM propionaldehyde and 15 µL of 0.2 M phosphate buffer pH 7.5 were dropped on screen-printed carbon working electrode (SPCE) with carbon

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counter and silver chloride reference electrode connected to the electrochemical analyser. The response of the sensor was measured at the steady state, when the current reached plateau phase. The response time was less than 2 min.

Inhibition assay

About 10 µL of enzyme and 10 µL of zineb were dropped on the electrode and incubated for 2 mins. 10 µL of 0.1 mM NAD^+, 20 µL of 50 µM propionaldehyde and 15 µL of 0.2 M phosphate buffer pH 7.5 was then dropped on the same electrode and the response of the sensor was measured when the current reach the plateau phase and the percent of inhibition were calculated.

Enzyme optimization

For enzyme optimization, the same procedures as above were used with different enzyme concentrations (0.25, 0.5 and 1 U) and different zineb concentrations (50 and 110 ppb).

Characterization of enzyme inhibition

Zineb was used to inhibit the enzyme. The same procedures as above were used with different concentrations of zineb (0, 30, 50 and 110 ppb).

Results and Discussion

This enzyme biosensor for dithiocarbamate detection was develop based on the inhibition of dithiocarbamate fungicides on enzyme-substrate reaction (Aldehyde dehydrogenase - Propionaldehyde) which will reduces the reduction current produces from the oxidation-reduction enzyme-substrate system using ß-nicotinamide adenine dinucleotide (NAD) as a cofactor (Figure 1). An electrochemical measurement was conducted using a screen-printed carbon working electrode (SPCE) with carbon counter and silver chloride reference electrode connected to the electrochemical analyser.

$$\text{Propionaldehyde} + NAD^+ \xrightarrow{\textit{Aldehyde dehydrogenase}} \text{Propionic acid} + NADH + H^+$$

Figure 1. Aldehyde dehydrogenase catalyses the oxidation of various aldehydes using NAD as a cofactor

Enzyme optimization was done to determine the best concentration of enzyme used in the reaction. The result showed that 0.5 U of enzyme was found to be the best enzyme concentration. This is based on the percent of inhibition assay using two different concentrations of zineb, 50 and 110 ppb where both exhibit similar results (Figure 2). The performance of enzyme biosensor was studied by exposure the enzyme with different concentration of dithiocarbamate fungicide. It showed that the reduction current reduces with an increase in the concentration of dithiocarbamate fungicides on the sensor surface (Figure 3). This is because dithiocarbamate fungicides have a shape that resembles the shape of the substrate, thus blocking the active center of the enzyme and inhibiting its activity (Gamal, 2010). More active site of the enzymes were blocked in the increasing concentration and reduces the reduction current value.

ISBN 978-967-10840-3-8
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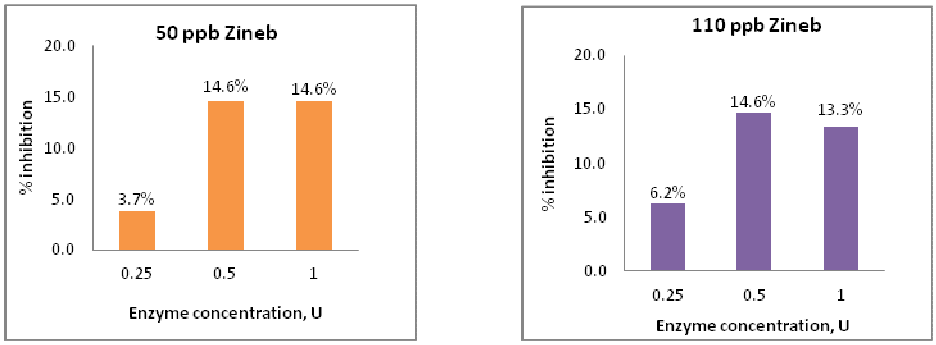

Figure 2. Enzyme optimization result in different concentration of dithiocarbamate a) 50ppb of zineb b) 110 ppb of zineb. Both showed similar results.

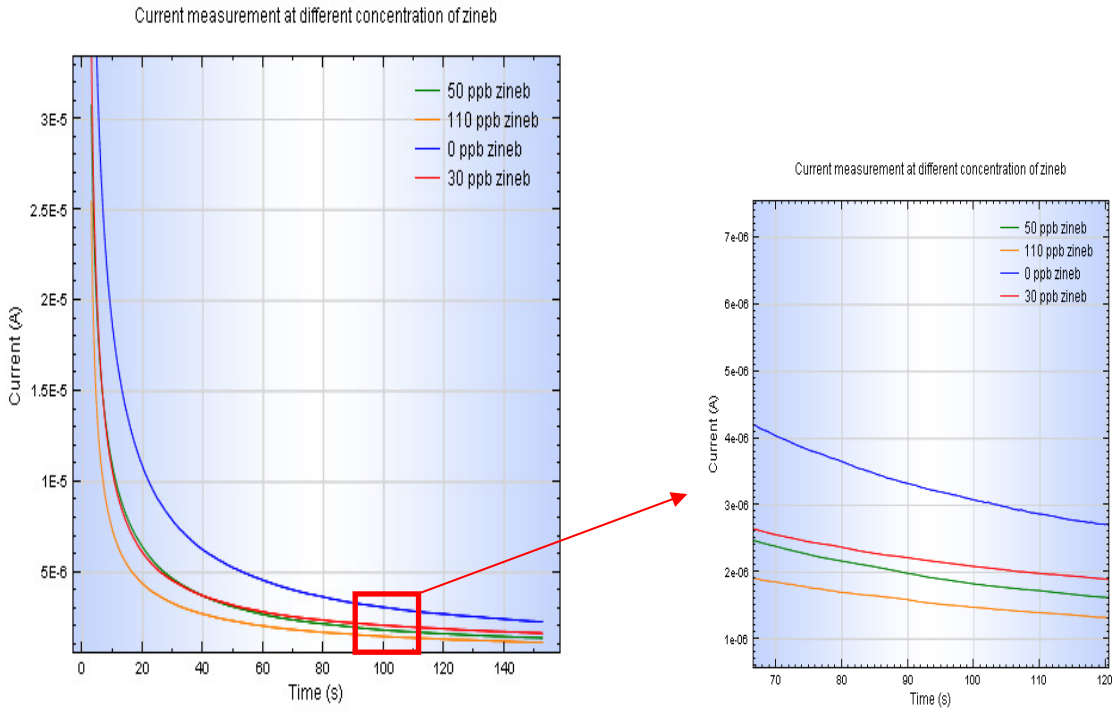

Figure 2. The performance of the enzyme biosensor towards exposures with different concentration of dithiocarbamate fungicides (Zineb). The reduction current reduces with an increase in the concentration of dithiocarbamate fungicide

Conclusions

In this study, we found that dithiocarbamate fungicide was possible to be detected by using biosensor based on inhibition of aldehyde dehydrogenase activity. Since each reaction only took less than 2 mins, it was much more rapid when compared with commonly used spectrophotometric and chromatographic methods. As this is only preliminary study, this sensor will be improved by enzyme immobilisation and using nano-particle for enhancing the current signal.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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Acknowledgments

This work was financially supported by Ministry of Science, Technology and Innovation (MOSTI) under Biosurveillance project (TP-RB-0002).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

The Effects of Polyamines on Growth and Biochemical Changes in Protocorm Like Bodies (PLBs) of *Spathoglottis plicata*

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Introduction

Spathoglottis plicata or also known as "orkid pinang" is one of the orchids that have captured horticulturist with its beautiful flower and good stature. This orchid has different morphological appearances when compared with other commercial orchids because its leaves are pleated and rather slim. The leaves can grow between 60 and 120 cm long and 7 cm wide. A shoot will grow from the older pseudobulb as a sheathing leaf and the other leaf will form later around 3 to 5 proper leaves on a long stalk. Its purple flower is self pollinating and can bloom for several weeks. *Spathoglottis plicata* 'alba' produce white colour flower with yellow lips while *Spathoglottis unguiculata* flower has a fragrance like a grape juice (McKinley, 2005). *Spathoglottis* is a slow growing type of orchid. Its multiplication frequency is also low. However, this problem can be overcome with the right amount of nutrient. There are some reports suggesting the ability of polyamines and amino acid improving the growth of this plant. But the knowledge on the effect of *in vitro* application of these chemical on tissue cultured plants is still limited. Polyamine is an organic compound having two or more primary amino groups. This compound can be found in plants both in free and bounded form. Putrescine, spermine, and spermidine are derived from the decarboxylation of amino acids arginine or ornithine (Kakkar et al., 2000). Polyamines appear to be important in cell division, plant growth, senescence as well as stress responses (Alcazar et al, 2006). Putrescine for instance, had been proved to increase the proliferationn of *Dendrobium Sonia* Protocorm Like Bodies (PLBs) (Saiprasad et al., 2004). No such study has been carried out using polyamine on growth and biochemical changes of *S. plicata* PLBs. Therefore, to investigate the effects of polyamine on the physical and metabolic changes of *S. plicata* PLBs, putrescine, spermine, and spermidine were selected as a potential polyamine.

