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Plant Ecophysiology for Sustainable Production

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Roohaida Othman
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Siti Hajar Ahmad
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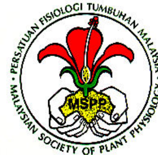

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Chapter 1

Plant Growth and Development

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Litterfall Assessment of Peat Swamp Forest (PSF) in Pekan, Pahang, Malaysia

Mohd Afzanizam, M.* **and Philip, E.**
Climate Change and Forestry Program, Forestry and Environment Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: mohdafzanizam@frim.gov.my*

Introduction

Tropical peat swamp forest (PSF) is a unique dual ecosystem i.e. tropical rainforest and wetland which may act as carbon sink and/or carbon source. In Peninsular Malaysia, the extent of peat swamp forest (PSF) coverage area is 255,080 ha (National Forest Inventory 5, 2015). From this total, 140,830 ha is located in Pahang, while Forestry Statistics show PSF coverage area under Permanent Reserve Forest by Forest type in 2014 as follow; Johor (5,429 ha), Pahang (140,830 ha), Selangor (82,890 ha), Terengganu (25,931 ha using digital satellite data). No value recorded in other states. To date, there were 93,477 ha of remaining virgin (VJR) peat Swamp in Peninsular Malaysia (NFI5, 2014). The Intergovernmental Panel on Climate Change (IPCC) has no formal definition of peatland, but it does include the concept of peatland in the 'land with organic soil' category (IPCC, 2013). The litter biomass pool comprises fallen leaves, twigs, flowers and inflorescence, fruits and seeds (Verwer and Van der Meer, 2010). Estimates of the litter carbon pool varies, ranging from 2.4 t C/ha (Delaney et al., 1997) to as much as 15 t C/ha (Chiti et al., 2010). Similar to dead wood/coarse woody debris, the litter biomass pool is the result of continuous input through litter fall and loss through decomposition. Through the decomposition of fallen leaves, these nutrients are taken up by plants and used for the production of biomass. Numerous studies on litterfall are available but generally limited to certain geographic regions such as temperate and boreal, forest types (lowland forest, mangrove, peat swamp. However biomass and carbon stock assessment in tropical peat swamp forest is still limited. Therefore, this research is crucial to determine the carbon pool especially in tropical country like Malaysia, as litter biomass contributed significantly in the peat swamp forests as compared to other forest types due to large quantity of decomposed leaves, roots and woody debris in the forest floor. As part of the Ramin Project, a joint effort between Malaysia and The Netherlands,where number of litter traps were setup in a mixed peat swamp forest (Phasic Community 1) at the Maludam National Park in Sarawak, Malaysia from 2004 to 2005 (JWG, 2005). The objectives of the present study were to (i) to quantify biomass and carbon stock from litterfall in peat swamp forest under pristine condition and (ii) to investigate monthly changes in litter production in relation to environmental factors.

Materials and Methods

Data collection from Ecological Plot

The study site is located at Compartment 75, Pekan FR, in the Southeast state of Pahang, Malaysia. According to District Forest Office, compartment 75 is designated as production forest. Ten plots of 20 m x 20 m were established for population profile assessments. All trees ≥10 cm dbh were measured for the stand structure and density (Figure 1). Sampling protocol following Standardized National Forest Inventory Manual/Manual Inventori Hutan Nasional, Forest Department of Peninsular Malaysia (FDPM).

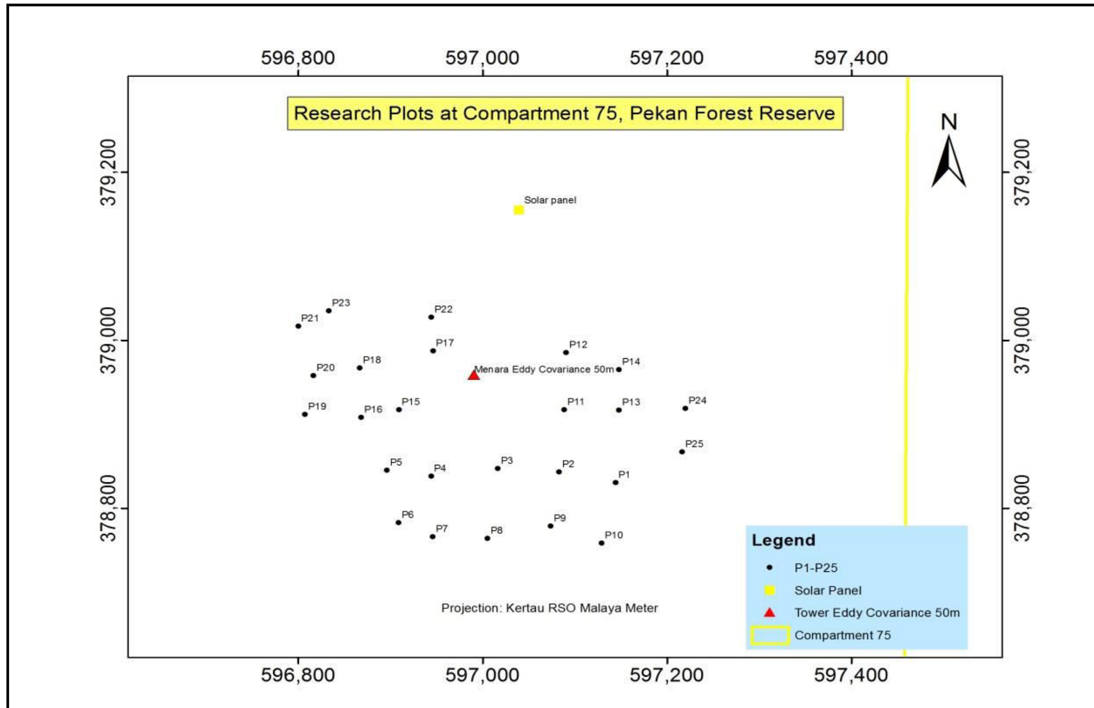

Figure 1: A 1-ha study area comprising of twenty-five ecological plot establishment (20 m x 20 m), Compartment 75 Peat Swamp Forest, Pekan Forest Reserve, Pahang.

Litterfall collection

Twenty five leaf litter trap (1 m x 1 m each) were established in every ecological plot as a representative sampes. Monitoring for litterfall production were carried out on monthly basis. Wet weight and dry weight were recorded for biomass and carbon stock calculation.

Simplified method for biomass and carbon stock for leaf litter pool

These include:
1. Wet weight (WWsample) = (Wbag +litter) – (Wbag)
2. Ratio dry to wet weight (DW ratio) = (DW subsample)/(WW subsample)
3. DW (gram) = DW ratio x WWsample
4. Scaling factor (SF) = 10,000m^2 / total area (m^2)
5. Conversion from biomass to carbon: Cplot (t C ha^{-1}) = DWplot x Carbon Fraction x 0.001
Carbon fraction used was 0.47 is a default value for conversion factor from biomass to carbon stock (IPCC, 2006)

Results and Discussion

A total of 465 individual trees from 61 species were present in Compartment 75, Pekan FR. Total biomass quantified using Manuri et al. (2013) were 248 and 317t/ha for 2016 and 2018 respectively. This value is almost twice higher than those obtained in oil palm plantation which ranged between 50-100 t /ha while carbon stocks between 31-62 t C/ha (for Malaysia based on 20 to 25 years old oil palm rotation.cycle) (Asari et al., 2013). Total components of carbon pool are as described in Table1 below:

Table 1: Dynamics of above ground biomass in a growing stand (1 ha) in Pekan Peat Swamp Forest.

Parameter Aboveground Biomass (t/ha)	2016	2018
Living trees	207.19	264.37
Belowground biomass	41.64	53.14
Leaf litter	0.12-0.69	0.08-0.89
Dead wood/Necromass (standing and downed)	0.028	0.06
Total biomass (t/ha)	248.86	317.57
Net gain (t/ha)		68.71
Change rate in total biomass (t/ha/year)		
Tree growth, Gm		34.35
Recruit, Rm		0.49
Death, Dm		0.03
Net gain (=GM+Rm-Dm)		34.81

Forest biomass fluctuations

Table 1 shows overall biomass assessment according to carbon pools. Any biomass change can also reveal that death of large trees dominated the total biomass decrease as the biomass increment in living trees and carbon stock also increase. This is because the contribution of forests carbon pool depends significantly on its successional stage. Forest biomass generally increases with stand age, and an indication towards a constant level at maturity age because of a decline in net primary productivity (Kira and Shidei1967; Whitmore 1984). However, this may not explain in our study plot since they are only a single growth plot. Further studies involving biomass fluctuation with time within the stand dynamics can be estimated by a repeated tree census such as changes in biomass/carbon stock) due to growth and mortality of trees can be accounted in a given area. The component of biomass change were analyze based on tree growth; recruitment and tree deaths. From our study, biomass gained by tree growth of 34.35 t/ha/yr is consistently higher than biomass loss by tree death of 0.03 t/ha/yr (Table 1) and only a minor fraction accounted for recruitment of 0.49 t/ha/yr. The biomass gained by growth and recruitment and that lost by mortality showed that there is little variation among the period between the trees census. This is however, yet to be proven as we have only 2-years of census data. The distribution of biomass loss in tree death showed a peak in larger class size of DBH>40cm despite very rare mortality of big trees. Early result suggests that a single treefall from the larger size classes has a greater role on the biomass change in the forest plots than frequently distributed mortality recorded from small trees. This similar trend has also been observed in the lowland dipterocarp forest of Pasoh Forest Reserve Negeri Sembilan.

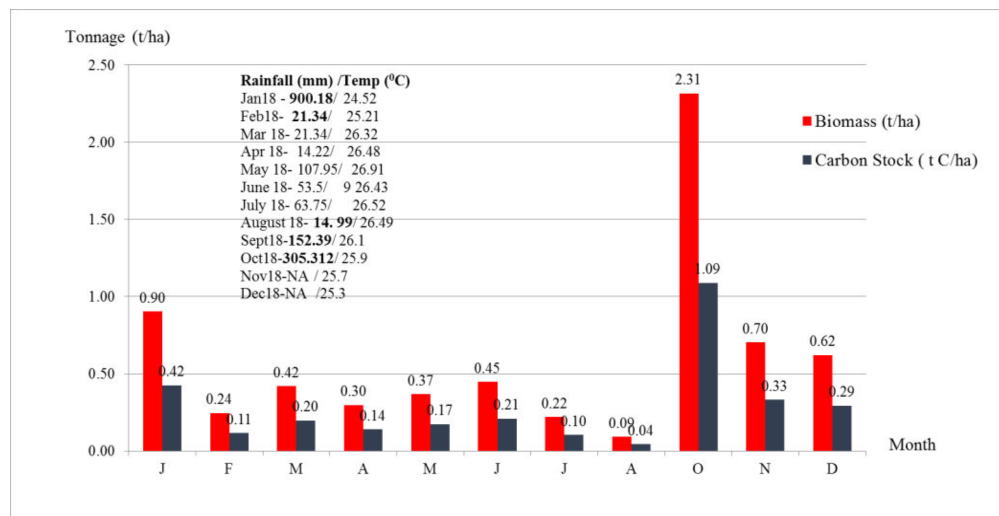

Figure 2: Leaf litter production in 2017.

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Leaf litter production

Leaf litter production range between 0.06 to 0.31 t C/ha in 2017 while in 2018, it ranged from 0.11 to 1.09 t C/ha (Figures 2 and 3). However, this is relatively low as compared to the earlier studies whereby the total average litter carbon production range from 2.27-5.93 t C/ha for a relatively undisturbed peat swamp forest after a two year study (2004-2005) at Maludam National Park in Sarawak (JWG, 2005). The values are within the same range compared to data from mixed swamp forest and low pole forest in Central Kalimantan, where the total litter carbon pool was 4.6 and 3.7 t C/ha/yr respectively (Sulistiyanto et al., 2007). In the mixed swamp forest at Lingga Water Catchment area the total litter carbon pool ranged from 1.82 to 4.75 t C/ha with an average value of 3.15 t C/ha. Results of litter trap data show that the total litter production was 7.62 t/ha/yr (~3.81 t C/ha/yr) in 2004 and 4.68 t/ha/yr (~2.34 t C/ha/yr) in 2005. Those values are comparable to the 7.17 t/ha/yr (leaf litter: 5.57 t/ha/yr, non-leaf litter: 1.6 t/ha/yr) found by Yiu-Liong (1990), but in a relatively undisturbed peat swamp forest.

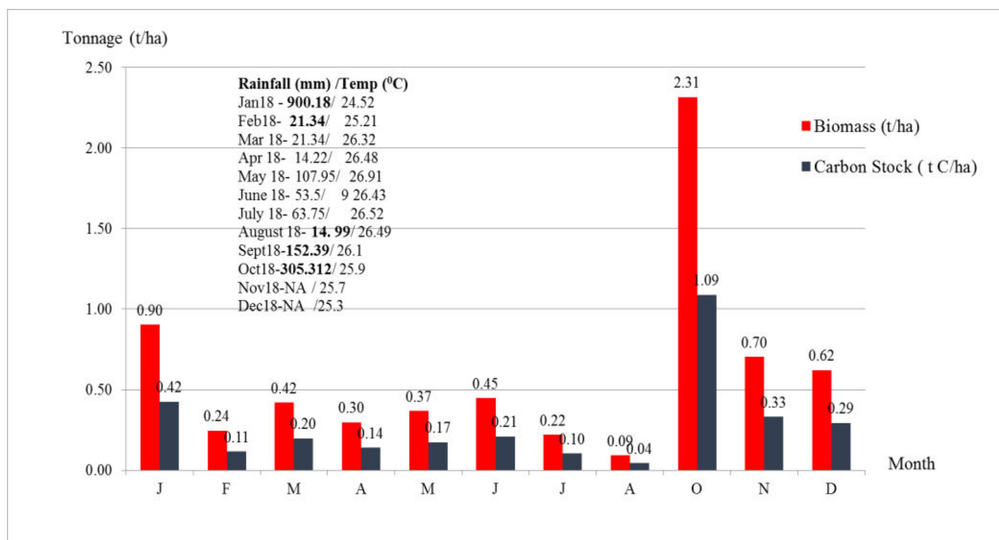

Figure 3: Leaf litter production in 2018.

Leaf litter production

It was observed that the rainfall volume was fairly high; 900.18 mm in January 2018 and decreased sharply in February was 21.34 mm due to the dry spell. This however, is contrast to the previous study where litter production quickly increases after the onset of the dry season, and declines towards the transition between dry and wet season (Wieder and Wright 1995). During El Nino season in 2018, the leaf litter production has significantly decreased from 942.75 kg/ha to 255.35 kg/ha in January and February (Figure 2) while a similar occurrence between January and February 2017 there was a declined in leaf litter production from 690 to 140 kg/ha. Similarly, leaf litter volume also gradually decreased beginning June 2018 until August 2018 (Table 2). We also found consistent evidence of litterfall seasonality between litterfall biomass and precipitation. It is thus strongly recommended that there is a strong correlation in climatic conditions and changes. Other factors including tree physiology and correlation between environmental factors (such as precipitation) with leaf litter production should be further examined. In general, precipitation and solar radiation may also dominate the litterfall seasonality at tropical forests (Haicheng et al., 2014) although radiation and temperature may dominate the seasonality at temperate and boreal forests. Hence, precipitation is a limiting factor for litterfall regulation, hence suggesting that litterfall helps plants reduce water stress during the driest periods for instance in Amazon rainforest (Tonin et al., 2017).

Conclusion

Seasonal fluctuations in leaf litter and their correlation between precipitation and leaf litter production is still being investigated in relation to climatic change/pattern. There should also be a continuous long-term litter production monitoring in the pristine peat swamp conditions to correlate with variations in environmental factors. Since litterfall undergo decomposition process in the forest, correlation with mean annual temperature (MAT), apart from mean annual precipitation (MAP) should also be considered to link between litter nutrients cycle and soil nutrients in belowground biomass (BGB) pool.

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Early Establishment of *Rhizophora* spp. on Mudflats using Organic Fertilizer

Mohd Ghazali, H.*, Tariq, M.H., Marryanna, L., Hyrul Izwan, M.H., Fatin, A.R. and Nur Aina, M.
Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: ghazali@frim.gov.my*

Introduction

Mangroves are a group of shrubs or trees that grows around coastal area. They play huge role for our ecosystems and diversity. As demonstrated by Raja Barizan and Farah (2018), seaward mangroves at Sungai Haji Dorani helps reducing natural disaster effects, soil erosion and preserve shorelines. Mangroves also give many valuable ecosystem services to human where its environment enhances fish and other marine production. Almost 10% household income in Delta Kelantan depends on mangrove yield area (Anonymous, 2014). Local communities able to harvest many types of mollusc and crustaceans that generate high market value for domestic consumption.

Planting the *Rhizophora* spp. for restoration in a specific area is a site specific activity. Thus, special care and attention is needed to ensure the success story. This is a case study carried out at Tanjong Piai National Park, Johor to determine the effect of fertilizer application towards growth rate of planted *Rhizophora* spp. This area has been severely disrupted and eroded caused by nearby physical development and strong waves generated by ships activities that passing through the Straits of Malacca almost every hour (Wan Rasidah et al., 2015). The reported rates of shoreline retreat were between 2 to 4 m/year and amplitudes over 1.5 m (Nor Aslinda et al., 2014). With the recent installation of offshore breakwaters structure, the erosion activities slowing down and mud flat established and natural re-colonization of mangrove seedlings can be observed. This study can perform as the recent case study for improved restoration effort to help mangrove re-colonization at local area.

However, these past few years, many researchers found that mangroves species are threaten with extinction especially for coastal development and agriculture (Polidoro et al., 2010). Various conservation strategies have been implementing to provide healthy mangrove ecosystem and enhance their growth. Similar to other plant, nutrient efficiency and absorption potential are vary among mangrove trees. Mangroves have saline type soil, acidic and frequently waterlogged. Moderate to low salinity treatment helps certain mangrove species achieve maximum growth and survival (Kodikara et al., 2018). The delivery of nutrient also highly depends on tidal cycle to circulate sediment containing organic materials.

Many mangrove soils have extremely low nutrient availability and normally, nutrient enrichment of mangroves contributed by common phenomenon which is decomposition of leaf litter (Keuskamp et al., 2015). Organic matter like phosphate (P) and nitrogen (N) are immobile where they are not ready use for plant, thus organisms that able to solubilize P have important role for plant growth. Manipulation of nutrient limitation by fertilization treatment applied may help in providing alternative to N- or P- fixing decomposer to enrich soils (Reef et al., 2010). Therefore, this study was made to analyse the plant growth rate aided by fertilizer compared to growth control without fertilizer.

Materials and Methods

Site description

Rhizophora spp. planting was carried out in the mudflat of Tanjong Piai Johor (Figure 1). It is located at the most southern tip of Asia continent. Tanjong Piai is part of the larger South-west Johor

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wetlands, which well recognized as Ramsar Site designated on 31st January 2003. Tanjung Piai has suffered from coastal erosion for many years and the area has been identified as one of the critical areas for coastal erosion under a National Coastal Erosion Risk Study. In recent years, the erosion has escalated significantly, where the mangroves are being uprooted thus exposing the shoreline.

Therefore, an effort to restore the mangroves here is much required to ensure ecological integrity and characteristics of this Ramsar Site remain lasting. Harsh and extreme environmental conditions at this areas required human intervention to enhance growth performance of planted trees especially at early stage. The objective of this study is to find a treatment to speed up an early growth of mangrove trees planted on the newly developed mudflats. This experimental plot can be used as a case study for future effort to enhance plant survival and better growth rate when mangrove trees planted on the mudflats. Based on the Figure 1, there are two experimental plot which is Block A (61˚27’N, 014˚02’E) and Block B (61˚28’N, 014˚01’E).

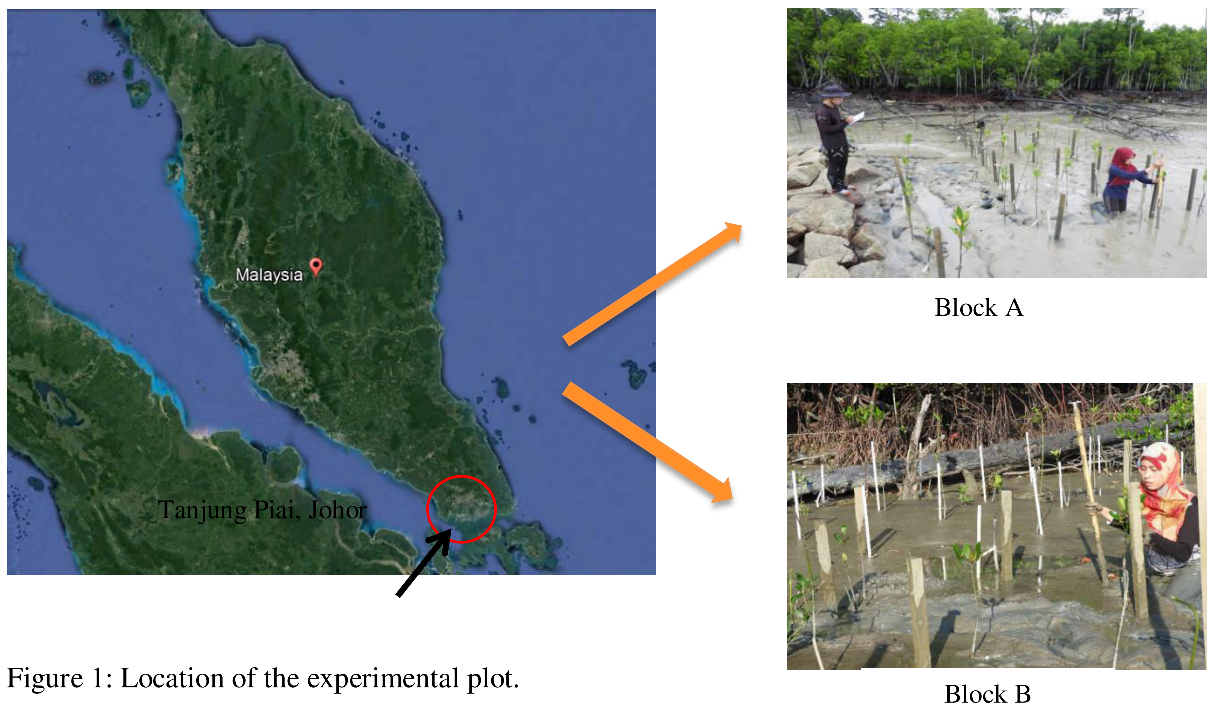


Figure 1: Location of the experimental plot.

Planting treatments

Two planting stocks, namely *Rhizophora apiculata* and *Rhizophora mucronata,* comprising of two of treatments were applied to the planting stocks which is fertilized (Treatment 1) and not fertilized (Treatment 2). For not fertilized treatment, the planting stocks were transferred into planting media consist of marine mud only. While, planting stock for fertilized treatment were transferred into planting media consist of mixture of organic materials (3 kg chicken dung) and marine mud. The fertilized treatments were applying into planting media once during the first planting on July 2018. These planting treatment were prepared in the biodegradable gunny sack and planted at 2 x 2 m spacing. Results were measured in terms of plant height increment (cm) and number of leaves. The data of plant height increment (cm) and number of leaves were collected once every two months, started from July 2018 until July 2019. Based on the Figure 2, showed the fertilized planting stocks in the mudflat.

Figure 2: Showcase study in the mudflat.

Results and Discussion

Results showed that plant height and new buds grow according to the treatments. The average mean height and number of leaves for the fertilized trees dominates those that are not fertilized trees in the species. These dominances are as shown in the form of graphs.

Mean height

The fertilized planting stock shows faster growth compared to not fertilize planting stock (Figure 3). The height of fertilized planting stock increased by 20% in average compared to the not fertilized planting stock. The mean height of fertilized planting stock in July 2018 is 45.14 cm then its increase until 104.43 cm in July 2019. Meanwhile, the mean height of not fertilize planting in July 2018 is 36.71 cm and also increased in a lower rate until July 2019 which is 77.29 cm. At the end of the study, the fertilization gives positive effect and the height dominance continues until the July 2019 with showed the higher mean rates of survival for fertilized planting stock (ranging between 50-56%). The lowest mean height rates of survival were observed in not fertilized planting stock.