Materials and Methods

Seed germination and induction of PLBs

The PLBs used in this experiment were initiated from seeds that were cultured on the basal media supplemented with 5 µM 2-4D to produce callus. The three weeks callus formed PLBs after four weeks of culture. The three weeks PLBs derived from the callus were subcultured into basal media added with 5 µM BAP for further use.

Media preparation

Half strength MS media supplemented with Vitamin B5, 2 % (w/v) sucrose and 0.3 % (w/v) Gelrite was used as a control and basal media. The pH of the culture media was adjusted to 5.75. Three polyamines were used with the same concentration, 50 µM of Putrescine, Spermine and Spermidine were added to basal media. Media with no polyamine were used as control in all experiment. All experiments were carried out by culturing 0.15 g PLBs to the inductive media. All cultures were maintained at 25 □C in the culture room condition under a 16 h photoperiod of 40 µmol m^{-s} s^{-1} light provided by cool white

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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florescent tubes. The results were collected after two weeks of culture period. The fresh and dry weight was recorded to determine the physical changes and total soluble protein and carbohydrate content of PLBs were determined.

Result and Discussion

The fresh and dry weight of *S.plicata* PLBs treated with 50 µM was lower compare to toher treatments and the control (1/2 MS) as showh in figure 1. However the PLBs culture with this treatment also produced highest total soluble protein but lowest carbohydrate content (figure 2). Polyamine can occur as a free form or bound to the phenolic acid or other low molecular weight molecule. Due to it cationic nature it is suggested that polyamine can act as scavenging free radicals, influencing nucleic acids and protein synthesis, RNAse, protease and other enzyme activities (Kaur-Sawhney et al., 2003). According to Wang et al., (2006) exogenous application of polyamines are able to increase the protein content of *Nymphoides peltatum* leaves under copper stress. Therefore the production of the protein in *S.plicata* might be affected by the polyamine. However according to Leon and Sheen (2003) Sugar signaling might be have a role in activation of ABA production during the osmotic stress. This might be the reason for the productions of carbohydrate in S.plicata PLBs were higher in certain treatment with polyamine.

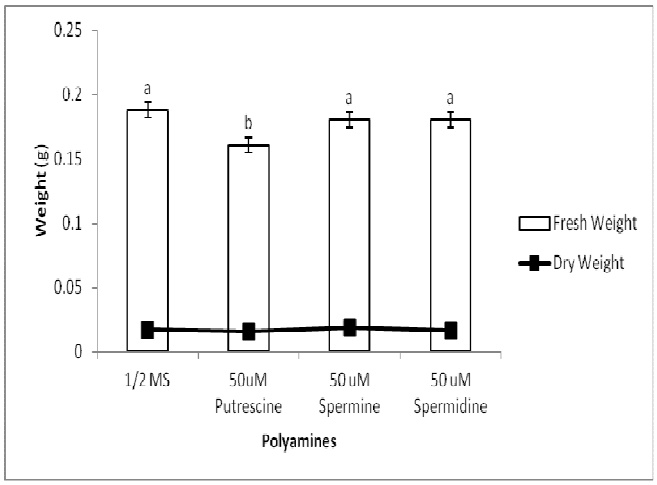

Figure 1. The average fresh weight and dry weight (g) of *S. plicata* PLBs after two weeks of culture onto ½ MS media supplemented with 50 µM of putrescine, spermine and spermidine. The similar letter shows insignificantly different according to Duncan's Multiple Range Test ($p<0.05$)

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

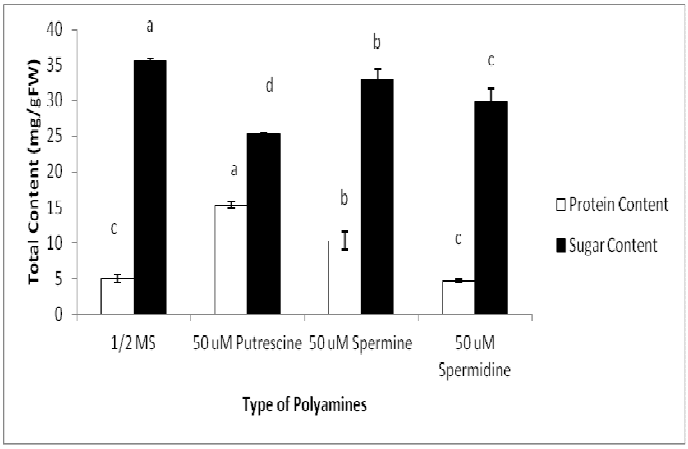

Figure 2. The total soluble protein and carbohydrate content of *S. plicata* PLBs after two weeks of culture onto ½ MS media supplemented with 50 µM of putrescine, spermine and spermidine. The similar letter shows insignificantly different according to Duncan's Multiple Range Test ($p<0.05$)

Conclusions

From this study we can conclude that polyamine is not suitable for the growth of *S.plicata* PLBs. However the result can be use for further experiment, to observe the effects of polyamine on the growth of other plant.

Acknowledgements

The author would like to thank Universiti Putra Malaysia for financial supported provide through Graduate Research Fund.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Toxicological Evaluation of Sambung Nyawa (*Gynura procumbens*) Extract Grown under Net House Condition

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Introduction

Herbs or medicinal plants have been used by human since ancient times and many of the primary sources of available drugs have been directly or indirectly derived from plants. For example, Metformin that are widely used to treat and to reduce the occurrence of diabetes was extracted from *Galega officinalis* (Bailey and Day, 2004). Approximately 800 plant species were reported to have anti-diabetic properties and plant derived compounds are mainly from the group of alkaloids, glycosides, galactomannan gum, polysaccharides, hypoglycans, peptidoglycans, guanidine, steroids, glycopeptides, and terpenoids which have demonstrated bioactivity against hyperglycemia (Mentreddy et al., 2005).

Gynura procumbens or locally known in Malaysia as Sambung Nyawa, is a small shrub tree that was used traditionally to treat fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus, and cancer. There are many of traditional claims on Sambung Nyawa have been validated through scientific research for it anti-virus, anti-inflammatory, anti-hyperlipidemic and anti-hypertensive activities. However, Sambung Nyawa was received much attention as anti-diabetes medicinal plant because of its ability to reduce the glucose level in diabetes patient. Recent study shows that the extract of Sambung Nyawa produce significant elevation in the fasting blood glucose (FBG) levels of normal rats, but a decrease in diabetic rats (Algariri et al., 2013).

The consumption of herbal remedies worldwide is recently increased and it is stimulated by several factors such products safety and effectiveness. However, in order to prevent life-threatening effect by the unknown toxicity in herbal remedies, scientific research in needed to validate the safety of herbal remedies. Through toxicity analysis using animal model, herbal extract or phytonutrients can be evaluated to ensure the component in herbs are safe enough for consumption and also have healing effect on certain disease. Therefore, this study aimed to carry out extensive toxicological evaluation of the methanol leaf extract of Sambung Nyawa using rat as a model system.

Materials and Methods

Production of Sambung Nyawa plant in net house

Sambung Nyawa plant was propagated through stem cuttings. The stem was cut at approximately 7 cm size and dipped into rooting hormone before immediately put the bottom of the cuttings into the soil (pot size: 25×100 cm). The plant was irrigated twice a day and after two months the plant is ready to be harvested for phytochemicals extract.

ISBN 978-967-10840-3-8
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Chemical profiling of phytonutrients from Sambung Nyawa extract

a. *Extraction of Sambung Nyawa extract*

Freeze dried sample of Sambung Nyawa were extracted with absolute methanol. After extraction, sample was dried and reconstitute with 30% methanol before being injected into LCMS-MS

b. *Phytochemicals profiling of Sambung Nyawa by LCMS-MS*

Separations of phytochemicals were achieved with the usage of reverse phase C18 columns (Thermo Hypersil GOLD C18) precede with a guard column. Electron spray ionization in trap mode was utilized to fragmentize the phytochemicals in negative mode.

Toxicology studies of Sambung Nyawa on laboratory rat
Sprague-Dawley rats (≈150 g) were acclimatized at a room temperature of 22 ºC for one week. Food and water were supplied *ad lbitum*. Rats were separated according to treatment and a control. The treatment consisted of 3 different dosages, low dose (100 mg/kg), medium dose (700 mg/kg) and high dose (1400 mg/kg). After a week of acclimatization, each rat was administered orally with either water or the treatment mixture according to each dosage by oral gavage technique. Control group received 1 mL of water. This procedure was repeated for 21 days. After 21 days, blood was withdrawn as much as possible via cardiac puncture for haematology and serum analysis. Rats were culled by cervical dislocation and organs (spleen, heart, kidney, lung, duodenum, pancreas, stomach) and muscle tissue were taken for histopathology analysis.