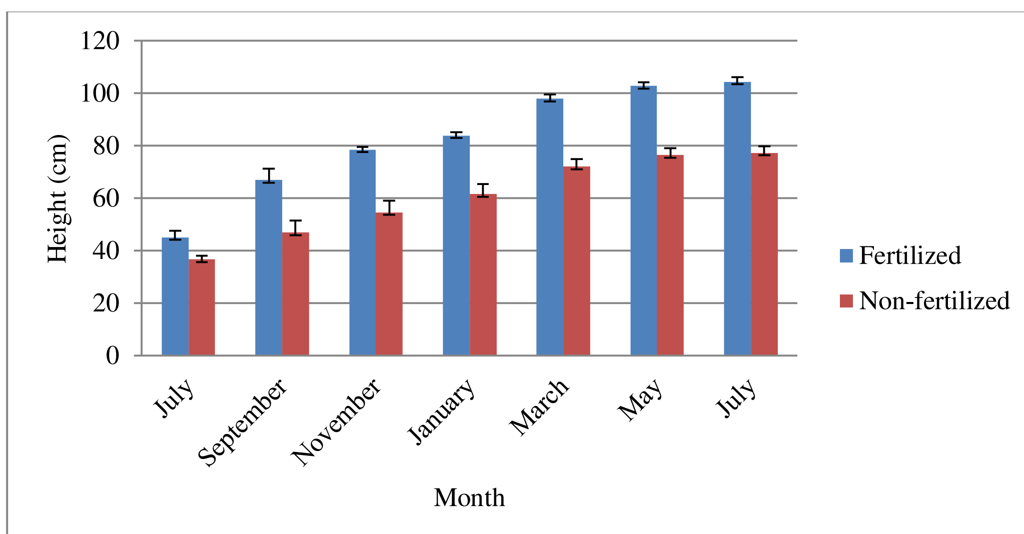


Figure 3: Mean height of *Rhizophora* spp.

Mean leaves number

Early observation showed that fertilized planting stocks had faster leaf formation compared to those not fertilized. The mean increment of fertilized planting stocks almost doubles after a month of

fertilization and it is expected to linearly increase the following month. At first measurement (Figure 4), the mean leaves number in July 2018 is 3.57 then it increased until 11.29 in July 2019. For the not fertilized planting stocks, the mean leaves number started with 1.43 then increased in a lower rate until July 2019 which is 5. The leaf formation is expected to be more prolific after two months treatment as the biological process reacted uniformly. The planting stocks without fertilizer also showing some increment but the progress is slower compared to a fertilized planting stocks.

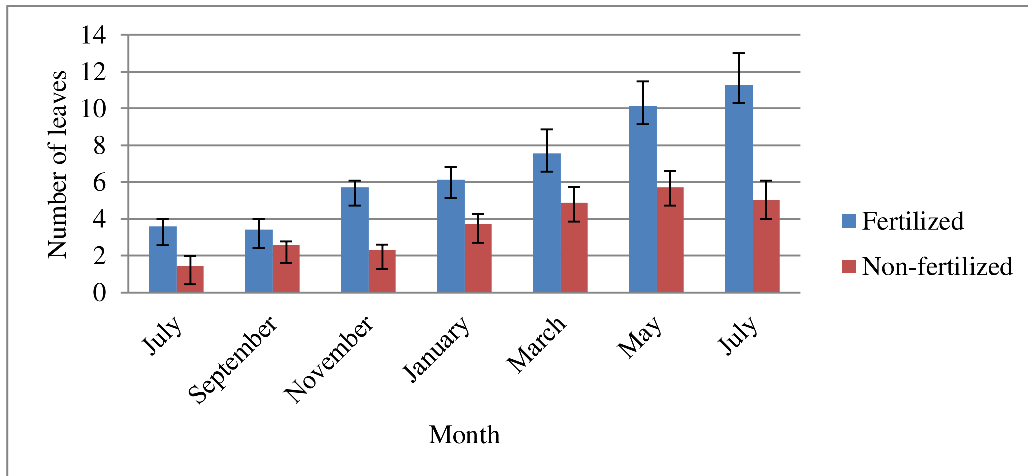
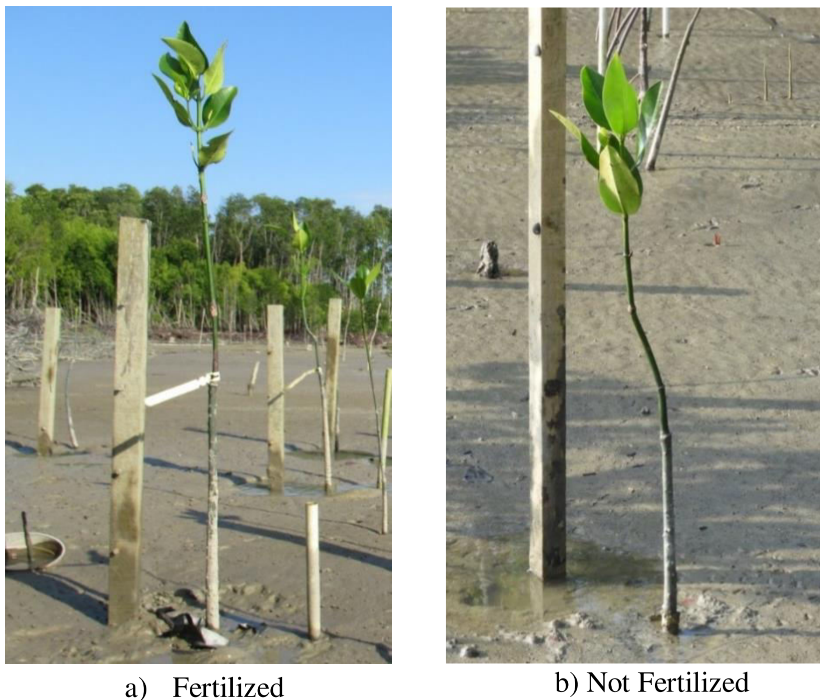


Figure 4: Mean leaves number of *Rhizophora* spp.

In Figure 5 shows the plant growth comparison between not fertilized and fertilized planting stocks after fourteen months of fertilizer application. The fertilized planting stock has more number of leaves and higher tree height compared to not fertilized planting stocks.



a) Fertilized b) Not Fertilized

Figure 5: Leaves of the planting stocks: a) Fertilized and b) Not fertilized.

Conclusion

This study showed that it is possible to enhance early *Rhizophora* spp. seedlings growth using organic fertilizer. However, it is still strongly suggested that for any early establishment of these planted trees, fertilizer treatments should be taken into considerations as this may help to promote rapid early leaves elongation and growth establishment. Furthermore, in order to ensure sustainable mangrove conservation, more restoration efforts such as using specific types of fertilizers to maintain sustainable release of nutrients are recommended.

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Induction of Flower in *Xanthosthemon chrysanthus* by the Combined Effects of Paclobutrazol and Potassium Nitrate

Ahmad Nazarudin, M.R.[1,*], Tsan, F.Y.[2] and Normaniza, O.[3]
[1]Forestry and Environment Division, Forest Research Institute Malasia (FRIM), 52109 Kepong, Selangor, Malaysia.
[2]Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia.
[3]Institute of Biological Sciences, Faculty of Science, University of Malaya (UM), 50603 Kuala Lumpur, Malaysia.
**E-mail: nazarudin@frim.gov.my*

Introduction

Paclobutrazol (PBZ) is a plant growth regulator that able to control vigorous growth of *Acalypha siamensis, Ficus microcarpa,* and *Syzygium oleina* (Ahmad Nazarudin et al., 2003), and *Leonotis leonurus* (Teto et al., 2016). PBZ also enhanced flowering of ornamental plants namely *Canna generalis* (Bruner et al., 2001), *Azalea japonica* (Meijón et al., 2009), and *Hibiscus rosa-sinensis* (Ahmad Nazarudin, 2012). Combination of PBZ and potassium nitrate (KNO_3) was also effective in improving flowering of fruit crops such as *Prunus armeniaca* (Arzani and Roosta, 2004), *Garcinia mangostana* (Omran and Semiah, 2006), and *Mangifera indica* (Burondkar et al., 2013). Thus, the objective of the study was to determine the effects of PBZ and KNO_3 on flowering response of *X. chrysanthus* under local climatic condition.

Xanthostemon chrysanthus (Myrtaceae) or known golden penda is native to tropical northern Australia, New Caledonia, New Guinea, Indonesia and the Philippines. In Malaysia, this species is grown in urban landscapes due to its bright yellow flowers. However, under local climatic condition, the flowering of the species is less in abundance. Therefore, the effects of PBZ and KNO_3 in inducing flowers of the species were explored.

Materials and Methods

Study location and plant materials

A total of 81 existing *X. chrysanthus* trees were randomly selected at Metropolitan Batu Park, Kuala Lumpur (3° 12' 49" N; 101° 40' 43" E). The trees have average height and diameter at breast height of 6 m and 12 cm, respectively.

Experimental procedures

Cultar® (250 g a.i. PBZ/L) and Krista™ K Plus (13.7:0:46.3) were applied as treatments. Nine treatment combinations (rate per plant) i.e. T1(0 g/L PBZ+0 g KNO_3), T2(0 g/L PBZ+100 g KNO_3), T3(0 g/L PBZ+200 g KNO_3), T4(0.125 g/L PBZ+0 g KNO_3), T5(0.125 g/L PBZ+100 g KNO_3), T6(0.125 g/L PBZ+200 g KNO_3), T7(0.25 g/L PBZ+0 g KNO_3), T8(0.25 g/L PBZ+100 g KNO_3), and T9(0.25 g/L PBZ+200 g KNO_3) were replicated nine times and arranged in a completely randomized design. Each dosage of PBZ was top up to 1 L volume with tap water prior to soil drench onto root collar of each tree while the control plants received same amount of tap water. Each rate of KNO_3 was equally divided and filled in four holes of 15 cm in depth dug under the drip-line of the tree canopy. PBZ was applied once at the start of the study, while KNO_3 was supplied at three months intervals.

Flowering response

Flower abundance (%), inflorescence diameter (cm), and number of flowers per inflorescence were recorded for a year. Flower abundance in each tree was expressed as estimated percentage of crown surface area with flowers in relation to the total crown surface area of the tree.

A total of five flowering trees for each treatment were selected randomly for inflorescence diameter, and number of flowers per inflorescence investigation. Five flowering branches, each with more than two inflorescences, were then randomly chosen from each tree for inflorescence diameter measurement. Two randomly selected inflorescences from each flowering branch were used to obtain ten inflorescences for the mentioned measurement. Measurement of inflorescence diameter by using a digital caliper was first recorded in April 2012 and repeated at two months intervals. On the other hand, the number of flowers per inflorescence was manually counted from five inflorescences per tree that were randomly selected at full bloom stage.

Statistical analysis

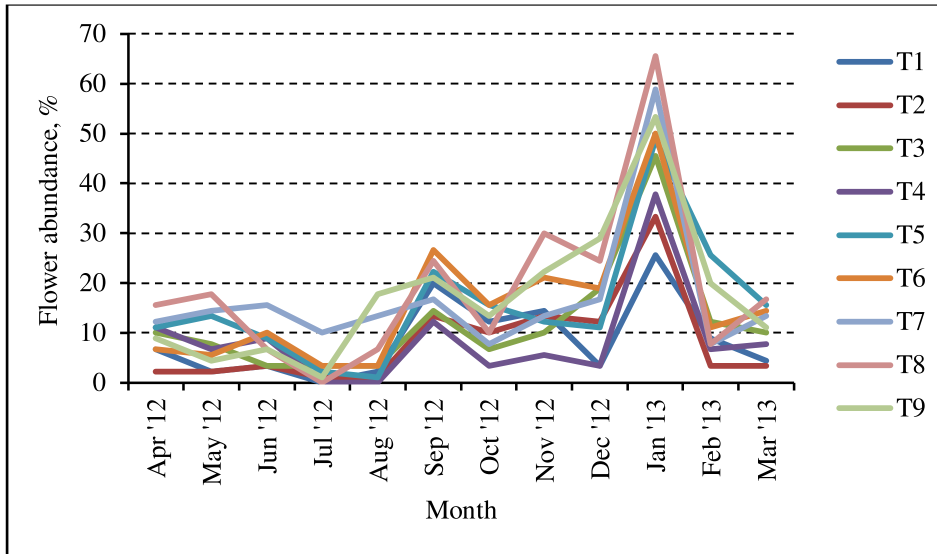
Data obtained were subjected to one-way Analysis of Variance and the treatment means were then compared using Duncan's multiple range test (DMRT) ($p<0.05$).

Results and Discussion

Observation on flowering pattern showed that *X. chrysanthus* flowers throughout the year with three distinct flowering occurrences observed in May 2012, September 2012 and January 2013 (Figure 1). The highest abundance of flowers (65%) was measured in January 2013 after being applied with 0.25 g/L PBZ+100 g KNO_3 (Figure 2). Previous studies indicated that PBZ increased the number of flowers in *Lupinus varius* (Karaguzel et al., 2004) and *H. rosa-sinensis* (Ahmad Nazarudin, 2012) while, combination of PBZ and KNO_3 gave higher amount of flowers and promoted earlier flowering in *M. indica* (Rebolledo-Martínez et al., 2008). Potassium had resulted in a better growth performance including translocation of carbohydrates, proteins, and photoassimilates in plants which indirectly produced more flowers (Akhtar et al., 2009). Besides affecting gibberellins, PBZ also increased abscisic acid and cytokinins concomitant with C:N ratio and leaf water potential in *M. indica* buds to stimulate flowering (Upreti et al., 2013).

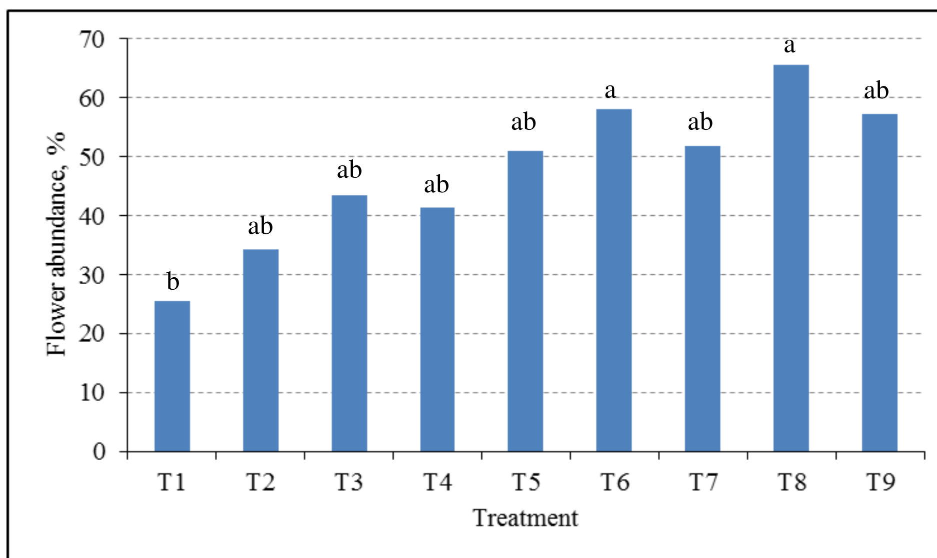
This study shows that, the peak flowering period occurred in early of the year. It could be due to the effects of higher amount of rainfall received in previous months which increasing the availability of water in the soil, thus promoting flowers in the following months. In Malaysia, greater volume of rainfall in the fourth quarter of each year is usually occurred. The Northeast Monsoon occurs from November till March offers greater capacity of rainfall than the Southwest Monsoon which carries relatively drier weather in May till September (MMD, 2014).

Application of PBZ alone resulted in smaller inflorescences size as compared to that of other treatments (Table 1). For example, application of 0.125 g/L PBZ gave a difference of 47.3% smaller inflorescence than that of PBZ supplemented with KNO_3. A similar trend was also observed with higher dosage of PBZ (0.25 g/L). On the other hand, both dosages of KNO_3 produced bigger inflorescences than that of the control, but smaller than those treated with combined treatment of PBZ and KNO_3 (Table 1). For instance, at first measurement, 100 g KNO_3 had 28.26% bigger inflorescence size as compared to the control. However, the inflorescence size was 31.33% smaller when compared to those inflorescence produced by trees treated with combined treatment of 0.125 g/L PBZ and 100 g KNO_3. These results suggested that combination of PBZ and KNO_3 would give bigger inflorescences than other treatments. Trees treated with combination of 0.125 g/L PBZ and 100 g KNO_3 also consistently produced greater inflorescences size ranged from 17 to 20 cm throughout the study period. It shows that aesthetic value of the species could be enhanced by applying suitable combination of PBZ and KNO_3.



T1(0 g/L PBZ+0 g KNO_3); T2(0 g/L PBZ+100 g KNO_3); T3(0 g/L PBZ+200 g KNO_3); T4(0.125 g/L PBZ+0 g KNO_3); T5(0.125 g/L PBZ+100 g KNO_3); T6(0.125 g/L PBZ+200 g KNO_3); T7(0.25 g/L PBZ+0 g KNO_3); T8(0.25 g/L PBZ+100 g KNO_3) and T9(0.25 g/L PBZ+200 g KNO_3).

Figure 1: Flower abundance of *X. chrysanthus* as response to different treatment combinations of paclobutrazol and potassium nitrate.



T1(0 g/L PBZ+0 g KNO_3); T2(0 g/L PBZ+100 g KNO_3); T3(0 g/L PBZ+200 g KNO_3); T4(0.125 g/L PBZ+0 g KNO_3); T5(0.125 g/L PBZ+100 g KNO_3); T6(0.125 g/L PBZ+200 g KNO_3); T7(0.25 g/L PBZ+0 g KNO_3); T8(0.25 g/L PBZ+100 g KNO_3) and T9(0.25 g/L PBZ+200 g KNO_3). Means with the same letter(s) are not significantly different ($p<0.05$) by DMRT.

Figure 2: Flower abundance of *X. chrysanthus* in January 2013.

Meanwhile, the number of flowers per inflorescence was significantly increased as response to combined PBZ and KNO_3 (Table 2). At initial stage, the number of flowers per inflorescence produced after 0.25 g/L PBZ+100 g KNO_3 treatment was 52.1% higher than the untreated control tree. At fourth measurement, the highest number of flowers per inflorescence (81 flowers) was recorded with the lowest rate of PBZ combined with 100 or 200 g KNO_3. The number of flowers per inflorescence for the control tree ranged from 35 to 38, whereas higher number of flowers per inflorescence ranged from 70 to 89 was recorded with 0.25 g/L PBZ+100 g KNO_3-treated tree, showing about twofold difference. Application of PBZ alone did not manage to induce more flowers as compared to that of

combined PBZ and KNO_3 in all studied rates. In other word, 0.25 g/L PBZ+100 g KNO_3 was beneficial for the species to consistently produced higher number of flowers per inflorescence.

According to Yayat et al. (2013) PBZ enhanced cytokinin synthesis in the roots and allocation of photosyntate utilization. Cytokinin from the roots will be transported to the upper part of the plant which further stimulated the growth of axillary buds (Yayat et al., 2013). These lateral buds will develop into either as leaves or flowers depending on other endogenous and environmental cues. In addition, reduction in vegetative growth due to PBZ inhibition effect was reported to increase the distribution of photoassimilates towards reproductive growth which encouraged the formation of flower buds and hence induced the flowering (Abou-El-Khashab et al., 1997).

In the present study, combined effects of 0.125 g/L PBZ and 100 g KNO_3 was dramatically increased the number of flowers per inflorescence which indirectly improved inflorescence size in *X. chrysanthus*. As a consequence, the flower abundance was much greater in trees treated with this combination than that of other treatments.

Table 1: Effects of PBZ and KNO_3 on inflorescence size of *X. chrysanthus.*

Treatment ID	Inflorescence size measured at two months intervals (cm)					
	1st Measurement	2nd Measurement	3rd Measurement	4th Measurement	5th Measurement	6th Measurement
T1	10.00^{e}	11.16^{f}	10.50^{f}	10.40^{e}	10.00^{f}	9.70^{f}
T2	13.94^{d}	12.34^{e}	12.30^{e}	13.10^{d}	12.96^{e}	13.20^{e}
T3	13.96^{d}	13.86^{d}	14.16^{d}	13.44^{d}	14.10^{d}	13.82^{e}
T4	10.70^{e}	10.66^{f}	10.62^{f}	9.94^{e}	9.54^{fg}	9.78^{f}
T5	20.30^{a}	17.44^{a}	20.68^{a}	18.96^{a}	20.34^{a}	19.70^{a}
T6	17.42^{b}	16.16^{b}	17.80^{b}	18.54^{ab}	19.50^{b}	18.80^{b}
T7	10.44^{e}	9.98^{g}	9.94^{f}	8.76^{f}	9.00^{g}	9.02^{f}
T8	15.98^{c}	14.64^{c}	16.80^{c}	17.92^{b}	16.46^{c}	16.98^{c}
T9	14.02^{d}	14.34^{cd}	14.12^{d}	15.68^{c}	16.28^{c}	14.68^{d}

T1(0 g/L PBZ+0 g KNO_3); T2(0 g/L PBZ+100 g KNO_3); T3(0 g/L PBZ+200 g KNO_3); T4(0.125 g/L PBZ+0 g KNO_3); T5(0.125 g/L PBZ+100 g KNO_3); T6(0.125 g/L PBZ+200 g KNO_3); T7(0.25 g/L PBZ+0 g KNO_3); T8(0.25 g/L PBZ+100 g KNO_3) and T9(0.25 g/L PBZ+200 g KNO_3). Means followed by the same letter(s) within column do not differ ($p<0.05$) by DMRT; n=50/treatment.

Table 2: Effects of PBZ and KNO_3 on number of flowers per inflorescence of *X. chrysanthus*.

Treatment ID	Number of flowers per inflorescence recorded at two months intervals					
	1st Measurement	2nd Measurement	3rd Measurement	4th Measurement	5th Measurement	6th Measurement
T1	37.04^{e}	38.64^{d}	35.84^{e}	36.32^{e}	37.24^{f}	35.68^{f}
T2	33.88^{e}	37.60^{d}	35.92^{e}	35.12^{e}	38.56^{ef}	39.68^{ef}
T3	39.64^{e}	41.60^{d}	46.60^{d}	42.40^{d}	43.00^{e}	43.96^{e}
T4	62.88^{c}	59.96^{c}	60.04^{c}	61.04^{c}	60.48^{d}	60.40^{d}
T5	70.68^{b}	72.36^{b}	75.92^{ab}	81.56^{a}	81.76^{a}	89.08^{a}
T6	72.8^{ab}	80.00^{a}	75.44^{ab}	81.68^{a}	77.28^{ab}	78.12^{b}
T7	53.36^{d}	55.12^{c}	62.24^{c}	62.08^{c}	60.20^{d}	57.32^{d}
T8	77.24^{a}	74.08^{b}	79.08^{a}	73.92^{b}	71.84^{c}	74.20^{c}
T9	76.28^{ab}	76.68^{ab}	71.80^{b}	72.08^{b}	74.04^{c}	72.16^{c}

T1(0 g/L PBZ+0 g KNO_3); T2(0 g/L PBZ+100 g KNO_3); T3(0 g/L PBZ+200 g KNO_3); T4(0.125 g/L PBZ+0 g KNO_3); T5(0.125 g/L PBZ+100 g KNO_3); T6(0.125 g/L PBZ+200 g KNO_3); T7(0.25 g/L PBZ+0 g KNO_3); T8(0.25 g/L PBZ+100 g KNO_3) and T9(0.25 g/L PBZ+200 g KNO_3). Means followed by the same letter(s) within column do not differ ($p<0.05$) by DMRT; n=50/treatment.

Conclusions

The effects of combination application of PBZ and KNO_3 had produced the highest flower abundance, biggest inflorescence size, and highest number of flowers per inflorescence in *X. chrysanthus*. Thus,

an approach by combining 0.125 g/L PBZ+100 g KNO_3 is recommended to be practiced for enhancing flowering of *X. chrysanthus* under local urban conditions.