Results and Discussion

Methanol extract of Sambung Nyawa was extracted from two month's old Sambung Nyawa plant which is grown under nethouse condition (Figure 1). Profiling of chemicals compound by chromatogram obtained via LCMS-MS have identified five main keys of phytochemicals existed in Sambung Nyawa methanol extract. It was found that Dicaffeoylquinic acid is the highest phytochemicals obtained from methanol extract of Sambung Nyawa.

Figure 1. Two months old of Sambung Nyawa plants propagated under net house condition

For the haematology analysis, the total white blood cell count, red blood cell values, platelet counts and lymphocytes readings were taken into highlight as they are important parameters that determine the health status relating to the safety and efficacy issues. Overall, the haematology analysis for total white blood cell count, red blood cell values, platelet counts and lymphocytes readings have shows no

ISBN 978-967-10840-3-8
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First Published, 2014

significant differences when compared to the control group (Figure 2). The haematology analysis results for low to high dosages of Sambung Nyawa extract treatment was detected in the range of normal rat value for Sprague-Dawley strain (Petterino and Argentino-Storino, 2006). However, the value of Lymphocytes for medium dosages treatment was lower than rat standard normal value. This abnormal reading is a bit doubtful since the reading for high dosages was in the normal range.

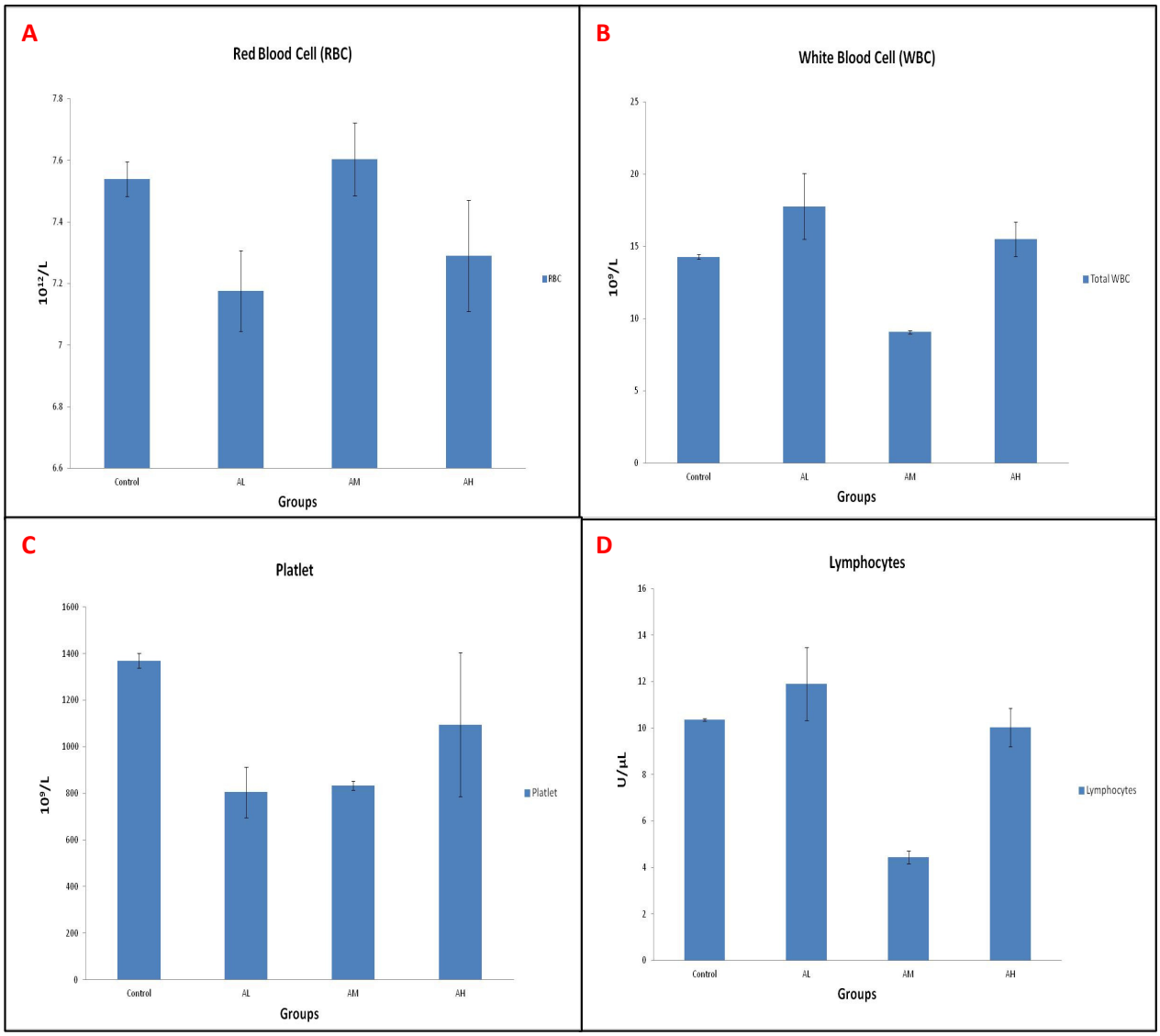

Figure 2. Comparison of Red Blood Cell (RBC), White Blood Cell (WBC), Platlet and Lymphocytes count between rats treated with different dosages of Sambung Nyawa methanol extract with a control group. A-Red Blood Cell, B-White Blood Cell, C-Platlet and D-Lymphocytes.

ISBN 978-967-10840-3-8
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Serum analysis for Sambung Nyawa treatment indicates that the reading for creatinine and ALT was found to have normal range for all dosages given (Figure 3). Only medium dosages give normal reading for the urea value. This abnormal reading for urea value is also a doubtful result since that low dosages gave abnormal reading. Furthermore, AST reading for all dosages of Sambung Nyawa treatment give abnormal reading including rat without treatment which is act as control group. This condition is clearly shows that there are others factor that influenced the abnormal reading for this experiment such as food and environment condition. Nevertheless, serum analysis depends much on the histopathology study as it supports the analysis. The morphology of cells can explain these results clearly. However, early deductions can be withdrawn from this analysis.

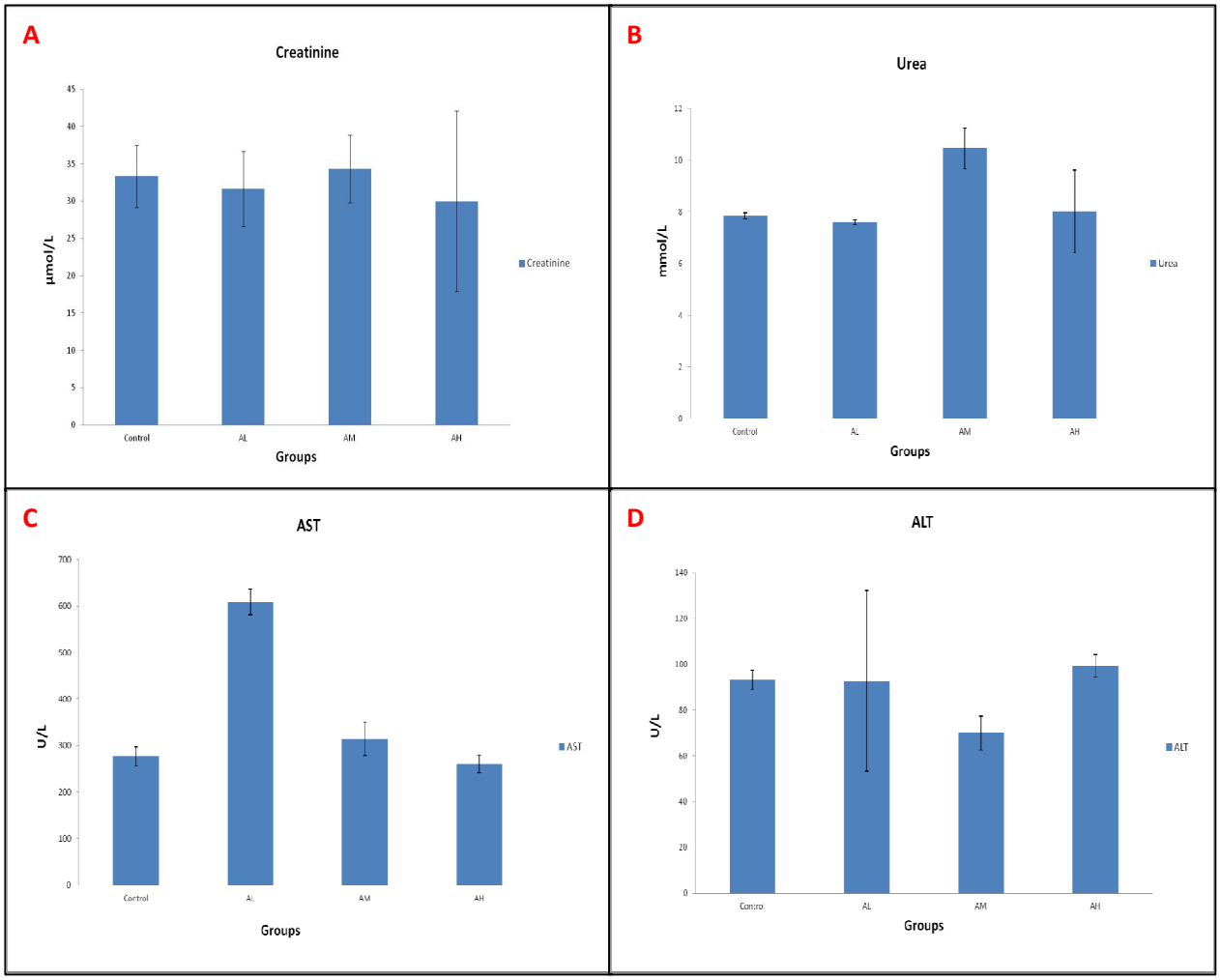

Figure 3. Comparison of Creatinine, Urea, Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) between rats treated with different dosages of Sambung Nyawa methanol extract with a control group. A-Creatinine, B-Urea, C-ASt and D-ALT.