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Effects of Different Growing Media on Seed Germination and Seedling Growth Performance of *Garcinia atroviridis*

Nurhazwani, M.[1,*], Saifuddin, I.[2] and Muhammad Syakir, B.[1]
[1]Industrial Crop Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Agrobiodiversity and Environment Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: wanie@mardi.gov.my*

Introduction

Garcinia is the biggest genus in the Guttifereae (Clusiaceae) family. In Malaysia, there are 49 species out of 350 species estimated worldwide (Whitemore, 1973). Some of the species are known to produce edible fruits and have been brought into cultivation such as *G. atroviridis* (asam gelugor), *G. prainiana* (cerapu), *G. forbesii* (rose-kandis), *G. cowa* (kandis), *G. dulcis* (mundu) and *G. hambroniana* (beruas). These species are usually consumed as food sources whereas some are also used in traditional medicine by rural folks. Underutilised *Garcinia* such as *G. atroviridis* are healthy fruits to consume because of its high antioxidant content and even better than common *Garcinia* which is mangosteen. *Garcinia atroviridis* or locally known as asam gelugor is native to Peninsular Malaysia. It grows well in wild vegetation and being cultivated most widely in northern states including Perak. The community planted the trees in orchard or home garden which is easier for them to harvest.

The quality of seed depends on seed management, including selection media for seedlings (Sakai and Atok, 2007). Generally, seedling media consist of top soil that contains macro and micro nutrients, sand that provides aeration, and organic materials such as peat moss, coco peat, chaff or ferns. Continuous use of top soil as seedling media will cause top soil degradation. Peat moss is widely and successfully used in different parts of the world as an environmental-friendly peat substitute for growing plants in containers. Media mixtures must be heavy to support the plant which is often provided by using topsoil or sand in the basic media mixture for planting. A good quality media for successful growing of a plant must sustain a long-range production of vegetative growth in good quality and quantity.

Media mixture is important when these constituents are combined and used as planting medium in the limited volume of a plant growing. There was no study on *Garcinia* sp. seeds using different growing media and their effect on seed germination and seedling growth. Therefore, the present study was conducted to evaluate the effect of different growing media such as peat moss and sand with respect to its aeration and moisture availability as well as their effects on the growth performance of *G. atroviridis*.

Materials and Methods

Planting materials

The *G. atroviridis* seeds were collected at Air Kuning, Perak on November 2018. Seeds were initially removed from the fruit, washed in running water and then placed to germinate in trays filled with planting media. Seed sowing was done about 1 cm depth in different media filled in container, prepared as per treatment. Planting materials used in these experiments are sand, peat moss and a combination of sand and peat moss (1:1).

Seedlings were grown under 50% shade and plant maintenance was carried out according to the normally recommended practices. The experiment was carried out in a greenhouse. Irrigation was

applied immediately after seed sowing and repeated twice every day till the final emergence. After seed germination, irrigation was applied twice every day.

Germination percentage and vigour index

Parameters such as seed germination percentage, seed vigour index and growth parameter were recorded. Germination percentage was scored at xx days after planting and calculated by the following formula:

$$\text{Germination (\%)} = \frac{\text{Total cumulative germination}}{\text{Total number of seeds sown}} \times 100$$

The length of seedlings was measured (from where to where) before seed vigour index was calculated by the following formula:

Vigour index = Germination (%) x Total seedling length (mm)

Growth measurement

Plants were sampled randomly from each treatment for determination of seedling length, seedling girth, root and shoot length, root collar diameter, leaf length and width, number of leaves and relative chlorophyll content. Measurement of seedling height was taken from the end tip of the main root to the highest shoot tip by using a measuring tape. Shoot length were measured from collar region to the highest shoot tip and leaf length and width was taken on selected fully expended leaves using Electronic Digital Caliper (Model SCM DIGV-6). Root collar diameter and root length was measured from the point where first root started up to the end tip of the main root using Electronic Digital Caliper (Model SCM DIGV-6) while the leaf number was manually counted based on fully expanded leaves. Relative leaf chlorophyll content of three leaves per treatment were measured and determined by using a chlorophyll meter (SPAD-502 plus, MINOLTA, Japan).

Experimental design and data analysis

This experiment was conducted in a complete randomised design (CRD) with three different planting media with four replications; 25 plants per replication. The data obtained were analysed using ANOVA in the SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Least Significant Difference (LSD) at p-value ≤ 0.05.

Results and Discussion

Germination percentage and vigour index

The germination and vigour of *G. atroviridis* seeds cultivated in different medium were observed at 30 days after planting (Table 1). Growing media significantly affected ($P \leq 0.05$) seed germination and vigour index. Germination percentage obtained at the end of the study were determined for sand (72.7%), peat moss (82.7%) and sand + peat moss (90.7%) media. The best germination percentage was observed in sand + peat moss media and the lowest germination was observed in sand media. Moreover, combination of sand and peat moss media gave highest seed vigour index compared to single media.

Growing media plays a vital role in growth and development of any plant species. It is an edaphic factor that acts as a precursor for initial stages of plant life. The supply of water and air to the growing plants can be greatly influenced by the physical composition of growing media (Beardsell and Nicholas, 1982) which may further affect the anchorage, nutrient and water holding capacity of the

medium. These characteristics directly influence the seedling emergence and vigour, and consequently the seedling quality (Baiyeri and Ndubizu, 1994). The reason for the best performance of combination of sand + peat moss are high organic matter content which increases the water and nutrient holding capacity of the medium, which improve the water utilization of the plant to germinate and seed vigour. Bachman and Metzger (2008) also reported these medium have bioactive principles which are considered to be beneficial for root growth and this related to result in greater root initiation, higher germination, increased biomass, enchanced growth and development.

Table 1: Effect of different growing media on germination percentage and vigour index on *G. atriviridis.*

Type of planting media	Germination percentage	Vigour index
Sand	72.7[c]	6899.23[c]
Peat moss	82.7[b]	8140.16[b]
Sand + Peat moss	90.7[a]	9852.74[a]

Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to LSD.

Vegetative growth measurement

After the seed germination status was determined, the vegetative growth of the *Garcinia* sp. seedlings was measured. Differences between the measured plant growth based on the media were determined by ANOVA. The results are presented in Tables 2 and 3.

Based on the observation on vegetative growth, the most successful planting media was the combination of sand and peat moss. It was shown that the least successful media was sand. The results displayed in Table 2 demonstrated that planting media significantly affects (P≤0.05) both root length and root collar diameter but no other parameters (seedling length and girth). In terms of root length and root collar diameter, the higher value was observed in combination of sand + peat moss, while the lower value was observed in the sand but no significant different with peat moss.

Table 2: Effect of different planting media on seedling height, girth, root length and root collar diameter of *Garcinia atriviridis* seedling.

Treatment	Seedling length (mm)	Seedling girth (mm)	Root length (mm)	Root collar diameter (mm)
Type of planting media				
Sand	94.90[a]	2.287a	16.50[b]	2.53[b]
Peatmoss	98.43[a]	1.983[a]	19.66[b]	2.59[b]
Sand + Peatmoss	108.63[a]	1.987[a]	23.32[a]	2.92[a]

Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to LSD.

Table 3: Effect of different growing media on shoot length, number of leaves, leaf length, leaf width and relative chlorophyll content on *Garcinia atriviridis* seedling.

Treatment	Shoot length (mm)	No of leaves	Leaf length (mm)	Leaf width (mm)	Relative chlorophyll content
Type of planting media					
Sand	77.46[a]	2.9[a]	29.15[a]	15.87[a]	19.61[a]
Peatmoss	78.76[a]	3.6[a]	31.86[a]	18.32[a]	29.54[a]
Sand + Peatmoss	83.66[a]	3.6a	35.43[a]	20.13[a]	27.81[a]

Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to LSD.

Growing media are designed to achieve high porosity and water retention while providing adequate aeration. By using peat moss and sand, it is providing the aeration, drainage and water holding capacity required and they no need to be pasteurized or fumigated to prevent diseases and weeds. The increase in seedlings growth recorded in this experiment could be a reflection of the effect of the combination of high moisture retention and significant air space in agricultural media. The results are in agreement with those obtained by Al-Imama and Al-Jubury (2011). In addition, growth media (peat moss and sand) contained adequate nutrients and the high-water retention capacity necessary for germination and growth of pistachio seedlings (Thanaa et al., 2019). These results are also in agreement with observations of (Poole and Conover, 1982; Hassan et al., 1994) that peat moss and sand increased nutrient retention, promoted vegetative growth, and improved growth overall through the activity of meristematic tissue (Kramer and Koziowski, 1979). In an experiment on the effect of aeration on rooting and growth of roses, Gislerod et al. (1996) proved that when changed from well aerated to unaerated condition, the root growth was reduced by 50%. It was advocated that different plant species have different root systems which enable them to grow under different oxygen conditions (Laan et al., 1987). Prasad (1979) discussed the physical properties of various media for container grown crops. de Boodt and Verdonck (1972), Abad et al. (2001) and Verdonck et al. (1973) also reviewed the physical properties of horticultural important substrates. The proper organic media has a several characteristics such as good aeration, high ability of storing water, high cation exchange capacity, and strengthen the seedling roots (Sukmana et al., 2005).

Conclusions

Combination of sand and peat moss was shown to be the most suitable growing media for seed germination and seedling growth of *G. atroviridis*. Germination percentage and vigour index were increased, as well as root length and root collar diameter. Based on the rest parameter for vegetative growth showed that sand and peat moss gave higher value compared to single media sand and peat moss.

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Assessment of Carbon Stock from Planted Mangrove Species in Perak, Johor and Kelantan

Salleh, M.*, Nurfarah Aqilah, M.N., Wan Mohd Shukri, W.A., Nur Hajar, Z.S., Mohd Danial, M.S., Abdul Aizudden, A.A., Aminudin, A.A. and Muhamad Khairul, E.
Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: sallehmt@frim.gov.my*

Introduction

Mangrove forest or vegetated coastal ecosystems play an important role as a substantial coastal carbon (C) sink. It was reported that a hectare of mangrove forest stores up to four times more C than most other tropical forests around the world (Donato et al., 2011). It is prominent that although plant biomass in the ocean and coastal areas comprises only 0.05% of the total plant biomass on land, it offers a comparable amount of C each year. Recently, great numbers of studies have revealed the importance of mangrove ecosystems in C stock (Alongi, 2004; Keith et al., 2009; Donato et al., 2011). Malaysian mangrove C stock has only been reported in shallow depth (Hong et al., 2017) and in production forest (Adame et al., 2018) but lack of studies on C stock from planted mangrove species. In 2005 to 2012, Malaysia has planted 6.3 millions mangrove trees and other spesies along the coastline. Therefore, a study was carried out in order to provide the information of C stocks in planted mangrove forests. In this study, the C stocks on the planted mangroves sites over the entire state of Perak, Johor and Kelantan were determined.

Materials and Methods

Study sites

Three planted mangrove plots along the coastline in Peninsular Malaysia including Perak, Johor and Kelantan were studied. The plots were planted earlier by the State Forestry Department in early 2000 - 2012 in order to minimize the impacts from tides, waves and winds to the land. Today, the tall, dense and steady trees of the planted coastal wetlands with the diameter at breast height (DBH) ranging from 2.0-12.0 cm naturally form a barrier or a buffer to protect coastal villages and communities, in addition to providing other vital ecosystem services. The two most common planted species of mangrove trees are *Rhizophora apiculata* (Bakau minyak) and *Rhizophora mucronata* (Bakau kurap). Details of the distributions of planted mangrove sites were shown in Table 1 and Figures 1-4.

Table 1: List of distribution of sampling sites.

States	Sites	Species	Year planted
Perak	Parit Haji Dollah, Mukim Lekir, Kinta/Merujung	*Rhizophora mucronata* and *Rhizophora apiculata*	2008
Johor	Sanglang,Gelang Patah	*Rhizophora mucronata*	2012
Kelantan	Pulau Kambing Tumpat	*Rhizophora mucronata* and *Rhizophora apiculata*	2007/2008
	Pulau Layang-Layang Tumpat	*Rhizophora apiculata*	2000/2004
	Pulau Tujuh Tumpat	*Rhizophora apiculata*	2006

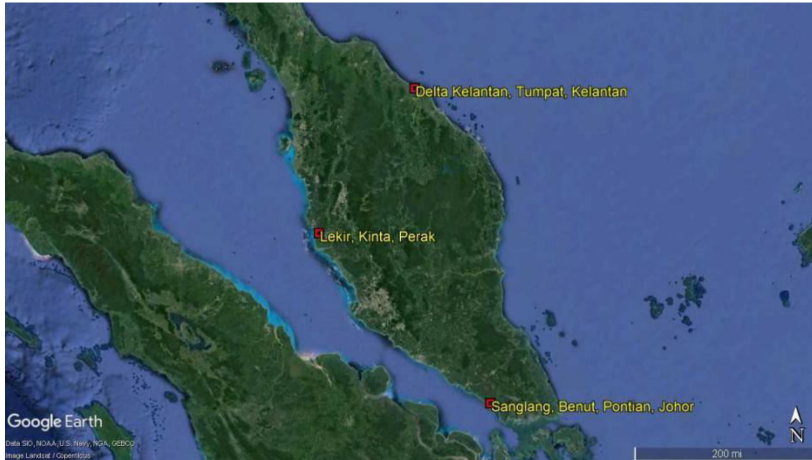

Figure 1: Map showing distributions of the studied three planted mangroves site in Peninsular Malaysia.

Figure 2: Lekir, Perak (P01, P02, P03, P04).

Figure 3: Sanglang, Benut, Johor (J01, J02).

Figure 4: Delta Kelantan, Kelantan (K01, K02, K03, K04, K05, K06, K07, K08, K09, K10).

Forest inventory

Planted mangrove trees measuring diameter of >2 cm in a square subplot measuring 20 × 20 m were established for each mangrove sites. Biomass of each tree was calculated by using allometric equation that was developed by Komiyama et al. (2008) for common species in the mangroves of Peninsular Malaysia. Correspond to the global wood density database by region of South East Asia (tropical), wood density used for *R. apiculata* is 0.85 (Martawijaya et al., 1992) and that for *R. mucronata* is 0.82 (Seng, 1951 in Soewarsono, 1990). The calculated biomass was converted into C by using a C factor of 0.47. The allometry of species-specific equation can be expressed as:

Above Ground Biomass for *R. apiculata* and *R. mucronata* = $0.1709\ p\ D^{2.516}$
where,

p is wood density (g/cm^3), D is diameter at breast height (DBH) (cm) (Rozainah et al., 2018).

Results and Discussion

Overall, based on the Table 2, Johor (27.61842 Mg C/ha) showed the least C production (Mg C/ha) followed by Perak (40.023455 Mg C/ha) and Kelantan (83.597618 Mg C/ha). This was due to the year of planting which led to the difference in DBH of the tree. Greater diameter will lead to greater C stock production. From the comparison table shown in Table 3, the results showed that the C stocks from planted mangrove areas were lower compared to natural forest.

Table 2: Summary of the measured C in three states.

State	(Plot, year planted)	Species	Above ground biomass (Kg)	C stock (Mg C/ha)	Average C stock (Mg C/ha)
Perak	(P01, 2008)	*R. apiculata*	3,737.083	43.91072	40.023455
	(P02, 2008)	*R. apiculata*	3,492.464	41.03645	
	(P03, 2008)	*R. mucronata*	3,629.117	42.64212	
	(P04, 2008)	*R. mucronata*	2,766.343	32.50453	
Johor	(J01, 2012)	*R. mucronata*	2,210.147	25.96922	27.61842
	(J02, 2012)	*R. mucronata*	2,490.86	29.26762	
Kelantan	(K01, 2000	*R. apiculata*	10,472.32	123.0498	83.597618
	(K02, 2000)	*R. apiculata*	8,235.321	96.76502	
	(K03, 2004)	*R. apiculata*	4,170.3328	49.00141	
	(K04, 2004)	*R. apiculata*	7,326.11	86.0818	
	(K05, 2006)	*R. apiculata*	7,424.864	87.24216	
	(K06, 2006)	*R. apiculata*	3,850.4348	45.24261	
	(K07, 2007)	*R. mucronata*	4,108.3947	48.27364	
	(K08, 2007)	*R. mucronata*	9,472.0186	111.2962	
	(K09, 2008)	*R. apiculata*	6,138.9142	72.13224	
	(K10, 2008)	*R. apiculata*	9,948.1987	116.8913	

Table 3: Comparison C stock in natural and planted mangrove.

Constituents	Omar et al., (2016)	Philip et al., (2011)	Hong et al., (2017)	This study
Condition	Reserved Forest	Natural Forest, 10 years; 20 years	Natural Forest, > 32 years	Planted mangrove 10 years; 6 years; 18 years
Sampling plots	Balok, Cherating, Kuantan, Pahang	Matang Mangrove, Perak	Kuala Selangor Natural Park, Selangor	Perak, Johor, Kelantan
Species	*Sonneratia alba* and *R. mucronata*	*R. apiculate*	*Sonneratia alba, Avicennia officinalis, Brugueira parviflora and R. mucronata*	*R. apiculata* and *R. mucronata*
C Stock (Mg C/ha)	165.51, 161.40, 215.47	45; 101	121.82	40.02, 27.62, 83.59

Conclusion

Overall the study has successfully determined the above ground biomass of mangroves from the three planted mangrove sites. The study also quantified the C stock in this planted sites. Results of the study showed that C stocks from planted areas were slightly lower compared with natural forest.

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Effects of Gamma Irradiation on Morphological and Flowering Characteristics of *Ruellia brittoniana*

Farah Zaidat, M.N.[1,*], Najah, Y.[1], Nurul Enanee, A.K.[1], Hanim, A.[2], Muhammad Aliff Fitri, A.[3], Zaiton, A.[4], Rozlaily, Z.[5] and Zulhazmi, S.[3]
[1]Breeding Program, Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Agronomy and Production System Program, Industrial Crop Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[3]Agronomy and Production System Program, Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[4]Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, 43000 Bangi, Selangor, Malaysia.
[5]Breeding Program, Industrial Crop Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: fzaidat@mardi.gov.my*

Introduction

Ruellia brittoniana or commonly known as Mexican petunia is a species of flowering plant in the family Acanthaceae (Afzal et al., 2015). It is native to Mexico and found abundantly in the Caribbean and South America. The plant has been known with multiple common names such as purple ruellia, Mexican bluebell, hardy petunias and purple shower. Today, the most widely accepted names for this species were *Ruellia brittoniana* and *Ruellia simplex. Ruellia brittoniana* is an herbaceous perennial shrub, quick to establish and can grow to 3 to 6 feet tall with equal spread. It's a flowering plant with lovely violet flowers that can last about one day but grow in abundance. Flower colors are mostly violet purple but also come in white or pink. They are heat-loving and can be planted under full sun as a landscape plant.

Gamma irradiation is one of the mutagens used in mutation breeding. Mutation breeding is an alternative and a complementary technique in plant breeding for the introduction of genetic changes. Up to 90% of released mutant cultivars were derived from the radiation-induced mutations (Pimonrat, Suraninpong and Wuthisuthimethavee, 2012). Gamma radiation is easy to use, safe to plant breeders and effective in many different plant species including flowering-plants, fruits, vegetables and herbs.

Ruellia brittoniana have been widely used as a landscape plant due to its durable nature plant and adapt to the tropical climate. But limited color choices and no new locally owned varieties lead to this mutation breeding research. Induce mutation breeding using gamma irradiation is the best and fastest technique to produce new varieties that are more attractive. Unlike other floral crops, studies on *R. brittoniana* for induced mutation have been not fully investigated. The objective of this study was to determine the effects of various doses of gamma irradiation and to investigate the optimum dose to induce mutation on *R. brittoniana.* This is a preliminary study and the results obtained will be used for further research.

Materials and Methods

The treatment of gamma irradiation was conducted at the Malaysian Nuclear Agency (MNA), Bangi. Cuttings of *R.brittoniana* var. Purple were irradiated with 8 different doses namely 0 (control), 10, 20, 30, 40, 50, 60 and 70 Gy. Ten cuttings were treated for radio-sensitivity test for each dose and 10 non-irradiated cuttings were used as control. The source of gamma rays was Caesium^{137} source (BioBeam GM8000). The cuttings were wrapped in a plastic bag and the Fricke's solution was attached together in order to measure the absorbed doses of gamma rays during irradiation. After irradiation, the cuttings were planted in a sand bed and watered once a day manually in the greenhouse at the Floriculture

Complex, Horticulture Research Centre, MARDI Serdang. Non-irradiated cuttings were also included as the control. No fertilizer was applied while the plants were growing. The experimental design was a completely randomized design (CRD) and significant treatment effects were analyzed by analysis of variance (ANOVA) using SAS Software. Weekly data were taken for plant height, new leaf emergence and number of flowers. Morphological changes of the plants were also screened for variations in flower development including changes in color and form. Quantitative data on flower morphology were also measured such as length of flowering stalks and inflorescence size.

Results and Discussion

The results of the experiment indicated that increasing doses of gamma irradiation effected the plant development. Analysis of variance showed highly significant differences ($p<0.001$) of plant height, new leaf emergence and number of flowers among different doses of gamma irradiation on *R. brittoniana* var. Purple. Mean of plant height, new leaf emergence and number of flowers decreased as the gamma dose increased from 0 to 70 Gy as shown in Table 1.

Table 1: Mean of plant height, new leaf emergence and number of flowers of *Ruellia brittoniana* var. Purple at eight different dose of gamma rays 8 weeks after planting.

Dose rate	Plant height	New leaf emergence	Number of flowers
0 Gy (control)	54.00[a]	18.89[a]	10.32[a]
10 Gy	44.88[b]	15.75[b]	5.40[b]
20 Gy	41.30[bc]	13.63[b]	5.17[b]
30 Gy	36.50[c]	10.75[c]	4.21[b]
40 Gy	34.25[c]	11.21[c]	4.13[b]
50 Gy	19.83[d]	7.27[d]	1.50[c]
60 Gy	17.40[d]	4.20[d]	1.27[c]
70 Gy	20.20[d]	4.77[d]	0.23[c]
Significance level	***	***	***

Means with different letter(s) within each column are highly significant between treatments by LSD test ($p<0.001$).

The result indicated that plant heights of *R. brittoniana* var. Purple were affected by the treatment. Plant height showed a decreasing trend as doses increased after 30 Gy (Figure 1). The highest plant recorded in this study was obtained at 0 Gy (control) with an average 54 cm while the shortest value was about 17.4 cm for cuttings irradiated at 60 Gy, both recorded at 8 weeks after planting. New leaf emergence also showed the same trend as observed in plant height which was inversely proportional to dose treatment (Figure 2). The effects showed that higher doses of gamma rays have an inhibitory effect on plants. Gamma rays has resulted production of free radicals which have destructive effects on physiological, morphological and anatomical aspects according to the irradiation level (Hanafy and Akladious, 2018). This is further supported by the fact that germination capacity decreases with a corresponding decline of plant growth and development when exposed at high dose of gamma rays (Marcu et al., 2013).

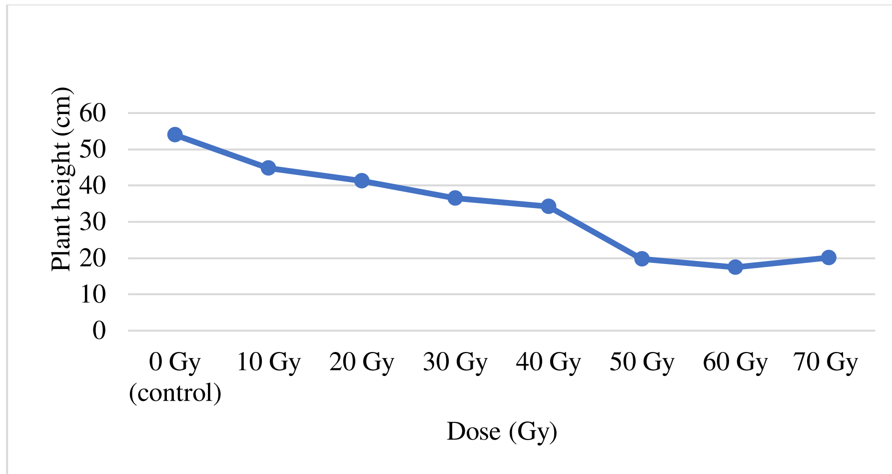

Figure 1: Effects of gamma irradiation on plant height of *Ruellia brittoniana* var. Purple.

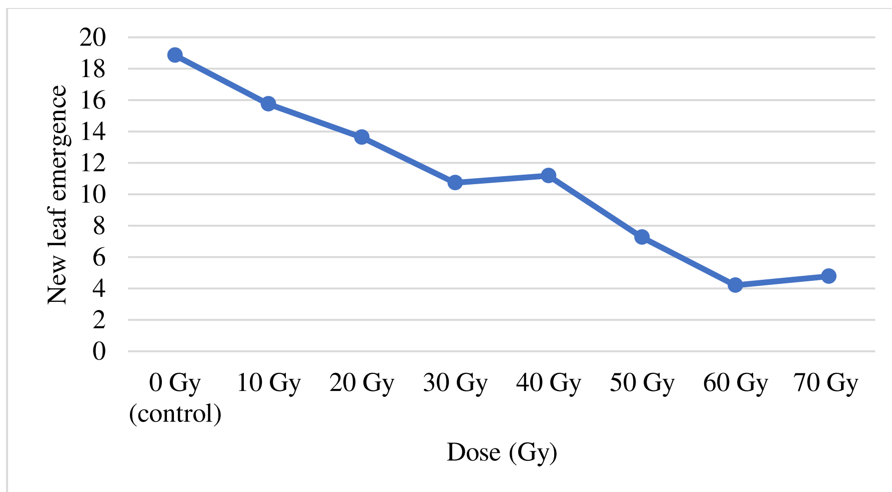

Figure 2: Effects of gamma irradiation on new leaf emergence of *Ruellia brittoniana* var. Purple.

Number of flowers showed decreasing trend as early at 10 Gy and highly significant reduction after 40 Gy (Figure 3). The results showed that flowering of *R. brittoniana* var. Purple were affected by the treatments and the plants barely produced flower at 70 Gy. High doses of gamma irradiation lowered the formation of flower due to the effects involving growth regulators of phytohormones such as auxins, cytokinins, gibberellins, abscisic acid, ethylene and brassinosteroids (Taheri et al., 2014). Exposure to gamma irradiation has influenced the amount of flowers interfering with the rate of flowering in *R. brittoniana* var. Purple. But all irradiated cuttings still survived until weeks 8 and were not totally inhibited by the radiation. Lethal dose (LD_{50}) values cannot be measured by using a simple linear regression (Excell) because all treated cutting was found to survive and flowering.

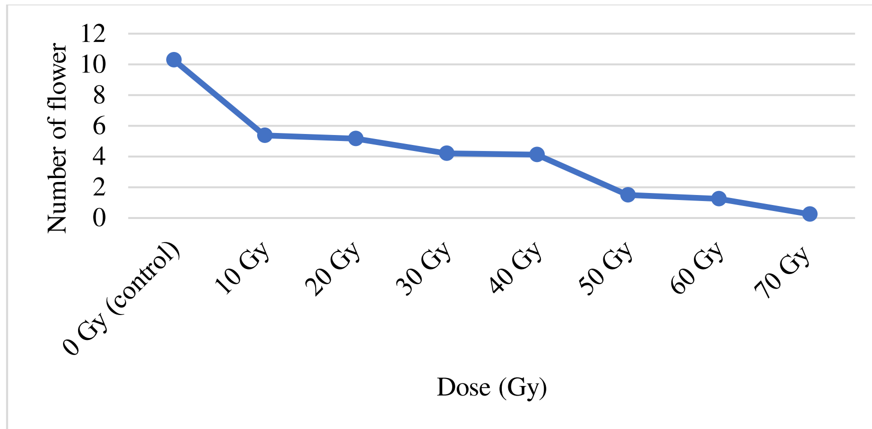

Figure 3: Effects of gamma irradiation on number of flowers of *Ruellia brittoniana* var. Purple.

Generally, the dose that reduces 50% of the growth is considered suitable for mutation induction. If the doses are too high, plants will be killed due to direct negative effects on plant tissue and became lethal. Gamma rays can give primary injuries which are retardation of inhibition of cell divison, cell death induction and changes in plant morphology (Abdullah, Endan and Nazir 2009). Induced mutation also created new genetic varieties within crop varieties. It can enhance the mutation frequency compared with the spontaneous mutation in nature. The alterations in plant morphology and the changes in color of leaves and flowers were observed after the irradiation. High doses of gamma irradiation lowered the production of flowers in *R. brittoniana* var. Purple, but the changes of flower color occurred at 20 Gy of irradiated *R. brittoniana* var. Purple. Data on flower color were investigated and color descriptions were made based on RHS (Royal Horticulture Society) color chart at full bloom stage (Ahmadi et al., 2012). There is flower in red purple 73D color (at 20 Gy) meanwhile the normal colour of *R. brittoniana* var. Purple was violet blue group 70A (Figure 4). There were no changes or mutagenesis in other flower morphology such as number of petals. There were no significant changes in leaf color, mostly showed color of yellow green 147A or green 138A. Various effects on the plant will be observed in later experiments in the different generations after mutation induction. Genetic effects or mutations may be transferred from M1 to the following generations.

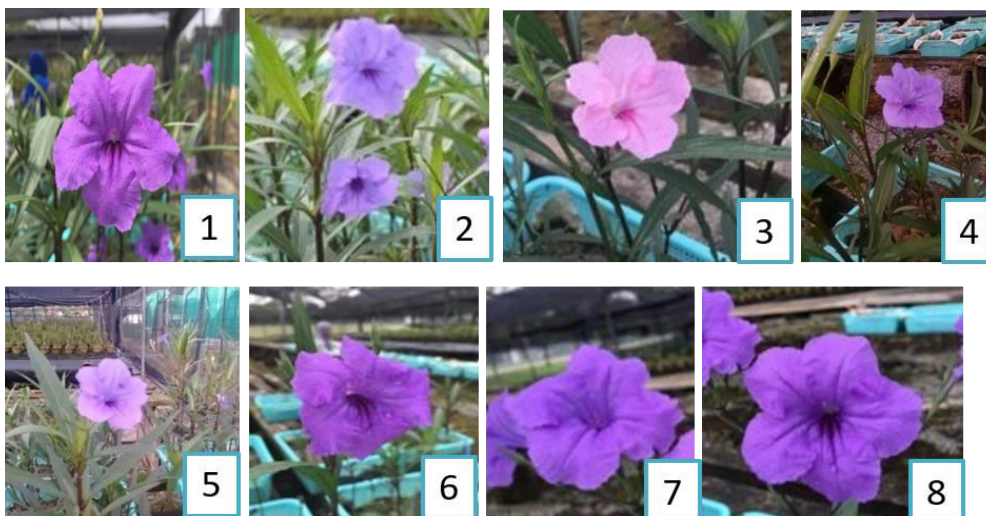

Figure 4: Effects of gamma irradiation on flower color of *Ruellia brittoniana* var. Purple at different dose of treatment: (1) 0 Gy- control, (2) 10 Gy, (3) 20 Gy, (4) 30 Gy, (5) 40 Gy, (6) 50 Gy, (7) 60 Gy, (8) 70 Gy.

Conclusions

The use of gamma irradiation has been shown to affect the growth and flowering of *R. brittoniana* var. Purple. Plant height, new leaf emergence and number of flowers decreased as the gamma dose increased from 0 to 70 Gy. Unfortunately, lethal dose (LD_{50}) values cannot be measured by using a simple linear regression (Excell) because all treated cuttings survived and were flowering. However, various effects of induced mutations on *R. brittoniana* var. Purple have also been demonstrated. Studies have shown that gamma irradiation affected the plant height, leaf and flower number, as well as modifications in plant morphology and flower color. This was a preliminary study and the results obtained will be used for further research to develop a new variety with improved commercial properties suitable for Malaysian landscape.

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Selection of Superior Genotypes of *Baeckea frutescens* with High Xanthine Oxidase Inhibitor Activity for Production of High Quality Planting Materials

Farah Fazwa, M.A.[1,*], Fadhilah, Z.[2], Mohd Zaki, A.[1], Fadzureena, J.[3], Siti Nur Aisyah, M.H.[3], Norhayati, S.[1], Syafiqah Nabilah, S.B.[1] and Masitah, M.T.[1]
[1]Forestry Biotechnology Division, [2]Innovation and Commercialization Division, [3]Natural Products Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: farah@frim.gov.my*

Introduction

Baeckea frutescens L. is a coastal shrub belongs to the family Myrtaceae. This plant is mostly present throughout Southern China, Australia, and South East Asia including Indonesia, Malaysia, and Vietnam. The leaves have been used to treat fever, headache, snake bite, and rheumatism. It is claimed to possess anti-bacterial, anti-dysentery, anti-pyretic, and diuretic activities (Group HMR, 2002). In Peninsular Malaysia, *B. frutescens* is found on mountain tops, quartz ridge, and sandy coast of the eastern parts (Kochummen, 1978; Wong et al., 2010). The leaves release an aromatic fragrance when crushed, the flowers are minute, solitary or in pairs with white petals (Kochummen, 1978). In Malaysia and Indonesia, *B. frutescens* is one of the ingredients utilized in traditional medicines during confinement and used in massaging postpartum women for the treatment of body aches and numbness of the limbs. An investigation by Fujimoto et al. (1996) reported that one of the ethanolic leaf extract isolates of *B. frutescens* exhibited strong cytotoxic activity against L1210 leukemia cells. The phytochemical study conducted by Makino and Fujimoto (1999) reported the three flavanones isolated from *B. frutescens* leaf extract also showed promising cytotoxicity against L1210 leukemia cells. A later report by Hwang et al. (2004) indicated anti-cariogenic, anti-malarial, and anti-babesial activities of *B. frutescens*.

The Forest Research Institute Malaysia (FRIM) has reported that methanol extracts of *B. frutescens* inhibited xanthine oxidase (XO) enzyme activity (Fadzureena et al., 2013). XO is an enzyme catalyzing the formation of uric acid from xanthine and hypoxanthine which in turn is produced from purine catabolism (Maiuolo et al., 2016). Therefore, any defect in the purine degradation pathway may result in an increase of uric acid level which recognized as the single most important risk factor for the development of gout (Fravel et al., 2014). At present, XO inhibitors are widely used for treating gout and hyperuricemia such as allopurinol, febuxostat and et cetera. However, most of the drugs were associated with adverse effects (Tsuruta et al., 2014; Rachiq et al., 2020). Later, phytochemical work has conducted and led to the isolation of new naturally occurring compound which is coded as BF6322, from the flavonoid group. This compound inhibited the activity of XO enzyme with IC_{50} value of 3.584 µg/mL. Besides that, BF6322 also showed less toxic against two normal cell lines which are liver and kidney with IC_{50} more than 20 µg/mL ($\geq$20 µg/mL) in *in vivo* cytotoxicity test (Patent filing PI 2014000187).

Looking at the benefits in pharmaceutical aspects of the species, an initiative has been taken by the plant breeder in FRIM to screen 84 genotypes of *B. frutescens* collected from three provenances in Peninsular Malaysia. The main attempt is to select high yielding genotypes of the species which contain high XO inhibitor activity for future production of high quality planting materials. The production of high quality planting materials of the species was studied as no work has done earlier. The selected superior genotypes may provide the plant breeder to use these basic planting materials to initiate a breeding programme for the species. Through selection, plants from different origins can be improved to develop new clone or varieties. The output derived from this study will support the herbal industries in getting quality raw materials and give added values for the production of pharmaceutical products in the market. To our knowledge, most of the raw materials used in producing herbal products and its development were being sourced from the wild, with little knowledge on the quality

of the raw materials were known. It is targeted that in the future, commercial plantation plot consisted of these high yielding planting materials will be established and may benefit the industries in commercializing the species.

Materials and Methods

Collection of Baeckea frutescens genotypes

A total of 84 genotypes of *B. frutescens* were identified from three provenances in Peninsular Malaysia such as i) Gunung Pulut, Perak, ii) Setiu, Terengganu, and iii) Sungai Baging, Pahang. A few phenotypically superior trees showing good growth, full of branches, superior height, and bole diameter were selected for the study. Only leaves and secondary branches were cut from the mother tree and packed into the plastics bags (Figure 1). All samples were labelled with different codes such as AA1 to AA30 (Gunung Pulut, Perak), TCA1 to TCA30 (Setiu, Terengganu), and CA1 to CA30 (Sungai Baging, Pahang) for easier identification. The topographic information such as coordinates, altitudes, and dates of assessment were also recorded.

Figure 1: Collection and preparation of *Baeckea frutescens* samples at field.

Extraction of Baeckea frutescens genotypes

Extraction was done using solvent extraction method, whereby fresh *B. frutescens* samples were cut into small pieces and dried in ventilated oven for three days before grounded into fine powder form. About 30 g of ground powder were soaked in a mixture of solvents at 1:10 ratio for a few hours at a set of temperature. The extract was filtered with suitable filtering method to remove unwanted fragments in the extracts. The excess of solvent was removed using rotary evaporator. The dark green crude extract was stored in a freezer at -20°C for further analysis. The average yield of *B. frutescens* crude extract was about 4%.

Screening of in vitro xanthine oxidase inhibitory assay

Method of *in vitro* XO inhibitory analysis was adapted from Noro et al. (1983) with a few modifications. The *B. frutescens* extract was dissolved with dimethyl sulfoxide (DMSO) at 20 mg/mL as extract solution. The potassium phosphate monobasic (KH_2PO_4) buffer solution, pH 7.5 was used as the main buffer in the system. The XO enzyme solution was freshly prepared. The 96-well microplate was pipetted in with main buffer solution, extract solution and XO enzyme before incubated for 15 min at 25°C. After incubation period, the reaction of enzyme was induced with addition of substrate solution. The microplate was then incubated again for 10 min before analysis. The production of uric acid was measured using spectrophotometer at 295 nm. The allopurinol is a common drug used to treat gout patient. Therefore, allopurinol was selected as positive control in this assay system.

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Results and Discussion

Screening of superior genotypes of Baeckea frutescens from three provenances

A total of 84 extracts of *B. frutescens* genotypes were assayed for XO inhibition. The results from all provenances show that all samples achieved the range of 40 to 80% inhibitory activity on XO at IC_{50} of 3.584 µg/mL using 70% methanol extract. The values are expressed as mean inhibition (%) ± S.E.M. of triplicate measurements from three independent experiments. Samples with more than 70% inhibitory activity were considered as high, whereas samples with 40-69% are moderate, and 0-39% are low. Population Setiu, Terengganu recorded the lowest value of inhibitory activity of 44.42%±0.67 from genotype TCA18 and the highest value of 78.12%±1.68 from genotype TCA8. It was observed that seven genotypes gave the inhibitory activity of more than 70% (Table 1). Provenance Setiu, Terengganu is located on the sandy coastal area of eastern part of Peninsular Malaysia where many *B. frutescens* plants can be found here (Ahmad and Holdsworth, 1995). The plants grown abundantly on infertile bris soil. Results indicated that many genotypes from this provenance gave higher value of inhibitory activity on XO may be due to its natural habitat that gave the best growth.

Table 1: Inhibitory activity on xanthine oxidase of *Baeckea frutescens* genotypes from Setiu, Terengganu.

No.	Genotype code number	Xanthine oxidase (%)±SEM[a]	No.	Genotype code number	Xanthine oxidase (%)±SEM[a]
1	TCA 1	66.28±0.62	15	TCA 15	74.14±5.11
2	TCA 2	63.37±1.27	16	TCA 16	57.67±3.66
3	TCA 3	68.69±3.08	17	TCA 17	61.41±0.67
4	TCA 4	64.69±0.78	18	TCA 18	44.22±0.67
5	TCA 5	64.18±0.84	19	TCA 19	62.27±0.23
6	TCA 6	72.24±3.24	20	TCA 21	61.45±0.01
7	TCA 7	63.80±4.02	21	TCA 17	57.01±0.85
8	TCA 8	78.12±1.68	22	TCA 23	55.21±1.08
9	TCA 9	62.68±0.31	23	TCA 24	56.45±1.04
10	TCA 10	68.54±2.00	24	TCA 26	57.32±0.92
11	TCA 11	70.12±2.02	25	TCA 27	57.65±4.64
12	TAC 12	73.39±2.98	26	TCA 28	58.39±0.06
13	TCA 13	71.10±0.93	27	TCA 29	63.22±0.88
14	TCA 14	72.00±1.95	28	TCA 30	61.80±0.29

[a]*Values are expressed as mean inhibition (%) ± SEM of triplicate measurements from three independent experiments.*

For provenance Sungai Baging, Kuantan, Pahang, it was observed that only one genotype (CCA9) gave the value of more than 70% inhibitory activity on XO. Majority of the genotypes from this area gave the value between 40-69% of which were categorized as moderate (Table 2). This is also a sandy coastal area and the *B. frutescens* plants grown at this area are relatively smaller compared to the provenance Setiu, Terengganu. The other provenances, Gunung Pulut, Grik, Perak is situated at the rocky mountain area. It recorded three genotypes (ACA4, ACA6 and ACA10) which having more than 70% of inhibitory activity on XO. Only two genotypes recorded value below than 50% which is ACA7 and ACA24 (Table 3).

Table 2: Inhibitory activity on xanthine oxidase of *Baeckea frutescens* genotypes from Sungai Baging, Kuantan, Pahang.

No.	Genotype code number	Xanthine oxidase (%)±SEM[a]	No.	Genotype code number	Xanthine oxidase (%)±SEM[a]
1	CCA 1	54.36±1.72	15	CCA 15	61.77±3.80
2	CCA 2	45.28±0.89	16	CCA 16	57.54±1.99
3	CCA 3	50.70±1.64	17	CCA 17	47.94±1.04
4	CCA 4	56.72±0.67	18	CCA 18	52.14±0.67
5	CCA 5	57.54±0.26	19	CCA 19	53.83±1.31
6	CCA 6	48.04±2.22	20	CCA 20	49.69±1.59
7	CCA 7	51.25±0.76	21	CCA 21	53.63±1.90
8	CCA 8	58.61±1.24	22	CCA 22	59.28±0.55
9	CCA 9	71.77±2.22	23	CCA 23	61.29±1.92
10	CCA 10	46.07±2.35	24	CCA 24	66.59±0.70
11	CCA 11	49.32±2.24	25	CCA 25	59.92±1.80
12	CCA 12	43.91±2.29	26	CCA 26	51.83±2.22
13	CCA 13	48.07±2.22	27	CCA 27	54.00±1.32
14	CCA 14	56.14±2.14	28	CCA 28	60.61±1.76

[a]*Values are expressed as mean inhibition (%) ± SEM of triplicate measurements from three independent experiments.*

Table 3: Inhibitory activity on xanthine oxidase of *Baeckea frutescens* genotypes from Gunung Pulut, Grik, Perak.

No.	Genotype code number	Xanthine oxidase (%)±SEM[a]	No.	Genotype code number	Xanthine oxidase (%)±SEM[a]
1	ACA 1	65.17±0.93	15	ACA 17	51.61±4.47
2	ACA 2	66.13±5.09	16	ACA 18	69.26±1.39
3	ACA 3	66.13±5.05	17	ACA 19	67.67±4.73
4	ACA 4	70.39±3.57	18	ACA 20	66.32±2.50
5	ACA 5	69.11±4.88	19	ACA 21	67.20±2.28
6	ACA 6	71.28±1.28	20	ACA 22	62.53±4.59
7	ACA 7	49.90±3.35	21	ACA 23	60.59±4.10
8	ACA 8	65.47±4.57	22	ACA 24	43.18±4.01
9	ACA 9	69.14±0.72	23	ACA 25	61.94±3.02
10	ACA 10	75.79±4.79	24	ACA 26	60.48±3.79
11	ACA 11	54.26±1.36	25	ACA 27	52.44±4.25
12	ACA 12	67.38±4.69	26	ACA 28	65.06±3.50
13	ACA 15	51.28±2.96	27	ACA 29	59.55±4.94
14	ACA 16	51.79±4.29	28	ACA 30	63.20±3.30

[a]*Values are expressed as mean inhibition (%) ± SEM of triplicate measurements from three independent experiments.*

Selection of superior genotypes of Baeckea frutescens

Based on the screening, it was recorded that 11 out of 84 genotypes of *B. frutescens* were selected as superior due to the value of more than 70% inhibitory activity on XO (Table 4). Genotype TCA8 from Setiu, Terengganu showed the highest value (78.12%±1.68) whereas TCA11 gave the lowest value (70.12%±2.02). It was observed that a total of seven genotypes were originated from provenance Setiu, Terengganu and three genotypes from provenance Gunung Pulut, Perak. Only one genotype from provenance Sungai Baging, Kuantan was categorized as superior. Beside *B. frutescens,* there were several herbs are proven to show as anti-gout potential mainly through XO inhibition assay. This assay is regarded as an essential standard for discovering anti-gout potential among medicinal plants (Bakar et al., 2018). It was reported that bitter melon (*Momordica charantia*) showed the highest percentage of XO inhibitory activity (96.5%) at 100 µg/mL using 70% methanol extract (Alsultanee et al., 2014). The highest XO inhibitory activity at 100 µg/mL (90.6%) was also discovered using the extract of aromatic ginger (*Kaempferia galangal*) (Nguyen et al., 2004). Superior genotypes usually referred to good growth characteristics and/or contained high quality active ingredients (Zobel and Talbert, 1984). High quality materials will be the added value to the end products. Therefore, it is

important to screen the genotypes which have better characteristics than the common one. A few investigations on selection of superior genotypes were previously conducted by FRIM on selected species such on *Citrus hystrix* (Farah Fazwa et al., 2005), *Citrus microcarpa* (Farah Fazwa et al., 2007), *Labisia pumila* (Farah Fazwa et al., 2012), and *Chromolaena odorata* (Farah Fazwa et al., 2019).

Table 4: Selection of eleven superior genotypes of *Baeckea frutescens* with high inhibitory activity on xanthine oxidase.

No.	Genotype code	Xanthine Oxidase (%) ± SEM[a]
1	TCA 8	78.12±1.68
2	ACA 10	75.79±4.79
3	TCA 15	74.14±5.11
4	TCA 12	73.39±2.98
5	TCA 6	72.24±3.24
6	TCA 14	72.00±1.95
7	CCA 9	71.77±2.22
8	ACA 6	71.28±1.28
9	TCA 13	71.10±0.93
10	ACA 4	70.39±3.57
11	TCA 11	70.12±2.02

[a]*Values are expressed as mean inhibition (%) ± SEM of triplicate measurements from three independent experiments.*

Conclusion

As a conclusion, the superior genotypes of *B. frutescens* were identified based on its value of inhibitory activity on XO which is more than 70%. It was concluded that provenance Setiu, Terengganu recorded the highest number of superior genotypes compared to Sungai Baging, Kuantan and Gunung Pulut, Perak. These selected genotypes will be further investigated to produce high quality clones in order to establish commercial plantation of the species in the future. The outputs from the study are not only beneficial for plant breeders in the aspect of producing new variety but also to the herbal industries in developing high quality products in the market.

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Effects of Rootstock on the Initial Growth and Development of Young 'Chok Anan' Mango

Abdullah, F.[1,*] and Tengku Maamun, T.A.M.[2]
[1]Director General's Office, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Arau, Lot PT 3747, Jalan Tambun Tulang, 02600 Arau, Perlis, Malaysia.
[2]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: fadhil@mardi.gov.my*

Introduction

In composite fruit trees, architectural structure and branching habit of scions can be modified by the genetic of rootstocks as early as the nursery stage (Seleznyova et al., 2007; Fazio and Robinson, 2008). A growing body of evidence also suggests that the effects of rootstocks mediated vigour control can be measured in the first season of growth in a range of fruit crops. In apple, Seleznyova et al. (2007) reported that scions on the M9 dwarfing rootstocks may grow similarly to scions on vigorous rootstocks in the first year of growth following grafting, but scion dwarfing was evident in the second year following grafting after increased flowering. Other studies reported that the number of sylleptic shoots, their length and number of nodes were reduced when grafted on dwarfing M9 rootstock; often in the first year (Volz et al., 1994; Costes et al., 2001; van Hooijdonk et al., 2010). In other fruit trees such as pear, rootstocks can affect the node neo-formation of primary and secondary shoots of the scion, contributing to the differences in the proportions of annual shoot types (Watson et al., 2012). In composite kiwifruit vines, recent study also demonstrated that the initial shoot architectural structure of 'Hayward' scions can be modified by the use of inter-specific hybrid kiwifruit rootstocks, and the rootstock effects can be detected in first- and second-growing year following grafting (Abdullah et al., 2015). Therefore, it can be concluded that the first and second year of growth following grafting are critical when the modifications of scions by the rootstock is first expressed.