Conclusions

As conclusion, Sambung Nyawa extract show no obvious toxicity to rat model even for high dosages intake. In order to support the haematology and serum analysis of rat treated with Sambung Nyawa extract, histopathology analysis is needed to verify the toxicity effect of methanol extract of Sambung Nyawa.

Acknowledgements

The authors would like to thanks Malaysian Agriculture, Research & Development Institute for the research funding for this project under WRM Megaproject research grant.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Recovery Study of Zineb Using Dehydrogenases Enzyme from *Bacillus* sp. for the Detection of Dithiocarbamate in Chinese Mustard

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Introduction

Dithiocarbamates (DTCs) are a group of organosulfur compounds that have extensively been used as pesticides in agriculture for more than 50 years (Szolar, 2007). Such compounds include the commonly used fungicides (zineb, maneb, mancozeb, ziram and thiram). These fungicides form the most important class of pesticides for broad spectrum control of various fungal diseases on seeds, fruit and vegetables (Noguer and Marty, 1997). They are non-systemic and non-selective pesticides.

Although acute toxicity of these compounds is low (Goranka and Wolfgang, 2009), there is an urgent need to find a sensitive and reliable method of detection since dithiocarbamates and their degradation products are suspected to be carcinogenic, goitrogenic, mutagenic and teratogenic. According to Malaysian Food Act 1983 (2009 edition), maximum residue limits (MRLs) for dithiocarbamate in vegetables and fruits is 10 ppm. The possibility of underground water contamination by dithiocarbamates is also of concern as these compounds have been reported to be easily transported in soils.

Treatment of laboratory animals with these dithiocarbamates may result in effects such as neuropathology, thyroid toxicity and central nervous system developmental toxicity. Based on a preliminary review, only the pesticides mancozeb, maneb, metiram, ziram, thiram and metam sodium were identified as candidates that may induce a common effect by a common mechanism of toxicity. Each of these dithiocarbamates has been suggested to induce distal peripheral neuropathy and, for most, this effect may be associated with the formation of a common metabolite, carbon disulfide (CS_2). CS_2 is known to induce distal peripheral neuropathy in laboratory animals (Mulkey, 2001).

Established protocols for dithiocarbamates residues have been done using chemical analysis such as gas chromatography (GC) which is time-consuming and laborious. Thus an enzyme assay method was specially designed as a screening tool to detect the residues of dithiocarbamates in vegetables using *Bacillus* sp. This bacterium is highly sensitive to some fungicides particularly dithiocarbamates.

Materials and Methods

Sample extractions

All samples used in this study were taken from local wet market. Chinese mustard was spiked with 100 mg/L stock of zineb according to a series of concentrations required (Table 1). Samples were incubated for 1 hr at room temperature. One millilitre EDTA was added to 1 g of chopped samples in a test tube. The samples were vortexed for 30 s and the extract was transferred to a new test tube.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
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Enzyme assay

The dehydrogenase enzyme assay consisted of 1 mL EDTA solution added with 50 µL of sample extract and 1 mL supernatant of *Bacillus* sp. After 10 min of incubation, 0.1 mL of triphenyl tetrazolium chloride (TTC) was then added to the assay and was then incubated for another 3 min. The optical absorbance was read at 484 nm. This result was then compared to the standard curve of zineb (Figure 1) to determine the exact amount of zineb recovered through this enzyme assay.

Results and Discussion

Using non-treated samples as 0 mg/L samples gave 132% recovery (Table 1). In fact, almost all samples showed more than 100% recovery in 5, 10 and 20 ppm which gave 144, 146 and 121% recovery respectively. This may be due to the existence of fungicides in the sample itself. Besides those samples, control, 15 and 25 mg/L gave more than 93% recovery which was acceptable value for this study.

Figure 1. Standard curve of zineb using dehydrogenase enzyme from *Bacillus sp*.

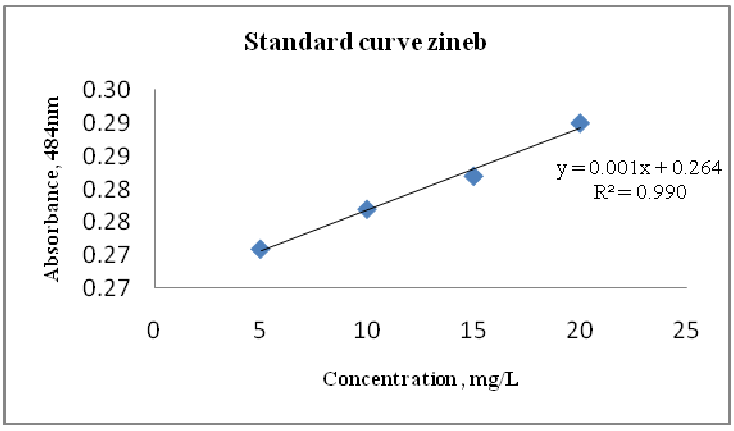

Table 1. Percent recovery of zineb in Chinese mustard in various concentrations

Zineb (mg/L)	Abs R1	Abs R2	Abs R3	Mean Abs	Abs from std curve zineb	Recovery (mg/L)	% Recovery
Control	0.268	0.255	0.257	0.260	0.279	0.00	93.19
0	0.364	0.366	0.366	0.365	0.350	0.00	131.89
5	0.411	0.404	0.406	0.407	0.366	7.22	144.33
10	0.417	0.432	0.42	0.423	0.277	14.59	145.86
15	0.378	0.42	0.378	0.392	0.282	14.82	98.82
20	0.353	0.351	0.352	0.352	0.290	24.28	121.38
25	0.404	0.389	0.371	0.388	0.397	24.45	97.82

Conclusions

There is an urgent need for the detection of dithiocarbamates in local and exported agriculture product. Based on this study, the dehydrogenase enzyme assay developed has been shown to allow rapid detection and on-site monitoring. More studies will be constructed to ensure the stability and sensitivity of the enzyme assay. The assay was designed to protect consumers from dithiocarbamates residues, but chemical analysis still serves as official and final confirmation process.

Acknowledgements

The author would like to thank all parties involved in this study especially MARDI officers and staff for their guidance and facilities provided. These studies were funded under Biosurveillance Project from MOSTI entitled Biosensor and Biodiagnostic in Agro-food Industries (RB5006BS10).

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Isolation and Identification of Bacterial Strains from Papaya Trees Antagonistic to Papaya Dieback Disease Pathogen, *Erwinia mallotivora*

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Introduction

Papaya fruit is of considerable economic importance in Malaysia accounting for 21% of the global export market in 2004 (Chan and Baharuddin, 2010). However since 2005, papaya production in Malaysia faced a major threat from Papaya dieback disease which caused rapid decline in production. The disease caused by *Erwinia mallotivora* affects all popular export varieties such as Eksotika, Solo and Sekaki and is characterized by leaf spot as well as greasy spot and water soaked lesions in all of the plant parts including leaves, fruits and stem. Later, dieback of the infected shoot occurs, leading to the destruction of the papaya trees (Noriha et al., 2011). In 2009, the disease has destroyed nearly one million trees or about 800 ha out of 2100 ha papaya growing area. Total yield loss was estimated to be 200,000 tons, equivalent to USD 60 million (Anon, 2009). The disease outbreak not only affects Malaysia reputation as an exporter but also farm output and income of the farmers. Currently, there is no effective method to control the disease with the exception of good agricultural practice as a preventive measure. Biological control using microorganisms to supress plant diseases offers a powerful and environmentally friendly alternative to the use of synthetic pesticide. Here we report the isolation and identification of several bacterial isolates from survived papaya plants in MARDI papaya germplasm which was heavily infected with papaya dieback disease.

Materials and Methods

Sample collection and bacterial isolation

Samples from different parts of papaya plants such as the petioles, leaves and roots were collected and processed. Briefly the tissues were placed in 10 mL of saline solution (0.9% NaCl) in 50 mL conical flasks and were shaken for 15 min. Serial dilution was then performed up to 10^{-4}. One hundred microlitres of bacterial suspension from each dilution was then spread on Luria Bertani and Nutrient Agar medium and incubated overnight at 28 ^{o}C and 37 ^{o}C. Colonies obtained were then streaked on a new medium for further test.