Grafting potential scion onto seedling rootstocks are common practices in propagating local mango cultivar such as 'Chok Anan' and 'Harumanis' (Tengku Maamun et al., 1996). To date, no standard clonal rootstocks were used in Malaysia and nursery practices is still rely on the seedling rootstocks (i.e. 'Telor' cultivar) for vegetative propagation (Tengku Maamun et al., 1996; Wahab et al., 2002; Abdullah et al., 2008). Until today, the initial modifications of scions growth by the mango rootstocks are not well understood. The biological processes particularly underlying physiological mechanisms of rootstock effects on scion architecture of mango are crucial to identify the precise stage of tree phenology when this occurs. Even though, a number of publications have clearly demonstrated the effect of rootstock on scion performance in mango (Avilán et al., 1996; Smith et al., 1996; 2003; Tengku Maamun, 2000; Ahmad Tarmizi et al., 2005), no detail studies have described the modification of scion architecture by the rootstocks during the initial stage of mango tree growth. Therefore, a replicated trial was undertaken to identify the effects of different type of mango seedling rootstocks cv. 'Telor' on the initial scion growth of 'Chok Anan' mango.

Materials and Methods

This study was conducted in year 2009 at Malaysian Agricultural Research and Development Institute (MARDI) Research Station, Sintok, Kedah. Two different types of seedlings from mango seed cv. 'Telor' were identified during the segregation processes. The first type of seedling was the seeds that only produced one single emerged plantlet (Type A), and secondly the seeds produced many plantlets (Type B) that can be up to five plantlets per seed (Figure 1). The seedlings were transplanted into 5 L polyethylene bags containing growing medium (top soil, sand and organic matter in 3:2:1 ratio) (Tengku Maamun et al., 1996).

Figure 1: Morphological characteristics of mango seedling rootstock cv. 'Telor' at full germination stage. Noted here, the seeds that produced only one single emerged plantlet (Type A) and the seeds that produced multiple plantlets (Type B).

Uniform healthy mango (*Mangifera indica* L.) cv. 'Chok Anan' budwoods with approximately 150 mm in length and 10-15 mm in diameter were collected during pruning season (in mid June 2009). The budwoods were veneer grafted at a height of 200 mm onto these two different types of 'Telor' rootstocks (Figure 1). At this time, the stem thickness of rootstocks was approximately 20 mm (rootstock age approximately 1-year-old). Grafted trees were planted into 35 L polyethylene bags containing growing medium in similar ratio. After a few weeks of establishment, the composite mango trees were placed 50 cm apart in rows at nursery and supported with the irrigation system consisted of 20 mm poly-tubing line attached with drippers (3 Lh^{-3} dripper per tree).

Measurement of shoot growth was conducted at the end of first growing season considering the first year of growth. Tree height (cm) was measured from the soil level in the polyethylene bags until the end of apex of primary shoots. The length of primary shoots (cm) was measured using a ruler from the shoot base until the tip of primary shoots. Stem diameter of rootstocks and scions (mm) were measured at 15 cm above the soil level and 10 cm from graft-union, respectively using a digital calliper (Mitotuyo, Japan) and converted to stem cross-sectional area (SCA). The total number of shoots including primary shoots was manually counted. The branching height was measured from the soil level in the polyethylene bags until the lowest branch of the trees. The width of canopy (cm) (North to South and East to West) and canopy height were measured using a measuring tape. Canopy volume (m^3) was calculated by multiplying the width and height of canopies. Grafted trees were placed in the nursery in a randomised complete block design with ten (10) single replicated trees arranged within four (4) blocks.

SAS application system was used to analyse and check the normality of the data. The differences between mean values were compared using T-Test at P=0.05.

Results and Discussion

Despite many potential impacts of rootstocks on qualitative and quantitative tree growth and yield of mangoes (Galàn Saúco, 2016), rootstock studies have been given less attention and scarcely explored

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in Malaysia. In order to control excessive vegetative growth, mango industry is still highly dependent on the horticultural manipulation such as pruning (Tengku Maamun et al., 1999), as there is still lacking of dwarfing rootstocks that can reduce scion vigour in ways similar to other fruit trees. Even though root restriction technique had shown promising results in controlling vigour of mango trees, the adaptation of this technique for commercial planting is still lacking (Mohammed, 1999).

Table 1: Rootstock effects on the initial tree growth and canopy characteristics of 'Chok Anan' scions.

	'Telor' seedling rootstocks		
	Type 'A'	Type 'B'	*P*-value
Tree growth and vigour			
Tree height (cm)	74.8^{b}	82.4^{a}	*P*=0.01
Branching height (cm)	23.9^{a}	17.4^{b}	*P*=0.01
Length of primary shoots (cm)	12.2^{a}	14.4^{a}	*P*=0.75
Total number of shoots	2.9^{a}	2.8^{a}	*P*=0.17
Stem cross-sectional area (SCA)			
Rootstock (mm^2)	177.6^{a}	195.1^{a}	*P*=0.11
Scion bud wood (mm^2)	37.0^{a}	40.0^{a}	*P*=0.44
Tree canopy characteristics			
Width of canopy (North - South) (cm)	45.1^{a}	35.8^{b}	*P*=0.05
Width of canopy (East - West) (cm)	45.6^{a}	37.2^{a}	*P*=0.07
Canopy volume (cm^3)	37.2^{a}	27.9^{a}	*P*=0.06

Means sharing same letters within a row are not significantly different at Pq0.05 according to T- Test.

In this study, the effect of rootstock on the initial growth and shoot architecture of 'Chok Anan' mango were clearly seen in the first growing season following grafting. The mean tree height of grafted 'Chok Anan' scions was significantly affected (*P*=0.01) by the type of rootstocks. Scion on rootstock 'Type B' produced significantly taller tree than scion on rootstock 'Type A' (Table 1), indicating that the vigour of 'Chok Anan' scion was controlled by rootstocks. Similarly, rootstock also had significant effect (*P*=0.01) on the mean height of scion branching with rootstock 'Type B' produced the lowest scion branching height compared to rootstock 'Type A'. However, no significant differences were recorded on the mean total number (*P*=0.75) and mean length of primary shoots (*P*=0.17) of grafted 'Chok Anan' scions. According to Hirst and Ferree (1995), shoot morphological characteristics may be one way in which rootstock influence productivity by affecting the individual length of shoots, and this can influence tree branching habits (Webster, 1995; Fazio and Robinson, 2008).

The mean SCA of rootstocks and scion budwoods was not significantly different between rootstock types (*P*=0.11 and *P*=0.44, respectively) (Table 1). However, there was a general trend that seedling rootstock 'Type B' may have had larger mean SCA compared with SCA of seedling rootstock 'Type A'. In fruit trees, initial stem calliper in nursery stage also has been thought to be correlated with the tree growth in the orchard (Reighard, 1990); for example in peach (*Prunus persica* L.), there is a correlation between stem calliper of tree in the greenhouse and planted in orchard (Reighard, 1990). While the diameter of the rootstock shank is not a good predictor of plant size and vigour (Fazio and Robinson, 2008), trunk or stem SCA is correlated with tree volume (Khatamian and Hilton, 1977; Webster, 1995) and is often used as a proxy measure for the total growth of a plant over its life. In general, the mean canopy width of grafted 'Chok Anan' scions may have been affected by the type of seedling 'Telor' rootstocks (Table 1). Even though the canopy width of the scions measuring from East to West was not significant, there was a strong trend that the canopy width of grafted 'Chok Anan' may be affected by the type of seedling rootstocks as the *P*-value close to significance (*P*=0.07). Similarly, the initial canopy volume of grafted 'Chok Anan' scions may also be affected by the type of seedling rootstocks (*P*=0.06). These results indicate that the rootstock may have an effect on the initial canopy characteristics of 'Chok Anan' mango during early stage of growth. We strongly

believe that this effect may have had significant influence on the future canopy architecture when planted in the field as demonstrated in many fruit crops (Fazio and Robinson, 2008).

Conclusion

A key finding of this study was that the different type of mango seedling 'Telor' rootstock appeared to modify the initial growth and vigour of young 'Chok Anan' mango at the early stage of growth. This was clearly demonstrated by the significant differences in the initial tree growth and canopy characteristics. Our findings also provide the first evidence that mango rootstock can affect canopy architecture of scion as early as in the first year of growth following grafting. These effects may have contributed to the variation in the performances of mango trees (tree canopy, yield, fruit quality etc.) when planted in orchard. Therefore, this growing evidence opens new research studies on rootstock for other mango cultivars, as well as others tropical fruit crops.

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Flowering and Fruit Development of Harumanis Mango on Different Soil Types in Agroclimatic Zone 1

Muhamad Hafiz, M.H.[1,*], Hartinee, A.[1], Nor Dalila, N.D.[1], Ahmad Mahdzir, A.[1], Muhammad Izzat, Z.[1], Shaidatul Azdawiyah, A.T.[2] and Mohd Aziz, R.[2]
[1]Horticulture Research Centre, MARDI Sintok, Bukit Kayu Hitam, 06050 Kedah, Malaysia.
[2]Biodiversity and Environment Research Centre, MARDI Headquarters, Persiaran MARDI-UPM 43400 Serdang, Selangor, Malaysia.
**E-mail: harfieyz@mardi.gov.my*

Introduction

Nowadays, the demand for Harumanis mango has increased drastically especially during the fruiting season between May to June every year. Usually, production area of Harumanis mango is specific to the northern region of Peninsular Malaysia, namely Perlis and Kedah, where the number of mango farms is seen to have increased with Perlis having 6048.29 hectares of mango (DOA, 2017). It has been reported by MARDI where agroclimatic zone plays an important role to promote the flowering and fruiting of Harumanis mango especially in Perlis and Kedah states (Tengku Ab. Malik et al., 1996). Mango is best planted in Agroclimatic Zone 1, an area characterized by 1-2 months dry period, with the temperature range between 7-10°C (Muhamad Hafiz et al., 2019). Northern region of Peninsular Malaysia as Perlis and Kedah states were located in this zoning area and received the drought season every year within 2 months. There are various factors that contribute for flower initiation and fruit development where agronomic practices, including pruning procedure, KNO^3 application, flower induction, pest and disease management play an important role in flowering and fruiting of mango (Davenport, 2003).

Generally, mango can be planted in any type of soil and able to grow vigorously when the vegetative stages take place. The reproductive stage has started when flowering of mango begins as the plant was exposed with drought period around 25-35 days (Muhamad Hafiz et al., 2019). Response to flowering may also be influenced by soil type which is related to the physical properties of the soil. Different soil physical properties may have different soil moisture characteristics which lead to a certain optimum level of drought stress or wilting point. It is similar to a condition where the reduction or low irrigation level at 0% or 25% evapotranspiration rate at flowering stage was found to be suitable for floral induction of Tommy Atkins mangoes (Leandro et al., 2016). Moreover, soil chemical properties also play an important role in the fruit development of mango. Basically, the production of Harumanis mango in Perlis and Kedah was performed in mineral soil consisted with clay particle as the main component. Thus, the leaching of nutrients is low on this type of soil. Ensuring proper range of soil pH and percentage of soil organic amendments may result in optimum fertility of the soil for fruit development of mango. Fertilizer application schedule on highest range of soil pH or calcareous soils may improve the nutrient use efficiency of the plant at each growth phase which ultimately provides good fruit production of mango (Raheel et al., 2011). Furthermore, with the increase of organic carbon content by the breakdown process of organic matter in soil may increase the soil pH and microbial activity which eventually increase nutrient availability and significantly enhance the flowering, fruiting, yield and quality of mango (Yashpal et al., 2017). Thus, this study was conducted to determine the effect of different soil types on the flowering performance and fruit development of Harumanis mango in the same agroclimatic zone.

Materials and Methods

An experiment was conducted at 3 different locations of Harumanis mango orchards in localities with different soil types in mineral soil category. All the locations selected are situated in the same Agroclimate Zone 1. Thus, the climatic condition of the locations is considered similar for the requirement for flowering and fruiting of mango. The soil types used were as clay soil in Arau, Perlis,

clay soil in MARDI Sintok, Kedah and sandy soil in Tanjung Sedili, Johore as shown in Figures 1A, 1B and 1C.

Figure 1: The types of soil at different locations as clay soil in Arau, Perlis (1A), clay soil in MARDI Sintok, Kedah (1B) and sandy soil in Tanjung Sedili, Johore (1C).

All farms were treated similarly adopting the agronomic practices of mango based on MARDI recommendations. The agronomic practices consisted of fertilization, pruning activity, flower induction and pest and disease management. Flower induction was done with the use of plant growth regulator, Paclobutrazol (PBZ) on the third week of October. The application was done by drenching at a distance of 30 cm from the main stem of the trees. A total of 30 trees with similar growth, age and size were chosen at each Harumanis mango orchard and were evaluated during flowering and fruiting stages. The data collected were percentage (%) of inflorescence trees, inflorescence shoot ratio and fruit to flower ratio. The number of fruits to flower ratio was counted after 8 weeks of fruit setting. Two types of soil analysis: physical and chemical characteristics were conducted, including pH and percentage (%) of organic carbon. For soil physical properties assessment, percentage (%) of clay, silt and coarse and fine sand were collected. Soil moisture content was also measured for all soil types. In terms of climatic environment data, total rainfall (mm) and maximum and minimum temperature (^{o}C) were recorded during 3-months period from December 2017 to February 2018, which included the flowering and fruiting stages.

Results and Discussion

Flowering stage

Flowering performance among locations differed (Figure 2). The flowering of Harumanis mango at Arau, Perlis started earlier in the fourth week of December 2017. It was followed by MARDI Sintok, Kedah and Tanjung Sedili, Johore with the same timing of flowering initiation at third week in February 2018. The flowering stage was initiated when the trees were exposed with a dry period of low rainfall distribution as 49.7 mm in Arau, Perlis, 22.4 mm in MARDI Sintok, Kedah and 208.3 mm in Tanjung Sedili, Johore. The range between maximum and minimum temperature were also observed during the flowering stage where the range measured at 10°C (Arau, Perlis), 12.6^{o}C (MARDI Sintok, Kedah) and 6.8^{o}C (Tanjung Sedili, Johore). Table 1 shows the reproductive measurements of percentage of inflorescence trees and inflorescence shoot ratio between locations. There is no significant difference among the locations for these parameters. Clay soil type represented in Arau, Perlis and MARDI Sintok, Kedah received rainfall amounts of 50 mm during the flowering period. It was also exposed with the range of maximum and minimum temperature around 12°C. In contrast, Tanjung Sedili, Johore with sandy soil received higher rainfall of 208.3 mm and lower range of temperature at 6.2^{o}C compared with the clay soil from the other two locations. However, the response of flowering with the parameters observed as percentage of inflorescence trees and inflorescence shoot ratio is still similar and not significant compared to the other locations. It was also seen that the mango orchard in Tanjung Sedili received higher rainfall distributions than the other 2 locations. This is due

to the fact that all of the mango orchards observed were situated in the same agroclimatic zone with a yearly occurrence of drought season that promotes flowering of mango plants. Low rainfall distributions and humidity is required by the mango trees for flowering (Sandip et al., 2016). Floral induction of mango trees is undeniably affected by cool temperatures which the range of temperature will be high (Normand et al., 2015). This condition is also similar with the finding where flowering of mango occurred for ‘Haden’ mango after 12 days being exposed with the maximum and minimum temperatures as 31°C/ 25°C (Shu, 1999).

Fruiting stage

Based on Table 1, the number of fruit to flower ratio reflected at the fruiting stage. In Tanjung Sedili, Johore the soil content of sandy soil was recorded significantly lowest compare to other locations. During flowering phase in third week of February 2018, there was high rainfall distribution recorded at 208.3 mm (Figure 2A) and significant value were recorded by relative humidity of 82.62% (Figure 2B). Unfavorable moisten condition may affect the flowering process at floral initiation and blooming. A prerequisite for successful mango production is the absence of rain during the flowering period. Moist and humid atmosphere washes pollen, disturbs the pollinators’ activity and encourages insect pests and diseases. It has been observed that blossom blight is a critical disease that interferes at this stage. High precipitations, heavy dew or foggy weather during the flower blooming season can stimulate tree growth but interfere with flower production and encourage diseases of the inflorescence (Rajan, 2012). Eventhough, the yield production of Harumanis in Tanjung Sedili, Johore was reduced due to this condition, there was still a produce of fruits. The fruits were harvested for grading and further studied was carried out with focusing on yield and quality verification.

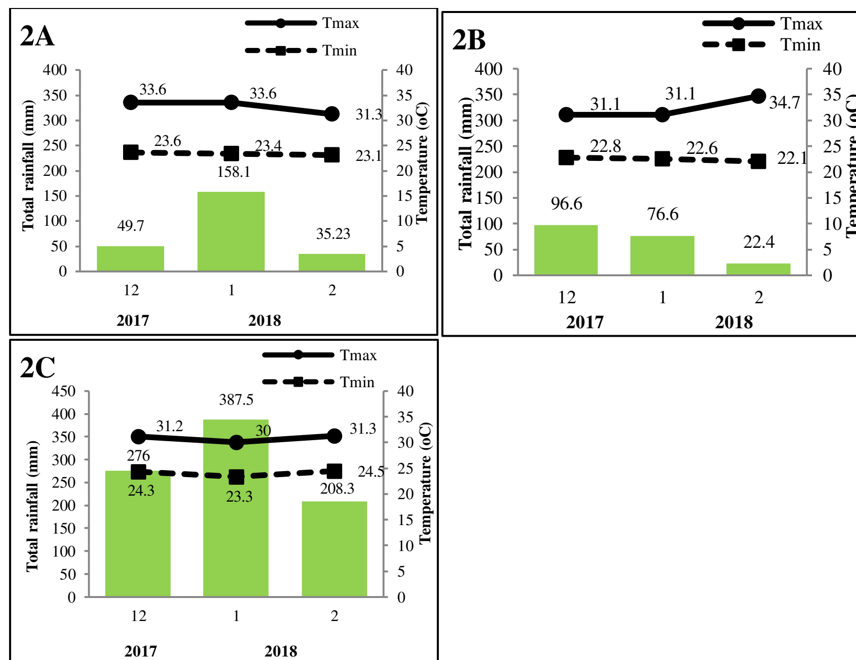

Figure 2: Total rainfall (mm), maximum and minimum temperature (°C) at 3 locations as Arau. Perlis (2A), MARDI Sintok, Kedah (2B) and Tanjung Sedili, Johore (2C) from December 2017 until February 2018.

Table 1: Flowering and fruit development of Harumanis mango from different locations and soil types.

Locations and soil types	Percentage (%) of trees with inflorescence	Inflorescence:shoot ratio	Fruit to flower ratio
Arau, Perlis (Clay soil)	93.3[a]	0.86[a]	0.47[a]
MARDI Sintok, Kedah (Clay soil)	90.0[a]	0.79[a]	0.39[a]
Tanjung Sedili, Johor (Sandy soil)	90.0[a]	0.81[a]	0.25[b]

Means with the different number in the same column is significantly difference at p≤0.05 according to DMRT.

The influence of soil physical properties to the flowering performances

Harumanis trees in Tanjung Sedili, Johore responded positively to flowering even though it received the highest rainfall distribution. This situation is believed to be influence by the characters of the soil physical properties. Soil physical properties were referred to the proportion of the percentage (%) of clay, silt, coarse and find sand. Based on the table 2, the location in Tanjung Sedili, Johore comprised of sandy soil, indicated the highest proportions of coarse sand particle (55.2%) among the other locations. Moreover, this type of soil shows the lowest and significantly different in terms of clay (9.2%) and silt (2.4%) content among the other locations. While, there is no trend amongst the location on the percentage of fine sand. From table 2, soil moisture content measured indicated that, the sandy soil in Tanjung Sedili, Johore (25.63%) was the highest and significantly difference compared to other location and soil types. According to this data, sandy soil has the ability to hold water at 25.63%. From here, there is a relation between the the level of water holding capacity with drought stress or wilting point of the plant. With high temperature exposure and decreasing level of rainfall, the soil continues to dry below than 25.63% of moisture level and enters the level of wilting point. The use of clay soil types in the other 2 locations indicated that, the Harumanis trees will only response for flowering if the soil dries below the range of moisture content or wilting point identified at 19.09%-19.38%. We could see from here, the sandy soil in Tanjung Sedili, Johore has an advantage where it is more conducive to receive the drought stress earlier than clay soil if the climatic condition is available. Sandy soil in Tanjung Sedili is considered to have poor water holding capacity due to the bigger particle of soil proportions. Then, the water may quickly run off or evaporated after raining with the exposure of high range of temperature. Thus, drought stress will be accumulated in the soil which later then enhances the trees for flowering. Flowering of mango was found dependent on drought stress, which took place earlier than unstressed trees. In tropical conditions flowering occurs after a period of drought (Tahir et al., 2003). Drought stress advanced floral bud break by nearly two weeks in about 40% buds of mango. It stimulated growth of floral buds and delayed the development of vegetative buds (Whiley, 1986).

Table 2: Soil physical properties as percentage (%) of clay, silt, coarse sand, fine sand and soil moisture content at different locations and soil types of Harumanis mango.

Locations and soil types	Physical properties (%)				
	Clay (%)	Silt (%)	Coarse Sand (%)	Fine Sand (%)	Soil moisture content (%)
Arau, Perlis (Clay soil)	30.4[a]	11.6[a]	21.4[b]	36.6[a]	19.09[b]
MARDI Sintok, Kedah (Clay soil)	16.0[b]	9.6[a]	33.8[b]	40.6[a]	19.38[b]
Tanjung Sedili, Johore (Sandy soil)	9.2[b]	2.4[b]	55.2[a]	33.2[a]	25.63[a]

Means with the different number in the same month is significantly difference at p≤0.05 according to DMRT.

The influence of soil chemical properties to the flowering and fruiting performances

Data presented in Table 3 shows that Tanjung Sedili, Johore, was significantly highest in terms of pH level (7.52) and percentage of organic carbon (4.78%) compared to other locations. Fruiting stage in Tanjung Sedili, Johore was suspected to be influenced by the significant level of pH and percentage of organic carbon. The optimum level of soil pH for mango was identified in the range of 5.5-7.0 during fruit development. Whereas, the percentage of organic carbon was required around 2-4%. In this case, flower and fruiting regulation of Harumanis mango was increased accordingly with the increase of soil

pH and organic carbon percentage. The level of pH and percentage of organic carbon was increased in sandy soil from Tanjung Sedili, Johore due to the existence of marine lives as the source of organic matter in coastal area. Breakdown process into organic matter that resulted to increase the soil pH and percentage of organic carbon. Thus, this process may increase the soil pH and microbial activity that eventually increases nutrient availability in the soil and significantly enhance the flowering, fruiting, yield and quality of mango (Yashpal et al., 2017). The usage of chicken manure and bokashi to mango cultivar as Tommy Atkin, Manila and Ataulfo was reflected to the high pH level and organic carbon content where it may contribute for flower and fruiting regulation (Antonio et al., 2014).

Table 3: Soil chemical properties as pH and percentage (%) of organic carbon at different locations and soil types of Harumanis mango.

Locations and soil types	Chemical properties	
	pH	Organic carbon (%)
Arau, Perlis (Clay soil)	5.74[b]	0.95[b]
MARDI Sintok, Kedah (Clay soil)	5.60[b]	0.57[b]
Tanjung Sedili, Johore (Sandy soil)	7.52[a]	4.78[a]

Means with the different number in the same month is significantly difference at $p \leq 0.05$ according to DMRT.

Conclusion

To summarize, sandy soil type from Tanjung Sedili, Johore was categorized as one of the most suitable soil types to enhance and facilitate the requirement for flower regulation of Harumanis mango. This is due to the availability of soil physical characteristics as soil proportion and soil moisture content which facilitated to receive drought stress faster and effectively. In terms of soil chemical characteristics, fruit development of Harumanis in sandy soil was supported by the high and appropriate soil pH and organic carbon formation, which the sources was available by the breakdown process of organic matter around the coastal area.

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Impact of Water Availability on Growth and Relative Water Content of Selected Open Pollinated Durian Rootstocks

Ahmad Hafiz, B.[1,*], Muhammad Najib, O.G.[2], Zul Helmey, M.S.[1] and Mohd Asrul, S.[3]
[1]Malaysian Agricultural Research and Development Institute, Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia.
[2]Malaysian Agricultural Research and Development Institute, MARDI Headquarters, Persiaran MARDI-UPM 43400 Serdang, Selangor, Malaysia.
[3]Malaysian Agricultural Research and Development Institute, Alor Setar, Jalan Alor Setar-Kuala Kedah, Malaysia.
**E-mail: ahafiz80@mardi.gov.my*

Introduction

Durian (*Durio zibethinus* Murr) is the leading fruit crop industry in Malaysia. Being the 'King of Fruit' in most countries in South East Asia, durian has become an important economic crop. The durian has various creamy pulp taste and texture, distinctive smell, high nutritional value and received widespread acceptance for fresh consumption and processed products, thus becoming one of the selected premium fruits in the Malaysian National Agro-food Policy (2011-2020). In 2017, durian was the largest planted fruit crop in Malaysia with a total of 73,000 hectares and an average annual production of 350,000 MT (Suhana et al., 2018).

This important fruit crop, however, is very sensitive to water availability, particularly in the stage of transplanting, and also throughout the vegetative and after flowering stage, where some orchards received prominent drought season in the area (Razi et al., 1994; Masri, 1999). In order to overcome this problem, most orchards and research are focused on improving the irrigation system (Jaafar, 1998). Apart from this approach, selection of rootstocks or varieties that are tolerant to drought or water deficit is also important (Reddy, 2013; Gessese, 2018).

Relative water content (RWC) is one of the important characters in determining water stress ability in plant. It indicates the water status in plants and reflects the balance between water supply to the leaf tissue and the transpiration rate (Dorota et al., 2015). Plants that are drought tolerant exhibit the capacity to maintain high RWC under water deficit and it also explain their capacity to accumulate great quantities of proline and other osmotic active compounds that are crucial in osmotic adjustment and also in reduction of osmotic water potential (Zlatev and Fernando, 2005).

The experiment focused on assessing the growth parameters and water relation performance of two open pollinated species of Durian viz. durian 'D99' (*D. zibethinus*) and durian 'Sempuh Api' (*D. lowianus*), a wild durian type under different water regimes. These varieties were selected due to their higher rate of self-compatibility and are listed among selected variety for durian rootstocks (Shamsudin et al., 2000). A previous study demonstrated their performance as grafted plants (Razi et al., 1994; Masri, 1999), while this study assessed these variety performances as seedlings.

Materials and Methods

Plant materials and growth conditions

The experiment was conducted under a shade house in MARDI Sintok, Bukit Kayu Hitam, Kedah, Malaysia. The mean daily air temperature and relative humidity during the experiment were 34°C and 60%, respectively. The experimental materials were young durian species of *D. zibethinus* (durian D99) and *D. lowianus,* DL, (durian Sempuh Api). Four months old of these durian seedlings, originated from MARDI Kuala Kangsar, Perak were raised in polybags of 20 cm x 30 cm containing soil mixture 3:2:1 (soil: organic manure: sand). Plants were watered accordingly after transplanting

until four months old and standard agronomic practices were carried out. Two levels of water regime were imposed on the plants, i.e. every day and every two days. Seven hundred milliliters of water were given twice a day during the watering session. The plants were grown for 32 days.

Measurements of growth and relative water content (RWC)

Plant height, stem girth, number of leaves, leaf area, fresh and dry weight of roots, stems and leaves were taken at the end of the treatment, after 32 days. SPAD readings were taken at $3^{rd}/4^{th}/5^{th}$ leaf from above using a chlorophyll meter (Konica Minolta SPAD-502). Leaf area readings were taken using a leaf area meter (Li-COR LI-3100C). RWC was determined according to the method of Weatherly (1950) with minor modifications. Ten leaf discs (1 cm in diameter) were floated for 4 hours in deionized water before the turgid weight were recorded. Measurement of the RWC was collected using the formula given below:

$$RWC = (FW - DW) / (TW - DW) \times 100$$

where FW was the fresh weight of sample, DW was the dry weight of sample and TW was the turgid/soaked weight of sample.

Experimental design and statistical analysis

The experiment was conducted in a split plot design with three replications, 6 plants per replication with watering regime as the main plot and species as the sub-plot. Data collected were analyzed using the analysis of variance (Statistical Analysis System) and the treatment means were compared using Tukey's honestly significant difference (HSD) at 0.05 probability.

Results and Discussion

Effect of water regime on growth parameters and relative water content of durian seedlings

The effect of the species and water regime were evaluated by analyzing the data obtained after 32 days of treatment (Table 1). Main factor species significantly affected plant height, stem fresh weight, relative water content ($P<0.01$), stem dry weight and root dry weight ($P<0.05$). However, effect of species will not be discussed here since the species are innately different even without watering regime treatments.

For main factor watering regime, only RWC and root dry weight were significantly affected ($P<0.05$) while the rest of parameters shown were not significantly affected by watering regimes. Significant interactions ($P<0.05$) between the main factors were recorded only for RWC while the rest showed no significant interactions (Table 1).

Regardless of the species, watering regime every two days significantly reduced root dry weight by 8.60%, compared to daily watering. Growth mechanism in plants involved several critical processes such as cell division, cell enlargement and differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. These processes are dependable on water deficit. Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure. Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells, resulting in the reduction of plant tissue growth (Farooq et al., 2009).

Table 1: Main and interaction effects of durian species and watering regime on growth, RWC and photosynthetic efficiency from two durian species.

Factor	Stem diameter (mm)	Plant height (cm)	Leaf no	SPAD	Leaf area	Leaf FW (g)	Stem FW (g)	Root FW (g)	RWC (%)	Leaf DW (g)	Stem DW (g)	Root DW (g)
Species												
D99	12.51[a]	67.78[b]	36.23[a]	26.27[a]	1010.8[a]	24.73[a]	31.78[b]	31.45[a]	65.67[b]	13.11[a]	15.90[b]	15.79[a]
DL	13.76[a]	92.51[a]	36.06[a]	27.22[a]	1274.5[a]	30.19[a]	44.12[a]	27.98[a]	84.33[a]	14.97[a]	19.85[a]	14.31[b]
Watering												
Everyday	78.63[a]	78.63[a]	34.75[a]	28.91[a]	1207.7[a]	28.56[a]	37.15[a]	30.80[a]	71.31[a]	14.36[a]	17.79[a]	15.60[a]
Every 2 days	84.44[a]	84.44[a]	37.80[a]	24.24[a]	1091.0[a]	26.68[a]	40.14[a]	28.07[a]	81.29[a]	13.84[a]	18.37[a]	14.25[b]
Species	ns	**	ns	ns	ns	ns	**	ns	**	ns	*	*
Watering	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	*
Species*Watering	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns

*Note:**Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean ± S.E; n=3). D99= Durian D99, DL= Durio lowianus.*

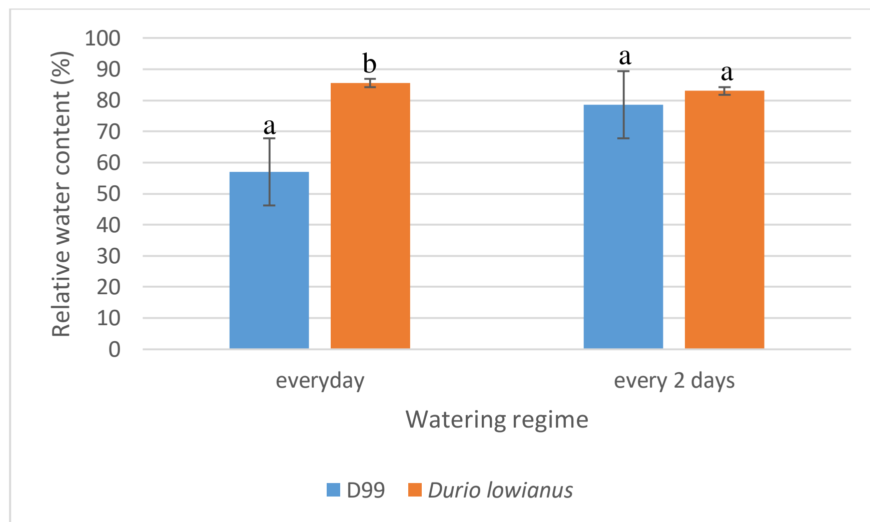

Figure 1: Relative water content (%) of 2 durian species for different watering regimes. Means in each watering regime with the different letters indicate significant differences at P≤0.05% level according to Tukey's HSD. (Mean±S.E; n=3).

Significant interactions between the main effects for RWC indicated that RWC between the species is highly dependable on the water status of the species. Although DL showed higher RWC compared to D99 in every two days of water regime, the values between the two species were not statistically different. For D99 however, there was a significant improvement of RWC in the mild water deficit treatment (Figure 1) compared to daily water regime. The variation in RWC capacity can be influenced by genetic factors of the species. Dorota et al. (2015) and Gessese (2018) reported variation of RWC capacity from 18 cultivars of "Katahdin" potatoes and Arabica coffee under different water deficit treatments. Mohd Asrul (2019) reported different RWC capacity in 'Harumanis' mango variants. Also, some plants exhibit improvement in growth and physiological parameter under mild water deficit due to oxygen and nutrient deficiency caused by leaching under excess watering regime (Luvaha, 2008). This can be concluded that, this two species exhibit equal performance in terms of this criteria.

Conclusion

Tolerance level among the durian seedlings is different according to the species. Based on the experiment, root dry weight and relative water content were significantly different according to the water regimes. In terms of the performance, both durian species (D99 and DL) exhibit individual special characteristic in RWC values. These particular candidates can be further tested for other criteria such as disease tolerant and other physiological parameter and rootstock-scion evaluation in order to develop high quality durian rootstocks.

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Effects of Different Fertilizers on the Growth Performance of *Endospermum diadenum* (Sesenduk) Tissue Culture Plantlets in Nursery Condition: A Preliminary Study

Mohd Saifuldullah, A.W.[1,*], Nor Hasnida, H.[1], Nazirah, A.[1], Muhd Fuad, Y.[1], Rozidah, K.[1], Sabariah, R.[1], Naemah, H.[1], Rukiah, M.[1] and Muhammad Afiqfuddin Shah, Z.[2]
[1]Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
[2]Kolej Vokasional (Pertanian) Teluk Intan, Jalan Changkat Jong, 36000 Teluk Intan, Perak, Malaysia.
**E-mail: saifuldullah@frim.gov.my*

Introduction

Endospermum diadenum known as sesenduk is belongs to Euphorbiaceae family. It is a fast growing hardwood species among timbers (Darus et al., 1990). Even though *E. diadenum* is a hardwood tree, the wood is light, coarse and floatable, and can be used for planks and clogs, match boxes, drawing boards and blackboards (Desh et al., 1940). *Endospermum diadenum* is also known as a mid-canopy tree that can grow up to 36 m tall with straight bole up to 25 m. The diameter of the tree can reach 13 to 60 cm. According to Chew (1980), sesenduk grows rapidly in open planting areas and suitable for afforestation program in Peninsular Malaysia. Planting materials of sesenduk is needed to cater the demand for forest plantation industry (Khairul Azwa, 2015).

The usage of fertilizer is to enhance the growth of the plants but intensive application of fertilizers can lead to underground water pollution, destroying microorganisms, and making plants more susceptible to pests and diseases, as well as reducing soil fertility (Abdelaziz et al., 2007). This study was carried out to determine the best fertilizers that would give better growth performance of *E. diadenum* tissue culture plantlets in nursery condition.

Materials and Methods

The experiment was conducted in the tissue culture nursery, Forest Research Institute Malaysia (FRIM), Kepong, Selangor. Eight months old of *E. diadenum* plantlets from the tissue culture laboratory were brought to the nursery for acclimatization process. The plantlets were planted onto jiffy-7 containing 60% of peat moss and 40% of coco peat. Those plantlets were acclimatized in an acclimatization chamber for four weeks prior to planting in polybags filled with a mixture of burned soil and peat moss in 1:1 ratio.

There were five treatments of fertilizer with six plantlets used in the experiment. The plantlets were placed under 50% of shade and arranged on a concrete bench (Figure 1). The treatments were control (T1), 0.2 g NPK 15:15:15 (T2), 0.3 g goat manure (T3), 0.2 g Complehumus 8:8:8+3MgO+10%HA+0.5%B+TE (T4), and 0.4 g teak leaf compost (T5). Fertilizers were applied once every two weeks, whereas watering was carried out three times a day using a sprinkler (10 min per watering).

Growth performances of the plantlets namely plant height (mm) and stem girth (mm) were measured weekly. Plant height was measured from the soil surface in the polybag until the terminal shoot using a ruler, while a caliper was used to record the stem girth at 2 cm height which has been marked on the stem at the start of the study. The growth data were collected until week 8.

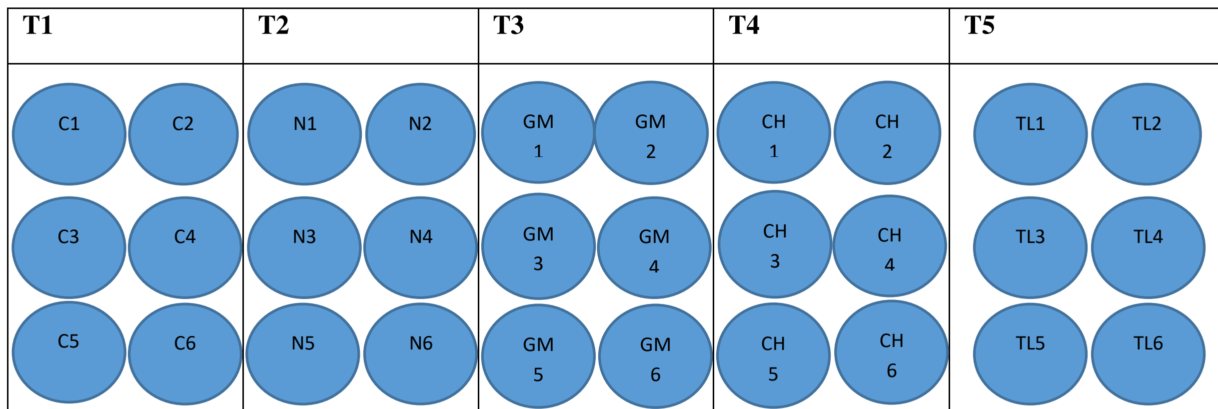


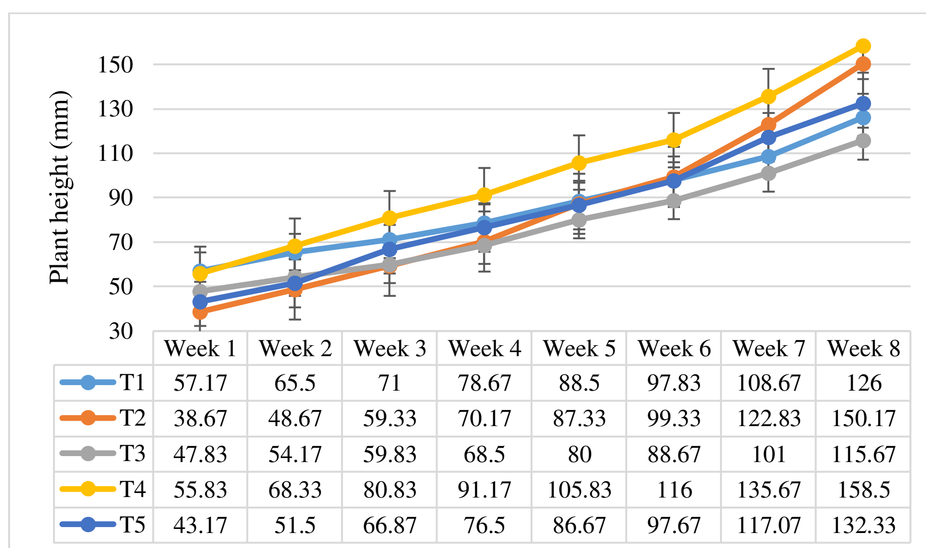
Figure 1: The arrangement of *E. diadenum* plantlets in nursery.

Statistical analysis

Data were subjected to Analysis of Variance (ANOVA) using SPSS Statistical Software and Tukey's post-hoc test was used to determine significant differences between treatments ($p<0.05$).

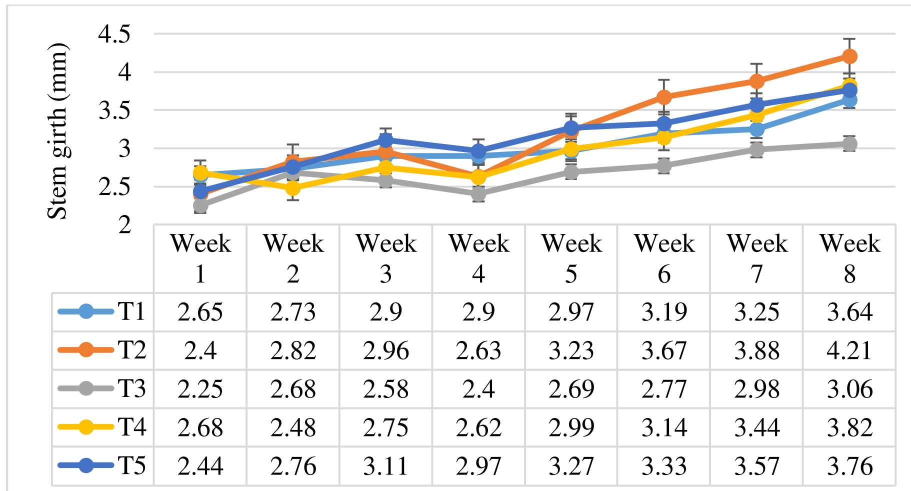
Results and Discussion

ANOVA showed no significant differences on plant height and stem girth among fertilizers throughout the study period. However, the plant height was found increasing over time (Figure 2). T4 gave a better growth pattern of plant height compared to others throughout the study. As for stem girth, T2 showed the highest growth pattern among those treatments towards the end of the study period (Figure 3). The application of fertilizer is crucial especially at nursery stage. It is because plants were grown in polybags with minimum amount of media. So that, over application of fertilizer into the polybags may cause of death of the plants. The presence of Nitrogen, Phosphorus and Potassium is also crucial for the expansion of plantlets. Inorganic fertilizer used in this study which is Complehumus 8:8:8 (T4) is suitable as it contains complete nutrients for plantlets in nursery stage. NPK fertilizer is commonly used in nursery because it can give faster effects on growth performance as compared to organic fertilizers.



	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
T1	57.17	65.5	71	78.67	88.5	97.83	108.67	126
T2	38.67	48.67	59.33	70.17	87.33	99.33	122.83	150.17
T3	47.83	54.17	59.83	68.5	80	88.67	101	115.67
T4	55.83	68.33	80.83	91.17	105.83	116	135.67	158.5
T5	43.17	51.5	66.87	76.5	86.67	97.67	117.07	132.33

Figure 2: Plant height of *E. diadenum* plantlets in different treatments for eight weeks in the nursery.



	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
T1	2.65	2.73	2.9	2.9	2.97	3.19	3.25	3.64
T2	2.4	2.82	2.96	2.63	3.23	3.67	3.88	4.21
T3	2.25	2.68	2.58	2.4	2.69	2.77	2.98	3.06
T4	2.68	2.48	2.75	2.62	2.99	3.14	3.44	3.82
T5	2.44	2.76	3.11	2.97	3.27	3.33	3.57	3.76

Figure 3: Stem girth of *E. diadenum* plantlets in different treatments for eight weeks in the nursery.

Conclusion

NPK which is inorganic fertilizer is essential for early growth of plants. It gave better growth effects than organic fertilizers at nursery stage. Inorganic fertilizers contain complete essential nutrients and easily absorbed by the roots. The optimum amount of fertilizers applications is crucial in nursery stage to ensure the survival of the plantlets after transplanted in plantation.

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Comparative Evaluation on the Growth Performance, Biomass and Chemical Compounds of Five Herbal Species Grown in Two Different Environment Conditions at FELCRA Berhad, Seri Iskandar, Perak, Malaysia

Syafiqah Nabilah, S.B.[1,*], Farah Fazwa, M.A.[1], Norhayati, S.[1], Zunoliza, A.[1], Samsudin, A.R.[2], Nadia, F.[2], Sari Indra Saputra, M.B.[2] and Azahar, A.[2]
[1]Cawangan Membaikbiak Herba dan Pokok, Bahagian Bioteknologi Perhutanan, Institut Penyelidikan Perhutanan Malaysia, 52109 Kepong, Selangor, Malaysia.
[2]Felcra Berhad, Wisma FELCRA, Lot 4780, Jalan Rejang, Setapak Jaya, Peti Surat 12254, Kuala Lumpur, Malaysia.
******E-mail:syafiqah@frim.gov.my*

Introduction

Malaysia is blessed with mega biodiversity that has high value potential for the herbal industry. The herbal industry has play a significant role in the economic growth of Malaysia to achieve the status of developed country by the year 2020 under the Economic Transformation Programme (ETP) (Rohana et al., 2017). The National Key Economic Area (NKEA) has highlighted herbs as the first Entry Project Point (EPP-1) under the scope of agriculture. To further develop the herbal industry, sustainable supply of the raw materials is important to ensure Malaysia can compete with other producing countries. Domestication and commercial plantation are the important elements contributed to the sustainable supply of raw materials which reduce the dependency on the natural forest resources.

To ensure sustainable supply of the raw materials for products development it is important to cultivate these species in commercial plantation. In Malaysia, land for agricultural activities is becoming more limited due to conversion for other uses such as industrial, residential and urban uses (Olaniyi, 2013). Therefore, there is a need to maximize land utilization. In recent years, integrated farming has been promoted among Malaysian farmers by incorporated short term crops using perennial crops with forest trees. However, it is important to identify the suitable selection of crops for integrated farming which can increase yield per ha and produce high quality plants.

In the Agriculture EPP-1, the government has highlighted several high value herbal products to penetrate the global markets. *Clinacanthus nutans* (belalai gajah), *Andrographis paniculata* (hempedu bumi), *Orthosiphon stamineus* (misai kucing), *Gynura procumbens* (sambung nyawa) and *Strobilanthes crispus* (pecah beling) were among the species listed. Each of this species has its own medicinal value and other associated secondary metabolites. Secondary metabolites are important compounds for the human beings as they are sources for food additives, flavours and pharmaceuticals (Ravishankar and Rao, 2000). The concentrations of various secondary plant products are strongly dependent on the growing conditions and physiology through altering the metabolic pathways responsible for the accumulation of the related natural products (Pradhan et al., 2017). Therefore, this study was conducted with the aim to identify the suitability and productivity of the five herbal species growing at the open field and shade field under oil palm plantation.

Materials and Methods

Planting materials

Five important herbal species listed in NKEA EPP-1 viz. *C. nutans* (belalai gajah), *A. paniculata* (hempedu bumi), *O. stamineus* (misai kucing), *G. procumbens* (sambung nyawa) and *S. crispus* (pecah beling) were collected from several provenances in Peninsular Malaysia and brought to Forest Research Institute Malaysia (FRIM) for cultivation. The herbal plants were propagated using stem

cuttings. Commercial rooting hormone (Serdix 1) were applied at each base of cuttings to enhance the rooting production. After four weeks, 500 rooted stem cuttings of the five herbal species were transferred to polybag and hardened at the nursery for three months.

Field plantation

The hardened plantlets were transported to R&D Centre, Felcra Nasarudin Belia, Seri Iskandar, Perak. The herbal plants were planted in two different environments; i) open field and ii) shade field under the oil palm plantation. The field plantations were established on December 2018.

Open field plantation

About 10 m x 15m area of land were plough using plough machine and two set of planting bed (1m x 15m) were prepared for each herbal species (Figure 1 (a)). A total of 150 herbal plants were planted in a randomized complete block design (RCBD). The planting distance was 1m x 1m and each planting hole were mix with leaf compost to enhance the soil condition.