Screening for growth inhibition of E. mallotivora

The bacteria were applied as four spots on LBsuc agar plates (with 5% sucrose) and grown overnight at 28 °C. *E. mallotivora* was grown for 24 hrs in LB broth and 0.2 mL of the bacterial suspension was mixed with 3 mL of LB soft agar (0.4%). The suspension was gently poured on top of the agar plate with the pregrown bacterial isolates. After incubation for 1 to 2 days at 28 °C, the plates were inspected for growth inhibition zones on the lawn of *E. mallotivora*.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Genomic DNA extraction and PCR amplification of 16S rRNA gene

Bacterial genomic DNA was isolated using GenElute Bacterial Genomic DNA Extraction Kit (Sigma-Aldrich, USA) according to the protocol provided by the manufacturer. PCR amplification was performed in a 25 µL reaction using thermostable DyNAzymeTM EXT DNA polymerase (Finnizymes, Finland) in a PTC-200 thermal cycler (MJ Research, USA). The reaction mixture consisted of 1 × PCR buffer; 2.0 mM of $MgCl_2$, 0.2 mM of dNTPs, 2 µM of forward and reverse primers, 100 ng of bacterial genomic DNA as template and 2.5 U of the enzyme mix. The 16S rRNA gene was amplified using the universal primer pair. Amplification reaction was carried out with the following cycling conditions: primary denaturation for 3 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 1 min at 55 °C and 2 min at 72 °C, and a final extension of 10 min at 72 °C. The PCR product was run through a 1% agarose gel and purified using QIA Quick Gel Extraction Kit (QIAGEN, Germany).

Cloning and sequencing of 16S rRNA gene

The purified PCR product was cloned directly into a vector using TOPO TA Cloning®Kit (Invitrogen, USA). Plasmid DNA of recombinant clones was prepared using QIAprep Spin miniprep kit (QIAGEN, Germany) followed by restriction endonuclease analysis to determine the presence of the cloned insert in the vector. The clones were sequenced commercially, and their nucleotide sequence comparisons were done using the Basic Local Alignment Search Tool (BLAST) on the non-redundant data bank of the National Center for Biotechnology Information (NCBI) (http://www.ncbi. nlm.nih.gov/BLAST/).

Results and Discussion

Bacteria isolated from different parts of the survived papaya plants such as leaves, petioles and roots formed colonies grown on LB and NA plates which often had a rough appearance. These bacterial isolates namely BS1, BS3, BS10 and BS11 grew fast at 37 °C, creamy white in colour and formed irregular shape with different margin and elevation (Figure 1). All four caused a growth inhibition zone on a lawn of *E. mallotivora* when grown on LBsuc agar plates. PCR amplification of the 16S rRNA gene from these isolates using the universal primers gave rise to the expected PCR products which were approximately 1500 bp in size (Figure 2). BLAST results analysis of the 16S rRNA gene showed that BS1, BS3, BS10 and BS11 belonged to the genus of *Bacillus* and were identified as *B. thuringiensis*, *B. cereus*, *B. thuringiensis* and *B. megaterium* with 99 to 100% nucleotide sequence identity.

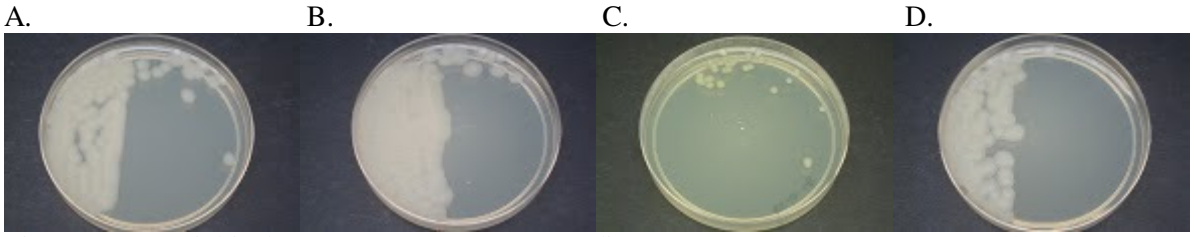

Figure 1. Bacterial isolates grown on LB Agar medium. A: BS1, B: BS3, C: BS10 and D: BS11

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

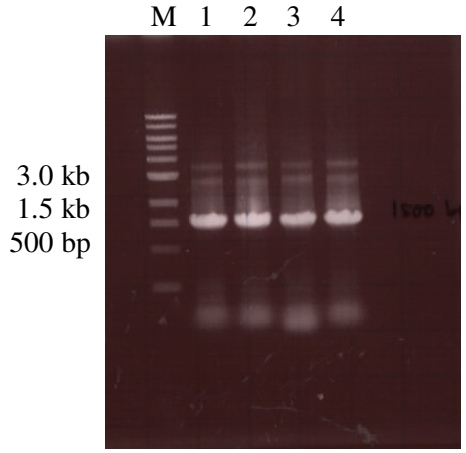

Figure 2. PCR amplification of 16S rRNA gene. The different lanes were M: 1kb DNA Ladder (New England Biolabs, USA). 1-4: PCR products from isolate BS1, BS3, BS10 and BS 11.

Members of the genus *Bacillus* are well known to produce several kinds of antimicrobial compounds such as peptide antibiotics and hydrolytic enzymes (Yu et al., 2002, Mawadza et al., 2000) as well as quorum quenching enzymes (AHL lactonase) which can control bacterial virulence (Dong et al., 2000; 2002, Morohoshi et al., 2009). Although several species of bacteria are known for antibacterial activity, non-pathogenic *Bacillus* spp. offer several advantages as they form endospores which can tolerate extreme pH, temperature and osmotic condition (Hendelsman and Stabb, 1996). BS1, BS3, BS10 and BS11 isolated in this study might be responsible for the slower development of papaya dieback disease in papaya by providing unfavourable conditions for *E. mallotivora* to colonize and invade papaya host.

Conclusions

Antagonist bacterial isolates from papaya plants were successfully isolated and identified using 16S rRNA gene cloning. Further study would be to screen and validate its quorum quenching activity against the papaya dieback disease pathogen *in vitro*. These bacterial isolates could potentially be used as biocontrol agents for controlling papaya dieback disease in the near future.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Screening and Mode of Action of Antagonist Yeasts against *Colletotrichum gloeosporioides*

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Introduction

Anthracnose caused by *Colletotrichum gloeosporioides* is a major postharvest disease that affects many tropical and subtropical fruit (de Capdeville, 2007), including papaya (*Carica papaya* L., Snowdon, 1990). Fungus normally begins the infection as early as flowering starts and remains latent until preferred postharvest environmental conditions for colonization of fruit tissue occurs. Therefore, disease symptoms usually emerge only after harvest, when fungal development continues due to favorable storage condition. However, postharvest infections also exist due to the availability of inocula in the processing environment and due to a large amount of wounds imposed to the fruit after harvest (de Capdeville, 2007).

Recently, biological control has been promoted as an option to synthetic fungicide treatment. Studies done by Rahman et al. (2007) showed that *Burkholderia cepacia* was able to inhibit the mycelial growth and spore germination of *C. gloeosporioides*. However, there are reservations on the use of *B. cepacia* as a biocontrol agent since some species of this bacterium under different genomovars are known to be human pathogens, particularly in cystic fibrosis (CF) patients. Among all antagonistic microorganisms, natural yeasts were reported to be an effective biological control agent (Irtwange, 2006). Previous studies also demonstrated that the modes of action of biocontrol yeasts showed less possibility of any risk to consumers (Arras et al., 1999).

Nevertheless, very few studies have been published on the control of papaya postharvest diseases relying on antagonistic yeasts especially in Malaysia. The main objective of the present work was to select and determine the mode of action for epiphytic yeasts isolated from papaya fruit peel in controlling the postharvest development of anthracnose disease.

Materials and Methods

Fungal culture and yeast isolates

C. gloeosporioides was isolated from infected papaya and cultured on potato dextrose agar (PDA) at 28±2 ^{o}C until pure culture was obtained and maintained at 4 ^{o}C. Spore suspensions were obtained by flooding 1 to 2 week-old PDA cultures of pathogen with sterile distilled water. Spore concentrations of the pathogen were determined by haemocytometer and adjusted with sterile distilled water to 5 X 10^4 spores/mL.

Yeast strains, namely yeast A and K previously isolated from papaya peel, were maintained at 4 ^{o}C on nutrient yeast dextrose agar (NYDA). Liquid cultures of yeasts were grown in 50 mL centrifuge tubes containing 25 mL of nutrient yeast dextrose broth (NYDB) on a rotary shaker with shaking at 150 rpm, 28±2 ^{o}C for 48 h. The medium were centrifuged at 2000 rpm for 10 min and the cells were resuspended

in sterile distilled water to wash the yeast cells from the nutritional compounds of the initial medium. The concentration of the yeast cell suspensions was obtained with haemocytometer and adjusted with sterile distilled water to 5 X 10^4 cells/mL, 5 X 10^6 cells/mL and 5 X 10^8 cells/mL.