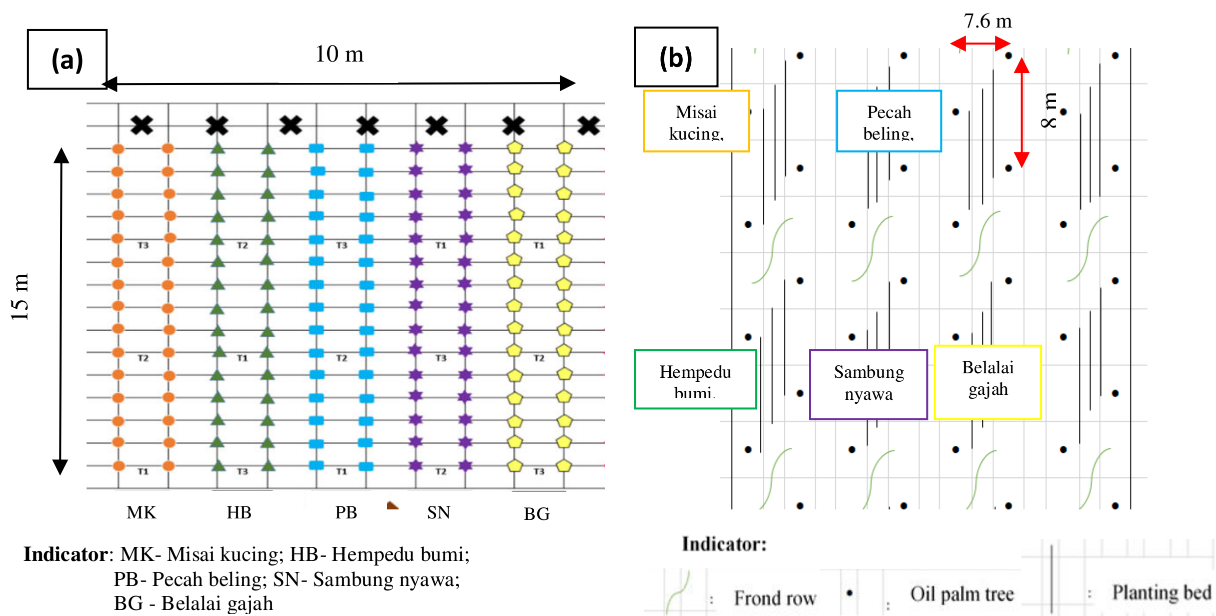

Figure 1: Planting layout of five herbal species; (a) Open field plantation; (b) Shade field plantation.

Shade field under the oil palm plantation

The experiment was conducted under the ten years of oil palm plantation. The oil palm plantation used triangular system of planting with 8 m x 8 m x 8 m spacing. The five herbal species were planted along the fronds row (composted) which involved 22.8 m x 17.6 m oil palm area (Figure 1 (b)). The experimental area was plough prior to plant and mixture of leaf compost were applied on each planting holes. A total of 150 plants were planted in 1 m x 1 m planting distance.

Plant maintenance

The plants were watered manually once per day at each field. Each plants were fertilized once a month with 10 g of NPK fertilizer (15:15:15). Weeding activity were conducted when needed.

Data collection

Among the data collected for plant growth performance were plant height, leaf length and leaf width and collar diameter. Growth data were recorded once per month until the plant reached the maturity age (16 weeks). The herbal plants were harvested randomly in each field at maturity age for biomass data collection. Fresh sample were weighted prior to drying process. The plants sample were oven dried at 60°C until it reach constant weight (approximately 72 hours). The environmental data such as temperature, percentage of humidity and light intensity were monitored at each field and tabulated in Table 1. All data were analyzed for Analysis of Variance (ANOVA) using IBM SPSS Statistics version 22.

Table 1: Mean value for light intensity, temperature and percentage of humidity recorded on December 2018 until March 2019 at two different fields.

Parameters	Open field		Shade field	
	Min	Max	Min	Max
Light intensity (lux)	516	1165	322	782
Temperature (°C)	31.8	37.5	28.6	33.5
Humidity (%)	61.2	67.5	84	89

Analysis of chemical compounds

A total of 20 g fresh leaves from each herbal species were harvested from two different fields. The leaves samples were washed with running tap water and proceed to oven dried at 60°C for 48 hours. The dried leaves samples were grinded into powder form. A total of 0.5 g of sieved powder material (500 µm) was added in 5 mL of methanol and the mixture was ultra-sonicated for 15 minutes. The resulting solution was filtered using 0.45 µm syringe filter prior to analysis. Samples were analysed using HPLC system (WATERS 2535 quaternary gradient pump, WATERS 2707 auto sampler and WATERS 2998 PDA). Two gradient system consist of two different solvent were used; A (0.1% Acid formic in water) and B (acetonitrile). The flow rate used was 1.0 mL/min and the injection volume was 10 µL. The retention times and UV spectra of the targeted compounds were analyzed at wavelength of 220 nm. Each herbal plant used different marker for quantitative analysis as shown in Table 2.

Table 2: Reference compound in each herbal plant for quantitative analysis.

Herbal species	Reference compound
Clinacanthus nutans	Shaftoside
Andrographis paniculata	Andrographolide
Orthosiphon stamineus	Sinensetin
Gynura procumbens	Chlorogenic acid
Strobilanthes crispus	Caffeic acid

Results and Discussion

Effects of different environmental condition on the growth performances and biomass of five herbal species

Environmental condition significantly influenced ($p<0.05$) the growth (Table 3) and yield (Table 4) of the five herbal species. Based on the results, different environmental condition shows significant difference on the plant height of *C. nutans* and *A. paniculata* where the plants become taller under high light intensity (open field). Meanwhile, the rest of herbal plants show similar plant height at both environmental condition. The open field plant developed greater collar diameter and more branches which measured as the crown width. This may be due to the higher absorption of photosynthetically active radiation (PAR) under sunny condition that has increased the photosynthetic rate that promotes plant growth and productivity (Kumar et al., 2014). Under the low light intensity, the herbal plants

tend to resemble etiolated plants which have unwanted tall appearance and are more apical dominant as no crown width were measured. These findings were similar with the studies of other medicinal plants such as sage (Zervoudakis et al., 2012) and damask rose (Thakur et al., 2019). The leaf size was also influenced by different light intensity as shade field plants produced greater leaf length and leaf width compared to open field. According to Lambers et al. (1998), shade-plants develop larger and thinner leaves to increase light harvest as under light starvation the photosynthetic yield will depend upon the efficiency of light energy capture by antenna pigments and its delivery to the reaction centers (Ruban, 2009).

Table 3: Growth performances of five different species grown in an open field and shade field.

Species	Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)	Crown width-*X* axis (cm)	Crown width-Y axis (cm)
Clinacanthus nutans	Open field	43.4±1.93[a]	10.1±0.28[a]	2.20±0.07[b]	6.35±1.39[a]	58.4±3.52	55.4±3.49
	Shade field	24.4±1.86[b]	9.77±0.39[a]	2.55±0.10[a]	3.49±0.23[b]	NA	NA
Andrographis paniculata	Open field	40.6±1.67[a]	6.13±0.13[a]	1.51±0.04[a]	7.26±0.25[a]	43.0±1.97	41.6±1.70
	Shade field	22.7±2.91[b]	5.68±0.47[a]	1.53±0.11[a]	2.07±0.11[b]	NA	NA
Orthosiphon stamineus	Open field	52.2±1.28[a]	4.47±0.09[b]	2.33±0.05[b]	6.99±0.35[a]	86.2±3.21	81.6±2.26
	Shade field	51.5±1.95[a]	8.06±0.20[a]	3.69±0.10[a]	3.99±0.13[b]	NA	NA
Gynura procumbens	Open field	25.6±1.54[a]	10.2±0.45[a]	3.93±0.14[b]	8.69±0.56[a]	32.9±4.73	43.8±6.35
	Shade field	20.7±1.76[a]	9.87±0.50[a]	4.43±0.19[a]	5.75±0.19[b]	NA	NA
Strobilanthes crispus	Open field	29.3±0.84[a]	11.9±0.20[b]	5.04±0.08[b]	8.58±0.34[b]	41.3±1.22	38.6±1.21
	Shade field	28.1±4.78[a]	17.2±0.41[a]	7.27±0.17[a]	4.05±0.36[a]	NA	NA

NA: not available.

Table 4: Mean value of fresh biomass, dry biomass and moisture content per plant of five different species grown in an open field and shade field.

Species	Open field			Shade field		
	Fresh biomass (kg)	Dry biomass (kg)	Moisture content (%)	Fresh biomass (kg)	Dry Biomass (kg)	Moisture content (%)
Clinacanthus nutans	0.1 ±0.01[a]	0.03±0.003[a]	73.1±0.11[b]	0.08±0.03[b]	0.02±0.002[b]	75.8±0.13[a]
Andrographis paniculata	0.18±0.01[a]	0.08±0.03[a]	60.9±0.10[b]	0.008±0.00[b]	0.001±0.00[b]	88.3±0.11[a]
Orthosiphon stamineus	0.25±0.02[a]	0.09±0.01[a]	60.0±0.13[b]	0.24±0.10[a]	0.06±0.006[b]	75.4±0.10[a]
Gynura procumbens	0.59±0.09[a]	0.09±0.01[a]	85.0±0.12[b]	0.05±0.01[b]	0.004±0.00[b]	92.0±0.11[a]
Strobilanthes crispus	0.23±0.13[a]	0.08±0.005[a]	65.2±0.11[b]	0.09±0.01[b]	0.02±0.004[b]	77.5±0.09[a]

Means followed by same value were not significant at $p<0.05$.

Data in Table 4 shows the yield of fresh and dry weight of the herbal plants in an open field and shade field. Different light intensity strongly affected the yield of the herbal plants as higher total of fresh and dry biomass were obtained at the open field. Reduction in light intensity reduces the rate of photosynthesis and rate of the growth which reflect the biomass of the plant (Baroli et al., 2008). Based on the results, herbal plants grown at the open field have lower percentage of moisture content than under shade plants. The open field plants might experience water stress due to high transpiration rate. For *O. stamineus,* no significant difference was observed on the fresh biomass at both fields. At the open field, the plants might produce larger quantity of leaves but smaller in size which could reflect the low biomass obtained.

Effects of different environmental condition on the chemical compounds of five herbal species

The quality and quantity of chemical metabolites in plants are influenced by several of factors, environmental is one of them. Different environmental condition significantly influenced ($p<0.05$) the concentration of phenolic compounds in five herbal species (Table 5). However, different plants had a different response to light intensity alteration and the resulting secondary metabolites production. In this study, the mean concentration of reference compound in *C. nutans, O. stamineus, G. procumbens* and *S. crispus* were found higher at the open field. In contrast, the reference compound in *A. paniculata* was higher under the shade field.

Table 5: Mean concentration of chemical compounds in five herbal species at different environmental condition.

Species	Reference compound	Treatment	Mean concentration±SEM
Clinacanthus nutans	Shaftoside	Open field	308.4±2.92[a]
		Shade field	39.3±2.52[b]
Andrographis paniculata	Andrographolide	Open field	1073.5±1.18[b]
		Shade field	1875.1±1.17[a]
Orthosiphon stamineus	Sinensetin	Open field	653.7±1.86[a]
		Shade field	317.5±0.46[b]
Gynura procumbens	Chlorogenic acid	Open field	92.4±0.43[a]
		Shade field	43.3±0.36[b]
Strobilanthes crispus	Caffeic acid	Open field	399.1±3.41[a]
		Shade field	351.3±0.74[b]

Light and temperature difference could be the factors that contribute to the concentration of the secondary metabolites in the herbal plants. Molmann et al. (2005) reported that the concentrations of quercetin and kaempferol in Alfalfa plant were higher at the warmer temperature and more putrescine content was observed under low temperature environment. Whereas, Ghasemzadeh et al. (2010) reported thet light has enhanced the biosynthesis of secondary metabolites *Zingiber officinale.* The highest biosynthesis of total flavonoid was found under low light intensity (310 $\mu mol\ m^{-2}\ s^{-1}$) while the total phenolic was high under high light intensity (790 $\mu mol\ m^{-2}\ s^{-1}$).

Conclusions

This study showed that the five herbal species produced better growth and yield planted in an open field area. These species could be integrated with non-shade plants such as in a coconut plantation. Whereas, the secondary metabolites production for each of the herbal plants can be influenced due to the environmental stress conditions. In future, the enhancement of bioactive compounds in the herbal plants can be optimized by exposing them to different kinds of stresses.

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Floral Development Stages of *Renanthera bella* J.J. Wood, an Endemic Orchid of Sabah

Nurul Najwa, M. and Nor Azizun, R.*
Unit for Orchid Studies, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400, Kota Kinabalu, Sabah, Malaysia.
**E-mail: azizun@ums.edu.my*

Introduction

Renanthera bella J.J. Wood is an epiphytic orchid that endemic in Sabah and produces beautiful, attractive and blotched pink to crimson red on a yellowish-cream to the apricot yellow ground colour of flower (Rice, 2003). This species had been widely over-collected and classified as endangered in Appendix I of Convention on International Trade Endangered Species of Wild Fauna and Fauna (Chan et al., 1994). Despite the vast literature on orchids, however little is known about their flowers, and the pattern of *R. bella* floral development itself. The floral development of four species of Cypripediaceae (Rasmussen, 1985) was studied by means of scanning electron microscopy, with special attention to the early development of the organs that constitute the gynostemium (Kurzweil, 1993). In the attempts to culture endangered of rare orchid species *in vitro*, most of the reports have been supported by anatomical and morphological of flower development observations (Burgeff, 1936; Arditi and Pridgeon, 1997). However, the study on flower development stages of wild orchid speciesis still lacking.

The scanning electron microscope is being used in this study to increase the accuracy on observation of plant and flower surface together with floral organ development. The use of electron microscopy begins with the development of electron optics (Bogner, 2007). Furthermore, it had high resolution in providing a detail view of images on small floral organ and plant surface (Polowick and Sawhney, 1986). The illustrations provide more advance in a viewing of inner floral organ and deep into the surface feature of stomata guard cell, epidermal and present of hairs. Biological specimens preparation for SEM viewing usually dehydrated with suitable drying method, and a thin metal layer are coated for achieving sufficient electrical conductivity (Ensikat et al., 2009). The use of SEM had been done previously on observation of stoma and micro character of the abaxial and adaxial surface of *Passiflora edulis* morphology surface on *in vitro* and *in vivo* leaves (Veeramohan et al*.,* 2013). The scanning electron microscope used in this study help in increasing the frequency to study the floral morphology in details (Chandra and Sawhney, 1984; Richards and Barrett, 1984).

An understanding of plant reproductive biology is essential to plant conservation effort. In orchid, the success of pollination event is strongly influenced by the floral development stages. This present study described the structure of *R. bella* flower initiation surface, morphology, and physiological changes in detailed.

Materials and Methods

Plant sources

R. bella plants were grown in Greenhouse at Institute for Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah. The observation and measurement of flower of *R. bella* were performed based on length, diameter, and morphology changes. The identification of *R. bella* was confirmed by Taxanomis and botanist from the Institute for Tropical Biology and Conservation (ITBC) and Poring Orchid Conservation Centre (POCC).

Flower bud initiation and development stages

Flower bud initiation and development of *R. bella* were recorded over two and half month started with bud initiation and opening stages. The method was carried out according to Smyth et al. (1990) with some modification by reducing the concentration of glutaraldehyde and ethanol. The use of glutaraldehyde and ethanol at high concentrations prevents absorption by small organs such as pollen and anther cap (Park et al., 2016). Furthermore, instead of using Critical Point Dryer, the desiccator were used to dry the sample before the observation under SEM. Data on length of bud, a width of bud, and the diameter of matured petal and sepal was recorded every weeks. Other than that, the changes in the development of flower are recorded from time to time.

Dissection of floral organ

Flower organ was dissected according to their flower part for further observation on their morphology and physiology under the scanning electron microscope. Both immature and mature floral organ was dissected for comparison of their development.

Scanning electron microscopy (SEM)

Inflorescence and the young bud of the *R. bella* were fixed in 1.5% glutaraldehyde (Figure 1A) and incubated in 4°C for 24 hours. The sample was rinsed in 0.1 M phosphate buffer for three times with and an interval of 10 minutes each. Then, the tissue material was dehydrated with a series of ethanol (35%, 50%, 70%, 90% and 100%) for 30 minutes of interval for each ethanol concentration. The sample was desiccated for 1 hour (Figure 1B) before mounted in metal SEM stub and coated with gold in Sputter Coater (Edwards) (Figure 1C) for further observation on scanning electron microscope (Zeiss) (Figure 1D) and the image was photographed.

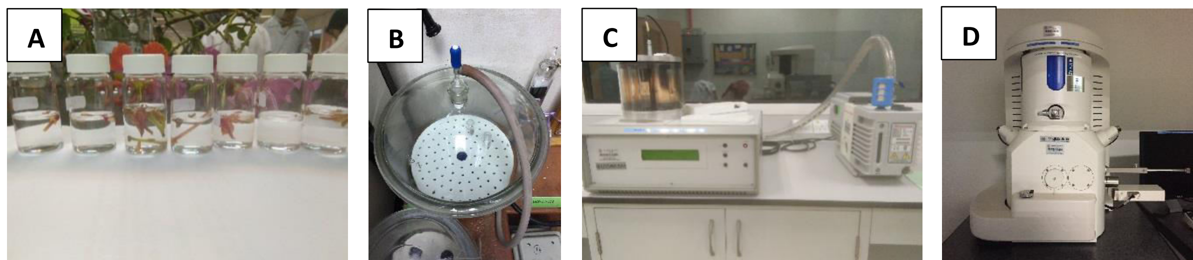

Figure 1: The protocol for scanning electron microscope observation. (A) Flower organ were dissected and fixed in gluteraldehyde. (B) Samples were dried in dessicator. (C) Samples were coated with gold in sputter coater. (D) Samples observation were done under SEM.

Results and Discussion

Scanning electron microscope observation on initiation of flower bud formation

Inflorescence and early flower initiation occur continuously until the opening of bud, and the development of *R. bella* was observed by days. Other than that, based on scanning electron microscope observation on flower development initiation process, it's can be classified into ten different stages (Table 1).

Table 1: Stages in *R. bella* flower development.

Stages	The development of flower bud pattern	Days of observation	Descriptions
1	Flower bud started to appear and form raceme type of inflorescence	7	The flower bud is creamy white while inflorescence stalk is light green.
2	Flower bud primordial started to develop	7	Flower bud white cream and brown
3	Sepal flower developed	12	Flower sepal red cream in colour
4	Development of immature column	16	Column red brown in colour
5	Lip developed and can be observed between lateral bud sepal.	16	Lip red cream in colour
6	Stigma papillae appear	16	-
7	Pollen not fully develop	16	Pollen is light yellow in colour and kidney like shape
8	Anther cap enclosed the pollen	16	Anther cap was dark brown in colour and had a straight line with a deep curve
9	Petal enclosed the flower organ	21	Adaxial sepal pale red
10	Bud started to open	25	Bud is red maroon in colour

Renanthera bella flower bud initiation begins with stage 1 when flower bud appears on apical meristem with an average diameter of 0.02 cm and form raceme inflorescence (Figure 2A). Furthermore, primordium bud is started to develop and become a stalk; this defines as the beginning of stage 2 (Figure 2B). Stage 3 begins when the sepal line formed, and by these stages, the diameter size of bud is about 0.25 cm (Figure 2C). The column started to arise and curved inward when they immature at which time stage 4 begins (Figure 2D).

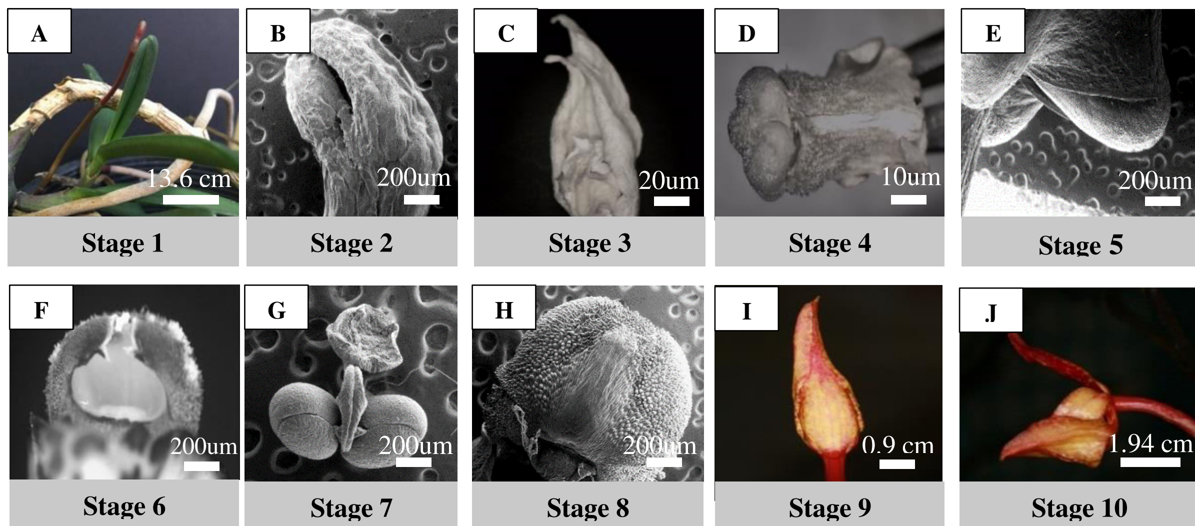

Figure 2: Flower development stages of *R. bella*. (A) stages 1: Raceme type of inflorescence; (B) stages 2: flower bud primordial developed; (C) stages 3: flower sepal developed; (D) stages 4: immature column development; (E) stages 5: lip development; (F) stages 6: stigmatic papillae arise; (G) stages 7: immature pollen developmet; (H) stages 8: anther cap development; (I) stages 9: petal fully enclosed the flower organ; (J) stages 10: bud started to open. (Notes: sepal and petal are dissected to reveal inner organ).

Then, stage 5 start with the development of the lip and the lip are started to appear below between lateral sepals of the flower bud (Figure 2E). Then, stage 6 showed the growth of papillae stigma for the pollination process (Figure 2F). In stage 7 (Figure 2G), pollen is formed while in stages eight, anther cap is seen to develop and enclosed the pollen for protection (Figure 2H). From the starting of stage 9, sepals cover the entire of bud at an average diameter 0.50 cm (Figure 2I). It can be seen the formation of primordial petal at the same time with sepal, and they are fast-growing to enclose the

bud. Stage 10 occurred when the sepals started to open and continue to elongate rapidly form the matured flower (Figure 2J).

The development pattern of *R. bella* bud followed typical acropetal chain by they start to mature from base to apex of plant and form raceme types of inflorescence (James and Melinda, 2004). Inflorescence was appearing from interstitial of leaf and can be elongate to 30 cm and above. Flower bud primordia were initiated at approximately seven day. There are about 7 to 15 bud of a flower bud in a single inflorescence. Flower bud size increase every week and started to open after 25 days observation. *Renanthera bella* inflorescences continue to develop with the suitable condition.

The estimation of transition development time from vegetative stage (Figure 2B and 2C) to reproductive stage (Figure 2D to Figure 2H) required approximately about 4 to 5 days. *R. bella* anther cap fused together at the front of column structure with the pollen located inside the anther for protection before pollination take place. The *R. bella* had gynandrous stamen where they fused with pistil to form reproductive structure called column. The column is androecium parts which known as male reproductive with modification of filament and style that fuse together in orchid flower (James and Melinda, 2004).

Furthermore, anther cap of *R. bella* were round and curve in shape with line pattern in the middle. Whole structure of immature anther was covered with puberulent hair. The observation on stigmatic papillae shows that they arise together with the column. In *R. bella,* lateral sepal arise at almost the same time with dorsal sepal while petal developed late in flower development which about most of the organ are fully developed. The development of the petal quickly precedes the anthesis process.

Since the flower development of orchid is still lacking, so it hard to compare the *R. bella* flower development with other orchid species. However, the overall development of this species is more simple compared to other plant species such as *Arabidopsis thaliana* in term of classification of their development stages (Smyth et al., 1990).

Conclusions

Floral development of *R. bella* follows a typical acropetal development. Definition of 10 stages of floral *R. bella* development which is related to entirely opening of the bud to formation of mature flower would take place within 25 days. A complete development of mature *R. bella* flower before form a capsule required about 35 days. This research is the pioneer for further study of floral development on other species of wild orchid.