Mycelial growth test

Six mm mycelial plugs of *C. gloeosporioides* were taken from 1 week-old culture by cork borer and placed into sterile glass vial containing yeast suspensions of the selected isolates at three different concentrations (5 X 10^4 cells/mL, 5 X 10^6 cells/mL and 5 X 10^8 cells/mL). The submerged fungal plugs were then incubated for 30 min at room temperature followed by air drying in laminar flow to remove the excess surface water. Treated mycelial plugs were transferred on petri dishes containing PDA. Fungal plugs dipped in commercial fungicide (Benomyl®) and sterile distilled water (SDW) served as positive and negative controls, respectively. The observation on radial inhibition of mycelial growth was carried out after 7 days of incubation at 28±2 ^oC (Dikin et al., 2002).

Production of diffusible antifungal substances

Production of diffusible antifungal substance(s) was tested using sandwich agar plates prepared according to procedure described by Dikin et al. (2002). A 100 µL of selected antagonistic yeast suspensions were then inoculated in the center of the petri dishes contained sandwich media. After incubation for 3 days at 28 ± 2 ºC, the NYDA media with the grown yeast isolates and filter paper layers were removed, and the plates were inoculated with a 6 mm mycelial disk of a 4 day-old culture of *C. gloeosporioides*. Plates were then incubated at 28 ± 2 ºC for 7 days and the radial growth of the fungus was measured. Control treatment was run by replacing the yeast suspensions with sterile distilled water and further inoculated with *C. gloeosporioides.* Results were expressed as means of percentage inhibition of growth of *C. gloeosporioides* in the presence and absence of yeast isolate.

Mycotoxin production by the antagonists

The potential of the selected yeast isolates to produce mycotoxin substances against *C. gloeosporioides* on solid nutrient medium were tested. One hundred microlitres aliquots of spore suspension of *C. gloeosporioides* were spread on the PDA plate with a sterile bent glass rod. Two sterilized filter paper discs (1 cm in diameter) were placed 3 cm apart on the agar surface and 50 µL of yeast suspension was pipetted onto each of the paper disc. In control plate, discs received 50 µL of sterilized distilled water. After 72 h of incubation at 28 ± 2 ºC, pathogen growth within 10 mm around the paper disc was examined.

Postharvest disease control

A total of 18 fully mature papaya fruits at color stage two were harvested from a papaya field. On arrival at the laboratory, the fruits were washed with tap water, immersed in sodium hypochloride 2% for 5 min, followed by immersion in 70% ethanol for 1 min, washed in distilled water and air-dried. Then, two wounds (6 mm in diameter, distance between wound 3 cm) were made on each fruit, and each wound was treated with 50 µL of yeast suspension and sterile distilled water as a control. After 2 h, 20 µL of spore suspension were inoculated on the wound. After the fruits were air-dried, they were placed in a commercial packaging held at room temperature (28 ± 2 ºC) for 6 days. Data on lesion diameter were taken on day 6.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Molecular and morphological identification

According to the results obtained from all the studies as described above, only yeast A was chosen to be identified. Molecular identification was done by Next Gene Scientific Pte. Ltd. Morphological characteristics of yeast A were examined by observing colony patterns described by Kurtzman and Fell (1998) to verify results obtained from yeast identification by the rDNA sequencing technique. Colony morphologies of yeast were examined in cultures grown on NYDA at 28 ± 2 ºC. The features of yeast culture on the plate namely color and texture were recorded 3 to10 days after incubation.

Statistical analysis

Data for experiments was analyzed using analysis of variance (ANOVA) and SAS version 9.0. The treatments were separated using Duncan's multiple range tests.

Results and Discussion

Mycelial growth test

The potential role of competition for nutrient and space in the biological control against *C. gloeosporioides* by antagonist yeasts chosen was examined by investigating the effects of different concentrations of yeast suspension on pathogen hyphal growth (Figure 1). The result showed that both yeasts, yeast A and yeast K, demonstrate suppression in mycelial growth when raising the yeast suspension concentration. However, yeast K showed a significant inhibition in each additional concentration compared to yeast A that only showed significant changes in 5 X 10^8 cells/mL compared to 5 X 10^4 cells/mL and 5 X 10^6 cells/mL.

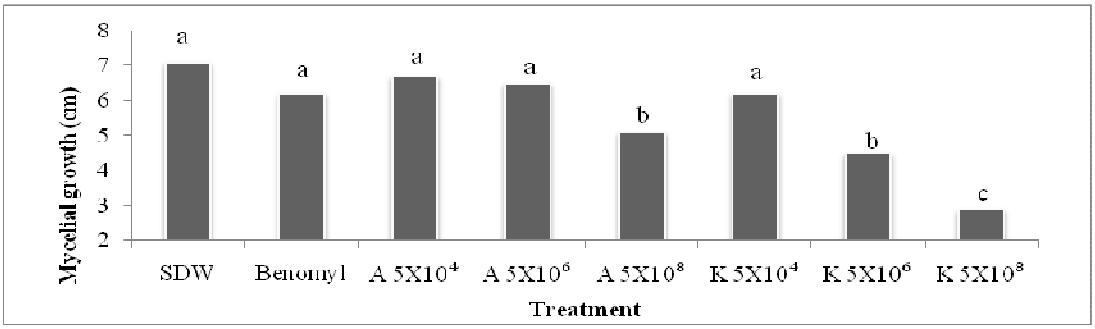

Figure 1. Effect of yeast A, yeast K and Benomyl® in controlling mycelial growth of *C. gloeosporioides* on PDA plate. Different letters above the bars indicate significant differences (P<0.001) according to Duncan test.

Production of diffusible antifungal substances

Based on the result of previous study, only one concentration was chosen for this experiment for both yeasts which was 5 X 10^8 cells/mL. From this study, yeast A showed a high suppression of pathogen growth compared to yeast K (Table 1). Yeast A totally suppressed pathogen hyphal growth while yeast K exhibited low antagonist activity if compared to control.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Mycotoxin production by the antagonists

Both yeasts did not show any suppression of the spore since it was still able to germinate on both yeasts (unpublished data). Therefore, it was suggested that no mycotoxin was produced by both yeasts to control *C. gloeosporioides.*

Postharvest disease control

After 6 days of incubation, wounds treated with yeast K showed low inhibition against *C. gloeosporioides* growth compared to yeast A (Table 2). However, yeast K showed significant antagonist activity compared to control (SDW) wound.

Table 1. Effect of diffusible antifungal substances production of different antagonist yeast on radial growth of *C. gloeosporioides* on PDA plate six days after inoculation at 28 ± 2 ºC.

Antagonistic yeast	Hyphal growth (cm)	Inhibition of radial growth (%)
SDW	6.9	-
A	0	100 a
K	5.7	18 b

Values with the different letters are significantly different (P < 0.001) according to Duncan test

Table 2. Effect of different antagonist yeast on radial growth of *C. gloeosporioides* on wounded papaya fruits 6 days after inoculation at 28 ± 2 ºC.

Treatment	Mycelial growth diameter
SDW	4.08 a
Yeast A	3.13 b
Yeast K	1.99 c

Values with the different letters are significantly different (P < 0.001) according to Duncan test

Molecular and morphological identification

BLAST results showed that yeast A was 99% to *Trichosporon asahii.* The result obtained from the molecular identification was compared with the morphological characteristic of yeast. According to Kurtzman and Fell (1998), *T. asahii* colonies are 16 to 24 mm in diameter after 10 days of incubation, white, farinose at the center, with a wide, dry often finely zonate margin with deep transverse fissures. Odor is lacking or faintly cheese-like. Therefore, the results from molecular identification correlated with morphological identification to suggest that yeast A was *T. asahii.*

Conclusions

This study showed that the production of diffusible antifungal substances appeared to be the mode of action of *T. asahii* against *C. gloeosporioides* which corresponded with El-Tarabily (2004) findings. The low antagonistic activity of *T. asahii* was indicated to be due to the competition for nutrient and space of yeast cell with the pathogen. Future studies should be done to discover possible induction of host tissue defences by antagonistic yeast to counter pathogen growth.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Specificity Test of the Polyclonal Antibody Produced against *Pyricularia oryzae*

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Introduction

Rice blast disease or Penyakit Karah, is caused by *Pyricularia oryzae* Cav. (previously known as *Magnoporthe grisea*). It is the most destructive of fungal diseases in rice crop and has caused significant yield losses worldwide including Malaysia with potential yield losses of more than 50 percent (Yang *et al*., 2012). Rice blast is classified into seedling blast, leaf blast, rice node blast, neck blast and corn blast. The cycle of this fungal disease begins when a blast infects and produces a lesion on all parts of the shoot, as well as stem rot and panicle blight and ends when the fungus sporulates and releases new airborne spores (Ou, 1980). Rice blast disease moves from disease plants to healthy plants in nearby fields by airborne spores. A visual examination is not sufficient to discriminate between the pathogenic and non-pathogenic spores (Smiley et al., 1992). Presently, pathogenic spores can only be confirmed in a minimum of five to six days later by the development of lesion on the rice plants. By the time visible lesions have developed on the rice plants, it may be too late to eradicate rice blast by the application of fungicide resulting in the destruction of all or large portion of the entire crop (Smiley et al., 1992).

Nowadays, one of the most widely used methods of controlling rice blast is by spraying fungicide However, intensive spraying requires an excessive amount of fungicide which increases cost, pollutes the environment and results in the development of multi-resistant fungal strains (LaMondia and Douglas, 1997). In some countries spore traps are used to predict rice blast epidemics. However the available traps are expensive and predictive data based on the number of spores trapped are applicable to the immediate area only.

Therefore, accurate and rapid identification of rice blast disease is essential for effective disease control. It enables more informed decisions to be made about cultivar choice and how and when fungicide can be used most effectively to control disease epidemics. Traditional approaches to rice blast diagnosis generally first involve the interpretation of visual symptoms (Stowell and Gelernter, 2001). This may be followed by laboratory identification, consisting of isolating the blast fungus from diseased tissue or trapped spores, culturing the fungus for spores and inoculating susceptible rice plants. But cultural isolation and identification is laborious, difficult and time-consuming (Ward et al., 2004). Other than that, several micro-detection based on molecular level such as polymerase chain reaction (PCR) and microscopy has also been developed. However, the major disadvantages of these methods are time-consuming and costly, requires skill personnel and impossible to be applied in real-time detection (Yang et al., 2012).

Recently, there has been a great progress in the development of very rapid diagnostic tests that are also simple. Therefore, the development of an inexpensive and accurate enzyme immunoassay screening kit for detecting *P. oryzae* in paddy field is highly desirable in deciding upon appropriate control for this fungus. In this work, we report on the production of polyclonal antibody against *P. oryzae* pathotype 7.0

using germinating conidial suspension as an antigen for immunization in rabbits for the production of polyclonal antibody. The specificity of the polyclonal antibody produced will be evaluated against different major pathotype of *P. oryzae* in Malaysia and other paddy fungal strains using the ELISA method.

Materials and Methods

Isolation and culture of fungi

The isolate of pathotype 7.0 of *P. oryzae* was isolated at MARDI Seberang Perai, Penang and was identified by lesion reaction on cultivars of rice. Oatmeal agar was used for culturing the fungal isolates. Mycelial colony on the oatmeal plate was cultured at 37 ^{0}C for 10 days. Large quantities of mass-produced conidia on oatmeal were collected by washing the colony surface on the oatmeal plate with 1 ml of ultra-pure water. Then this was filtered with cheesecloth.

Preparation of germinating conidia for antigens

The spore suspension was centrifuged at 1000 g for 10 min and re-suspended about 1 x 10^8 conidia/ml in phosphate buffer saline (PBS). The suspension, used as immunogen was aliquot and stored at -20 ^{0}C until use.

Immunization procedures

The spore suspension was prepared following the step above, and mixed with an equal volume of Freund's Complete Adjuvant and emulsified. The antigen-adjuvant mixture was injected into the New Zealand Rabbit. The first injection was followed by two booster injections at a three-week interval. For the second injection, spore suspensions were mixed with Incomplete Adjuvant. The third injection was done with equal volume mixture of suspension and PBS.

Serum preparation

Blood sample was collected in tube which then exposed to room temperature for 5 min. Anti-serum was prepared by centrifugation at 3,000 rpm for 10 min and subjected to titration by ELISA.

Cross-reaction test

Specificity of polyclonal antibody for *P. oryzae* form paddy against other isolates was tested against different pathotype of *P. oryzae* such as 1.0, 9.0, 15.0 and two other paddy fungal strains *Helminthosporium oryzae* (Brown spot disease) and *Rhizoctonia solani* (Sheath blight disease). All fungal strains were obtained from MARDI Seberang Perai, Penang.

Indirect ELISA

Indirect ELISA was performed for specificity test on the produced polyclonal antibody against P. oryzae pathotype 7.0. Aliquots of conidial suspension (*P. oryzae* pathotype 1.0, 7.0, 9.0, 15.0 *Helminthosporium oryzae* and *Rhizoctonia solani* were dissolved in carbonate coating buffer (10^7 spore ml^{-1}), dispensed about 100 µl into each well, and kept for 2 hours at 37 ^{o}C. The coated wells were washed four times with PBST and the uncoated well surface blocked with 1% BSA at 37 ^{o}C overnight. After a further washing of four times with PBST, well plate was incubated at 37 oC for 1 hour with 100 µl per well of antibody at the concentration of 1:4000. The plate was washed a further four times with

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

PBST again and then incubated at 37 °C for 2 hours with secondary antibody. After final washing for four times, 100 µl of the substrate solution *p*-NPP was added per well and incubated for 15 min followed by absorbance reading in an ELISA reader at 405 nm.

Results and Discussion

The specificity of the produced polyclonal antibody against *P. oryzae* was evaluated using a variety pathotype of *P. oryzae* and different fungal strains. Based on the result obtained using ELISA, cross reaction studies of the developed polyclonal antibody against *P. oryzae* to several pathotypes showed moderate cross reaction (43%) to pathothype 15.0 while two other pathotypes 1.0 and 9.0 exhibited low cross reaction of 18.5 and 16.2 %, respectively, compared to control (Figure 1). Other fungal species (*H. oryzae* and *R. solani*) showed extremely low cross reactivity to *P. oryzae* with absorbance values not exceeding control (Figure 2) indicating the developed polyclonal antibody is very specific.

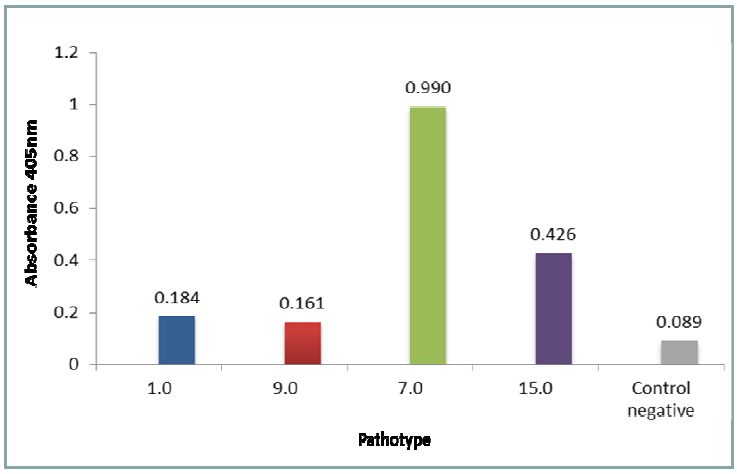

Figure 1. Cross-reaction against different *P. oryze* pathotype

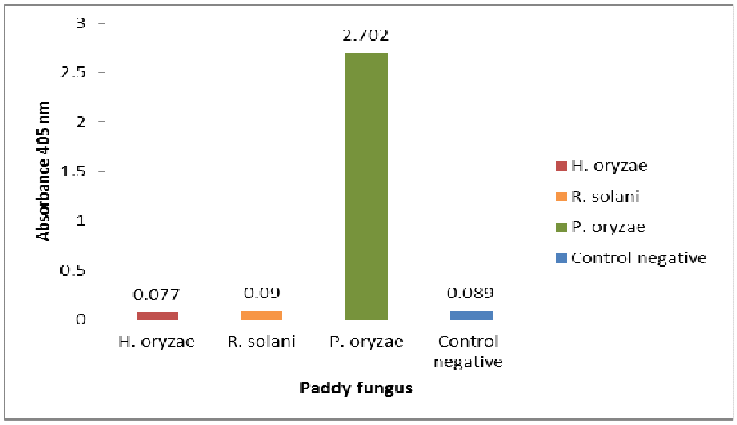

Figure 2. Cross-reaction against different paddy fungus

The results confirm that the production of polyclonal antibody against *P. oryzae* using conidia as antigen is specific judging by the results obtained. In particular the low cross reaction against *H. oryzae* is important since this strain exhibited very similar symptoms of infection to *P. oryzae* (Yang et al., 2012).

This would ensure that any diagnostic test used in the field would not have false positive results due to the present of *H. oryzae*.

Theoretically, the choice of antigen is based on application of polyclonal antibody to specific fungal structures to be detected as well as on consideration of resulting specificity of the polyclonal antibody (Dewey et al., 1991). According to Xia et al. (1993), the immuno-gold labelling studies done by them shows that the epitope recognized by monoclonal antibody was found only in the cytoplasm of conidial cells but not in or on cell walls of conidia or in hyphae. Thus the choice of conidia as an antigen in this work is appropriate for production of polyclonal antibody.

Conclusions

In conclusion, the produced polyclonal antibody is proven to be very sensitive and specific to the target pathogen, *P. oryzae* and related strains. Other genus of fungal pathogen showed very low cross reactivity. The development of early detection kit for this fungal pathogen could save thousands of tonnes of rice lost yearly and the results obtained in this work is a promising start in realising this goal.

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ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Determination of Antibody-Antigen Interaction of Rice Tungro Viruses Using Surface Plasmon Resonance

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Introduction

Rice is one of the world's most important cereals. More than 90% of the world's rice is produced and consumed in Asia (Hossain and Pingali, 1998). Of all viral diseases on rice, Tungro Disease is the most economically devastating in South and Southeast Asia, where epidemics of the disease have occurred since mid-1960s (Azzam and Chancellor, 2002). Rice Tungro Disease (RTD) is reported to be responsible for 5-10% annual losses of rice yield in Asia and about 2% in India. RTD is also called by various names in different countries where in Indonesia, it is known as Mentek (Ou, 1985) or Penyakit habang, in Malaysia is known as Penyakit merah and known as yellow-orange leaf in Thailand.

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

Since rice is a staple food for Asians and world's important cereals, early detection is important for a successful disease control which can reduce disease spreading and yield loss. Although symptoms of Tungro infected plants can be seen by visual or occasionally by insect transmission of the viruses to assay plants, but diagnosis of the disease by the symptoms alone is not reliable. This is because other disease and non-pathogenic disorders such as excess water after drought, insect injury or nutritional deficiencies also can show similar symptoms (Nath et al., 2000, Boltovets et al., 2004). PCR (polymerase chain reaction) is a reliable and accurate technique for viral detection but it is a destructive technique. DNA or RNA of the viruses needs to be extract before the PCR can be carried out. One of the most efficient, reliable and non-destructive techniques, which are used for the rapid detection of biospecific interactions is by using Surface Plasmon Resonance (SPR) (Boltovets et al., 2004). Therefore, the aim of this study is to determine the antibody-antigen interaction of Rice Tungro viruses which use intact viral particles using SPR.

Methodology

Infected plant samples

Two rice varieties (Y1286 for RTSV and MR81 for RTBV) were planted in soil filled tray. The plants were applied with fertilizer and water during this period. Green leafhoppers (GLH) in plastic cages were used to transmit the viruses from an infected plant. Virus inoculations were conducted at 20 day after planting. After 24 hours inoculation, the GLH were killed and the infected plants were grown until 50-60 days.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Rice tungro antibodies

Three types of antibodies were developed using recombinant coat protein as the antigen (RTBV coat protein, RTSV coat protein 1 and RTSV coat protein 2). Three months old rabbits were immunized with the antigen. Blood samples were taken for antibody purification using two step procedure involving ammonium sulphate precipitation and Protein A affinity chromatography. One mg/ml concentration of antibodies was used for the test.

Surface plasmon resonance assay

Gold chip sensor was coated with mercapto-undecanoic acid for overnight (11-MUA). Each chip was inserted into an Autolab ESPRIT SPR system. Healthy and infected paddy leaves were grinded and dissolved with buffer. 100 µl samples were directly flowed on the sensor surface which was already immobilized with antibody. Analyses were performed using the Autolab ESPRIT with pre-programmed parameters. Data were analyzed using Autolab ESPRIT Kinetic Evaluation version 5.0.

Results and Discussion

From the SPR results, the antigen-antibody interaction between the antibody immobilized on the gold-sensor surface with the healthy and an infected plant showed a different response. Infected samples showed higher response compared to the healthy sample for all 3 types of antibodies tested. These were due to the binding of antigen to antibody leads to the formation of the large shift of the resonant wavelength. The SPR detects and measures changes in refractive index due to the binding and dissociation of interacting molecules at or proximity to the gold sensor surface. The change in refractive index (proportional to the concentration of the interacting molecules) causes a shift in the angle of incidence at which of the SPR phenomenon occurs (Guidi et al., 2001). For RTSV CP1 antibody, response unit for healthy plant is 384 RU while for infected is 451 RU (Figure 1). For RTSV CP2 antibody, response unit for healthy is 193 while for infected is 239 (Figure 2) and for RTBV, response unit for healthy is 315 RU while for infected is 376 RU (Figure 3). These results suggested that the antibodies are capable to interact with the Tungro viral antigen using surface Plasmon resonance.

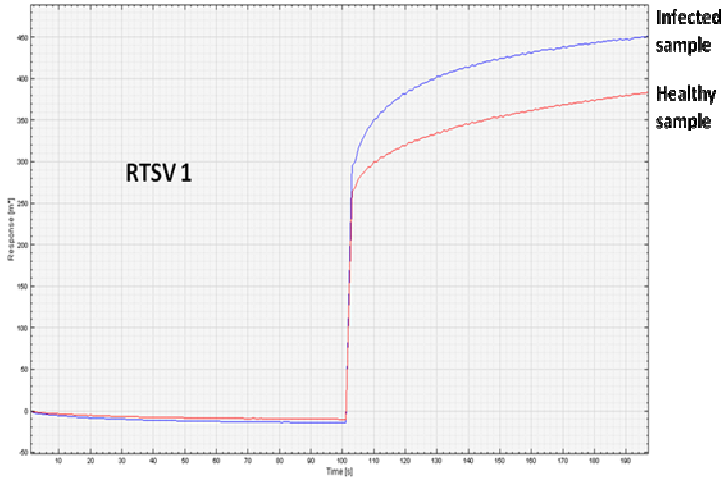

Figure 1. Interaction between antibody produced from recombinant RTSV coat protein 1 with healthy and RTSV infected plant sample

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

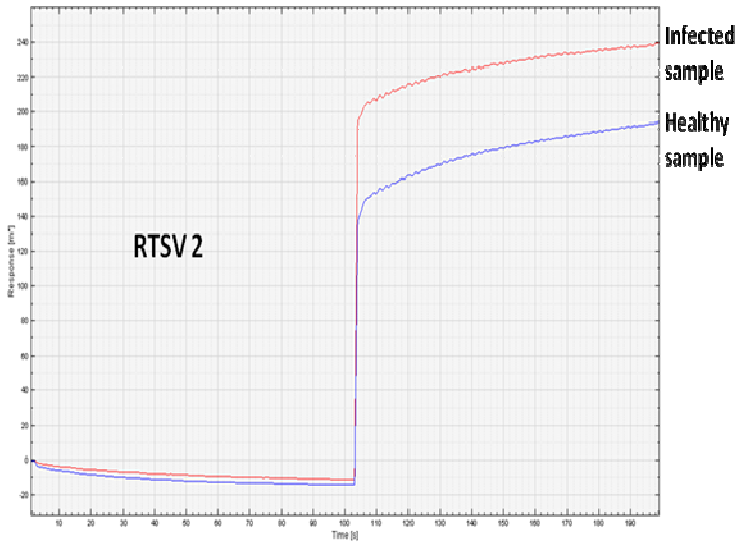

Figure 2. Interaction between antibody produced from recombinant RTSV coat protein 2 with healthy and RTSV infected plant sample

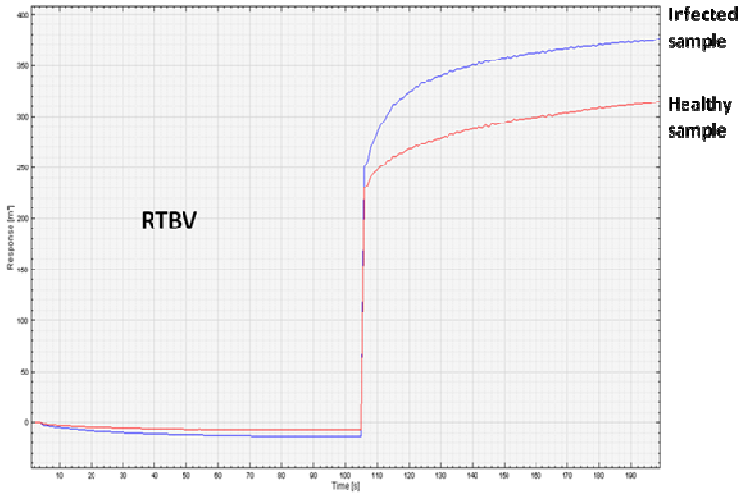

Figure 3. Interaction between antibody produced from recombinant RTBV coat protein with healthy and RTBV infected plant sample

Conclusions

All three types of antibodies tested showed positive results where they exhibit significant different in response unit measurement between healthy and infected plant samples using surface plasmon resonance. Therefore, since all the antibodies produced are capable to interact with Tungro viral antigen, these antibodies will be proceed for further optimization, so then it could be used in the diagnosis of suspected Rice Tungro Disease plants.

ISBN 978-967-10840-3-8
Trans. Malaysian Soc. Plant Physiol. 22
First Published, 2014

Acknowledgements

We are grateful to Dr Norliza Abu Bakar (Biotechnology Research Centre, MARDI); for supplying the antisera at the initiation of this project. This work was supported by the Ministry of Science, Technology and Innovation (MOSTI) under the Top Down Nano Directorate Fund (RB5023NF10).

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