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Chapter 2

Ecophysiology and Stress Biology

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Growth and Physiological Responses of True-to-type Durian Seedlings from Two Species as Affected by Different Cyclic Water Stress

Muhammad Najib, O.G.[1,*], Siti Zainab, J.[2], Ahmad Hafiz, B.[3], Zul Helmey, M.S.[3] and Izyani, R.[1]
[1]Industrial Crop Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, 43400 Serdang, Selangor, Malaysia.
[3]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) 06050 Bukit Kayu Hitam, Kedah, Malaysia.
**E-mail: najibo@mardi.gov.my*

Introduction

In 2015, the world population had reached 7.3 billion and with approximately increased by 83 million annually whereby the current population is projected to reach 8.5 billion by 2030 (United Nations, 2015). Agricultural productivity in contrast, is not increasing at a required rate to keep up with the food demand. Many factors contributed to this problem such as availability of agricultural land, fresh water resources, ever-increasing biotic and abiotic stresses, and low economic activity in agricultural sector (Athar and Ashraf, 2009). It is believed that abiotic stresses are considered to be the key source of yield reduction (Munns and Tester, 2008). Water stress is one of the major abiotic stresses that affect production in agriculture. Drought continues to be an important challenge to agricultural researchers and plant breeders. It is assumed that by the year 2025, around 1.8 billion people will face absolute water shortage and 65% of the world's population will live under water-stressed situations (Mekonnen and Hoekstra, 2016). Drought, as an abiotic stress, is multidimensional in nature, and it affects plants at various levels of their organization. It affected a wide range of physiological processes such as stomatal behaviour, photosynthesis, transpiration, translocation and partitioning of assimilates. Changes in all these processes are of particular importance due to their direct relationships with crop growth and productivity. Durian (*Durio zibethinus* Murr.) is one of the crops most sensitive to drought (Masri, 1999). Lack of water has been the main limiting factor in determining the success of transplanted durian plants. Usage of drought tolerant rootstock will be advantageous in overcoming water stress problem in both nursery and transplanting stage. However, since durian flower is highly self-incompatible, seedlings derived from the fruits are genetically heterogeneous, hence having variables in level of water stress tolerance. Production of clonal drought tolerant rootstock will secure genetic uniformity in seedlings, with regard to drought tolerance. In order to evaluate durian species that is drought tolerance, other external factor particularly genetic heterogeneity among the rootstocks has to be removed, and is achievable via DNA fingerprinting. Thus, using genetically homogenous seedlings of *D. zibethinus* (clone 99) and *Durio lowianus*, a study was carried out in order to evaluate growth and physiological responses of these two durian species subjected to different watering cycles at seedling stage.

Materials and Methods

Plant materials and growth conditions

Durian seeds from both species were collected at Malaysian Agricultural Research and Development Institute (MARDI) Kuala Kangsar from July-August 2018. Seeds were sown in sand bed and were transplanted into 8 x 12-inch polyethylene bag after 1 month. The growing media used was mixture of soil, processed chicken dung and sand with ratio of 3:2:1, respectively. Genetically homogenous seedlings from the corresponding species, determined via DNA fingerprinting were separated from other seedlings after 2 months of transplanting. The true-to-type seedlings from both species were grown under 50% shade and plant maintenance was carried out according to the normally recommended practices (Zainal abidin et al., 1991).

Watering cycle

At five months after transplanting, three different levels of watering cycle; every day, every 2 days and every 3 days were imposed to the true-to-type seedlings from both species. The plants were watered around 9-10 am with approximately 350 mL of water for each watering cycle. The soil moisture (%) was determined using the HH2 moisture meter (Delta T Devices V4.2, Delta-T, United Kingdom). The field capacity for each watering cycle was then calculated based on the soil moisture for each watering cycle which were 100% (control), 38.3% and 14.5% for every day, every two days and every three days watering cycle, respectively.

Measurement of growth

At 29 days after treatment where the symptoms of water stress prevailed, plants were sampled at random from each treatment for determination of plant height, stem diameter and leaf number. Measurement of plant height was taken from the surface of the soil in the polyethylene bag to the highest shoot tip by using a measuring tape. Stem diameter was measured at the lowest part of stem using Electronic Digital Caliper (Model 500-196-30, Mitutoyo, Japan) while the leaf number was manually counted based on fully expanded leaves. The whole plants were then separated into leaves, stems and roots and the dry weight of each part was determined after 72 hours at 75°C in a drying oven. Plant dry matter was expressed as total dry weight of leaf, stem and root. Leaf areas were measured and recorded as total leaf area per plant using automatic leaf area meter (LI-300, LI-COR, USA).

Relative water content (RWC)

At 31 days of treatment, samples from the top fully expanded leaves were taken for RWC determination. Fresh weight (FW) of five leaf discs were measured before floated on deionized water for 4 hours. The wet surface of the turgid leaf discs was blot dried quickly before weighing (TW). The leaf discs were then dried for 72 hours at 70°C in oven and dry weight (DW) was then measured. The RWC was calculated and expressed in percentage based on the following formula:

$$RWC = (FW - DW / TW - DW) \times 100$$

Net photosynthesis and stomatal conductance

At 30 days of treatment, net photosynthesis (A) and stomatal conductance (gs) were measured on the third fully expanded leaves by using a portable close photosynthesis measurement system (Infra-Red Gas Analyzer, Li 6400, Li-Cor Inc., a USA). The measurements were taken around 11:00 am using five measurements for each replication with an irradiance setting of 1000 µmol m^{-2} s^{-1}. Irradiance was provided by an LED RGB (Red Green Blue) light source (LI-6400-02B, Li-Cor Inc., USA).

Experimental design and data analysis

This experiment was conducted in a split-plot design with species as main plot and watering cycle as sub-plot with three replications; 6 plants per replication. The data obtained was analyzed using analysis of variance (ANOVA) in the SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Least Significant Difference (LSD) at $P \leq 0.05\%$.

Results and Discussion

Effects of cyclic water stress on vegetative growth

Water stress had considerable effect on the plant vegetative growth. Watering cycle significantly affected (P<0.05) leaf and stem dry weight while the rest of the parameters were not significantly affected. No significant interactions were recorded between the main effects for all the parameters (Table 1). Effect of species on vegetative growth variables will not be discussed here since the species are innately different even without watering cycle treatments.

Regardless of the species, watering the plants every 3 days significantly reduced leaf dry weight and stem dry by 16.39 and 7.53%, respectively, compared to watering every day. Growth is accomplished through cell division, cell enlargement and differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit (Farooq et al., 2009). Water availability to cells is low because of poor hydraulic conductance from roots to leaves caused by stomatal closure and eventually resulted in decrease of nutrients supply to the shoot.

Previous studies reported that water stress limits the size of individual leaves (Tiaz and Ziger, 1998) and leaf number (Pellegrino et al., 2005). The results in this study however did not show any significant effects of watering on leaf area and leaf number, albeit the decreasing trend of these parameters as field capacity reduced. Presumably, duration of treatment need to be prolonged for this effects to be profound.

Table 1: Main and interaction effects of durian species and watering cycles on vegetative growth.

Factor	Stem diameter (mm)	Plant height (cm)	Leaf no	Leaf area	Leaf DW (g)	Stem DW (g)	Root DW (g)
Species							
D. zibethinus (D99)	9.53[a]	71.09[b]	42.17[a]	1191.17[a]	7.61[b]	9.22[b]	4.01[b]
D. lowianus	9.77[a]	101.75[a]	37.34[a]	1335.78[a]	9.64[a]	14.04[a]	4.77[a]
Watering							
Every day (100% F.C)	9.98[a]	86.98[a]	42.94[a]	1335.65[a]	9.21[a]	12.21[a]	4.79[a]
Every 2 days (38.3% F.C)	9.87[ab]	86.92[a]	40.82[a]	1279.85[a]	8.97[a]	11.40[ab]	4.35[a]
Every 3 days (14.5% F.C)	9.11[b]	85.34[a]	35.50[a]	1174.94[a]	7.70[b]	11.29[b]	4.02[a]
Species	ns	**	ns	ns	**	**	*
Watering	ns	ns	ns	ns	*	*	ns
Species*Watering	ns	ns	ns	ns	ns	ns	ns

***Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to LSD (Mean ± S.E; n=3).*

Effects of cyclic water stress on leaf water status and gaseous exchange

Watering cycle significantly affected (P<0.05) relative water content (RWC) while the rest of the parameters were not significantly affected. No significant interactions were recorded between the main effects for all the parameters. Regardless of the species, watering the plants every 3 days significantly reduced RWC by 9.45% compared to watering every day (Table 2). This result is in agreement with result reported by Mohd Razi et al. (1994), where RWC of durian seedlings significantly reduced when the field capacity was reduced from 80% to 10%. Leaf RWC strongly relates with cell volume

and it can accurately indicate the balance between absorbed water by plant and that lost though transpiration (Lugojan and Ciulca, 2011), hence it is an important indicator of water status in plants. Furthermore, the close correlation of RWC with plant physiological activities and soil water status (Ozkur et al., 2009) qualifies it as a reliable trait, for assessing plants tolerance to drought stress.

Photosynthesis is the main driver for crop productivity, which is negatively influenced by water deficit conditions. Stomata closing in response to moisture stress results in a reduction in leaf photosynthetic capacity resulting in chloroplast dehydration and decreased CO_2 diffusion into the leaf (Reynolds-Henne et al., 2010). Generally, photosynthesis rate for plants subjected with water stress will reduced significantly. Previous study of water stress on grafted durian planting materials done by Mohd Razi et al. (1994) reported that leaf photosynthesis rate of grafted D99 seedlings reduced significantly from 7.7 µmol CO_2 m^{-2} s^{-1} to 1.6 µmol CO_2 m^{-2} s^{-1} as the field capacity reduced from 80% to 10%. Even though the leaf photosynthesis rate recorded in our study are around the figures as reported by Razi et al. (1994), results from our experiment however did not show any significant reduction in terms of leaf photosynthesis rate. This might be explained by the difference between the samples tested in the studies. Mohd Razi et al. (1994) used D99 planting materials grafted on D8 rootstocks whereas in our study, we used homogenous, true-to-type seedlings of D99 which were verified using DNA finger printing. Plant root systems are essential for adaptation against different types of biotic and abiotic stresses (Wasaya et al., 2018) and the amount of root and its distribution in the soil affects the ability of root to absorb water (Faust, 1989). Phenotypically, root structure is different between species (Antos and Halpern, 1997) and to some extent, between varieties. Rootstocks derived from D8 seeds were heterogeneous genetically, thus might have different root morphology. This heterogeneity led to different responses in water stress tolerance levels; particularly in terms of the root capability to absorb water.

Table 2: Main and interaction effects of durian species and watering cycles on RWC, gaseous exchange and SPAD.

Factor	RWC (%)	A (µmol CO_2 m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	Relative chlorophyll content (SPAD)
Species				
D. zibethinus (D99)	71.65^{b}	5.62^{a}	0.08^{a}	33.21^{a}
D. lowianus	80.05^{a}	5.43^{a}	0.06^{a}	33.05^{a}
Watering				
Every day (100% F.C)	79.10^{a}	5.73^{a}	0.074^{ab}	33.40^{a}
Every 2 days (38.3% F.C)	76.83^{ab}	5.97^{a}	0.085^{a}	32.74^{a}
Every 3 days (14.5% F.C)	71.62^{b}	4.89^{a}	0.060^{b}	32.66^{a}
Species	**	ns	ns	ns
Watering	*	ns	ns	ns
Species*Watering	ns	ns	ns	ns

***Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to LSD (Mean ± S.E; n=3).*

Conclusions

Watering cycle every three days significantly reduced plant water status and vegetative growth of *D. zibethinus* and *D. lowianus*. Based on the reduction pattern observed in vegetative growth and relative water content, both species have the same tolerance level against drought stress. Analysis on biochemical contents of oxidative stress need to be conducted in order to further differentiate tolerance levels among the species.

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Effects of Light Wavelength Exposure in Reducing Chilling Injury and Maintaining Postharvest Quality of Pineapple (*Ananas comosus* L. cv. Morris)

Nurul Afina, A. and Wan Zaliha, W.S.*
Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
**E-mail: wzaliha@umt.edu.my*

Introduction

Pineapple (*Ananas comosus* L.) is one of the most important tropical fruit that cultivated and produced globally in significant quantity after banana and citrus. In 2018, world pineapple production was dominated by Costa Rica (2,930,661 MT), Brazil (2,694,555 MT) and Philippines (2,612,474 MT) (FAO, 2018). In Malaysia, Johor produced the highest production of pineapple (274,284 MT) followed by Sarawak (25,664 MT) and Sabah (11,154 MT) (Department of Agriculture, 2017). The production of pineapples are primarily for fresh consumption and industrial processing products (Hossain and Bepary, 2015). For export market, Malaysia receives high demand from Singapore, Saudi Arabia and United Arab States (Halim, 2016). Most of the pineapples were exported by using sea freight at storage temperature of 7°C to 8ºC for ripe and 10ºC for unripe fruit. However, pineapple is susceptible to chilling injury (CI) when stored below 13ºC where CI is one of the most significant contributors to the postharvest losses. The CI usually happened after being stored for a long period at temperatures below 10°C to 15°C. Chilling injury occurred on fruits because they unable to perform normal metabolic process which results to tissue became weaken. CI is physiological disorder in fruit occurred when the fruit is exposed to low temperature below its optimal level during storage. In response to chilling stress, many physiological and biochemical changes and dysfunctions of certain cellular occurred which includes stimulation of ethylene production, increase in respiratory rate, interference in energy production, increase in activation energy, slowing of protoplasmic streaming, increase in permeability, reduction in photosynthesis, enzyme inactivation, membrane dysfunction, and alteration of cellular structure (Wang, 1982). The development of chilling injury symptoms such as surface lesions, internal discoloration, water-soaking of the tissue, off-flavour, decay and failure to ripen normally will be started after been exposed to low temperature for a long period (Saltveit and Morris, 1990).

Many postharvest treatments have been designed to overcome CI problem i.e. methyl jasmonate (Gonzalez et al., 2000), salicylic acid (Sayyari et al., 2009), heat treatment (Sayyari et al., 2011), nitric oxide fumigation (Singh et al., 2009), wax edible coating (Qiuping and Wenshui, 2007) and 1-methylcyclopropene (1-MCP) (Salvador, 2004). However, the above treatments might be expensive and toxic to the fruit thereby affects human health (Stohs and Miller, 2014). In line with that, one of the effective approaches to reduce CI at low temperature storage could be by using Light Emitting Diodes (LEDs). LEDs have been widely used in horticultural due to its advantages such as small in size, has lower energy consumption, high durability and long lifespan (Mori et al., 2007). Hassan et al. (2017) reported that LED light able to induce bioactive compound synthesis for crops, enhance antioxidant properties, improve nutritional traits of the postharvest produce and offer protection against food spoilage and crop loss. Therefore, this study aimed at evaluating the reduction of CI and maintaining the postharvest quality of pineapple as it will be more convenient and easy to handle. In addition, currently, no information available on the effects of LEDs in reducing CI and maintaining postharvest quality performance of pineapple particularly Morris cultivar stored at 10°C. Thus, this warrants investigation.

Materials and Methods

Planting materials, experimental location and experimental design

The experiment was conducted at the Post Harvest Technology Laboratory, Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu. A total of 96 Morris pineapple fruits at maturity index 3 were used in the experiment. The pineapples were sorted based on similar size, colour and the absence of physical injuries. The experiment was arranged in Complete Randomized Design (CRD) with four different light treatments wavelenghts viz. i) fluorescence light (serve as control, 400 nm-700 nm), ii) white light LEDs (390 nm-700 nm), iii) blue light LEDs (430 nm-470 nm) and iv) red light LEDs (630 nm-640 nm). Every treatment consists of three replicates with single pineapple treated as one experimental unit. All fruits were stored in chiller 10°C and exposed to the assigned LED lights with different wavelength for 17 days. The quality and chilling injury incidence were assessed at five day intervals (10ºC±3ºC) and following two days at ambient temperature sets at 25±3ºC to stimulate marketing condition viz; 0 day and 2 days shelf life (0+2), 5 days and 2 days shelf life (5+2), 10 days and 2 days shelf life (10+2), 15 days and 2 days shelf life (15+2).

Parameter evaluation

Postharvest parameters evaluation were CI score, flesh colour and firmness, soluble solids concentration (SSC), total phenolic content (TPC) and ascorbic acid (AA) concentration. The CI of pineapple was evaluated visually according to the score proposed by Nilprapruck et al. (2008). The CI of the flesh was rated on a scale from 1 to 5. Scale 1: no browning symptom in the flesh, scale 2: light chilling injury with browning lesion less than 25%, scale: 3 medium chilling injury with browning lesion 26-50%, scale 4: severe chilling injury with browning lesion 51-75%, scale 5: chilling injury with browning symptoms more than 76% of the flesh. The flesh colour was measured by using Kinoca Minolta CR-400 (Minolta camera Ltd, Japan) as according to CIELAB colour parameter. Fruit colour data were expressed in lightness (L*), chromaticity value a* and chromaticity value b* (McGuire, 1992). The flesh firmness was determined using TX plus texture analyser (Stable Micro Systems, United Kingdom). The probe of P/2 stainless needle was used to penetrate the flesh of the pineapple wedges with a test speed of 1 mm/sec. The pre-test speed and the post-test speed were set as 5 mm/sec and the target distance was adjusted to 10 mm. The firmness readings were taken on the upper, middle and bottom part of pineapple wedges and the reading expressed in Newton (N). SSC was measured individually by means of a hand-held refractometer (AOAC, 1990). The samples were sliced into smaller pieces and squeezed into juice by using muslin cloth and the readings were expressed as % Brix. Total phenolic content of fresh cut pineapple determined according Wan Zaliha and Koh (2016) by using standard curve obtained and expressed as milligrams of gallic acid equivalents per 100 grams of fresh weight (mg GAE $100g^{-1}$ FW). Ascorbic acid concentration was being determined by using indophenol titration method as according to AOAC (2004). The concentration of ascorbic acid was determined by using the standard curve obtained and expressed as mg per 100g fresh weight.

Statistical analysis

The data were subjected to the analysis of variance (ANOVA) using GLM (General Linear Models) procedures and further separated by Tukey's HSD (Honestly Significant Difference) test at $p \leq 0.05$ (SAS Institute Inc., 1999).

Results and Discussion

Pineapple is a non-climacteric fruit which need to be harvested ripe since the ripening process discontinues after harvest. In order to maintain its freshness and quality especially for export markets, low temperature storage is a critical parameter to be managed. Common postharvest physiological disorder of pineapple due to low temperature is CI. Under CI, observable symptoms include internal browning and black heart conditions (Hassan et al., 2011). In this study, CI incidence score increased

in all treated pineapples except for pineapple treated with blue LEDs which maintain at score 1 throughout the experimental period (Figures 1 and 2). However, flourescence and LEDs treated fruits had similar CI score except on fruits stored for 15 days in chiller and following two days at ambient temperature (15+2). On day 15+2, blue LEDs light has tendency in reducing CI of pineapple (score 1) by showing pure yellow colour flesh (score 1) with only a very mild CI throughout the experimental period. According to Ouzounis et al. (2015), blue light contains more energy compare to red light due to its higher frequency and shorter wavelegth. It also used in postharvest preservation, due to their low heat irradiance and higher efficacy (Mohidul Hasan et al., 2017). In line with that, blue LEDs might be suitable to be applied in reducing chilling injury as it give much larger spectrum, higher frequency and shorter wavelength to terminate the changes in kinetics of enzyme activity. Based on the report of Xu et al. (2014), blue light enhances anthocyanin accumulation in strawberry fruit which might be result from the activation of key enzymes in the pentose phosphate, shikimate, phenylpropanoid, and flavonoid pathways. However, the exact biological mechanisms of how CI is induced through light are not fully understood. In addition, to the authors' best knowledge, this is the first report showing that LEDs able to reduce CI in pineapple fruits stored at low temperature.

CI during cold storage of many fruits occurs due to oxidative stress (Sala, 1998; Mao et al., 2007; Zhao et al., 2009) which develops as a consequence of reactive oxygen species (ROS) generation exceeding the capability of the antioxidant system in the cell (Hodges et al., 2004). The reduction or failure of antioxidants to protect against the ROS can cause oxidative damage leading to the loss of membrane integrity in the tissue (Hodges et al., 2004) such as CI. A positive relationship exists between the antioxidant enzyme activity and chilling tolerance of harvested fruit was also reported by Cao et al. (2009). In this study, LED has been used to reduce or delay the CI incidence in pineapple cv. Morris. Possibly, light quality affects the photo-oxidative properties of plants by modulating the antioxidant defense system, resulting in the rise of antioxidative enzyme activity. In addition, many researches have shown that different LEDs treatments result in the accumulation of bioactive compounds and antioxidants in crops including varieties of lettuces (Li and Kubota, 2009), pea seedlings (Wu et al., 2007), Chinese cabbage (Avercheva et al., 2014) and tartary buckwheat (Tuan et al., 2013). Such improvements in the antioxidant characteristics may arise due to the induction of β-carotene, glucosinolates, free radicals, scavenging activity, ROS-scavenging enzymes, phenolic compounds, and vitamin C (Cao et al., 2011). To date, no research has been conducted on the effect of LEDs in reducing CI of cold-stored fruits. However, numerous reports of LEDs in improving plant biomass and nutrients were easily found. Thus, the science behind LEDs reducing CI could only be based on its impact on whole plants.

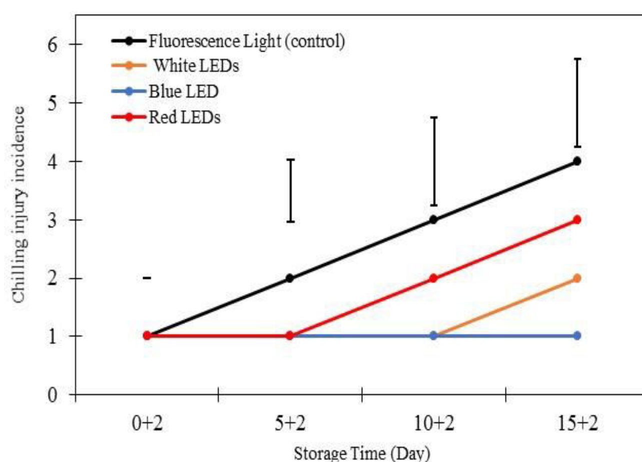

Figure 1: Effects of different lights wavelength exposure on chilling injury index of pineapple cv. Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=0, Day 5+2=1.067, Day 10+2=1.509 and Day 15+2=1.509).

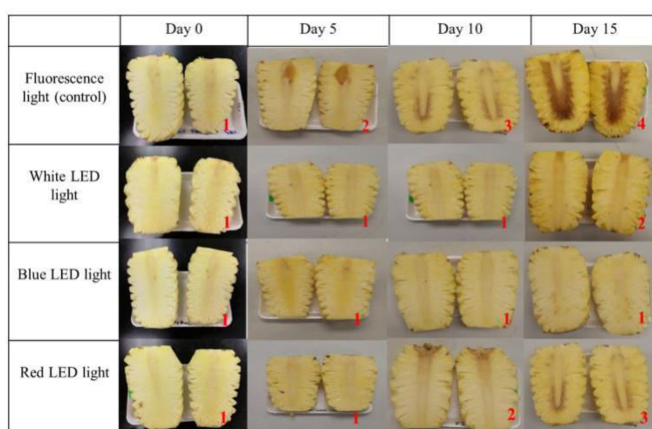

Figure 2: Effects of different lights wavelength exposure on chilling injury index of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Number in the denoted to chilling injury score.

Lightness coefficient indicates differences in lightness and darkness where positive value shows lighter while negative value shows darker. Chromaticity b* is difference in yellow (+) and blue (-). Degree of browning incidence can be determined by measurement of lightness coefficient (L*) and chromaticity b*. The L* value is represent brightness and b* value represent loss of yellow colour of browning incidence. In general, high value of L* and b* indicates lower extent to chilling injury in pineapple. Overall, there were no differences between treatments recorded on flesh colour of pineapple cv. Morris (Figures 3, 4, 5 and 6). The declining of L* value means the changes of flesh colour from bright yellow into browning occured. This was supported by Promyou et al. (2012) and Supapvanich et al. (2011) studies where the declining of L* value associated by browning index in jujube fruit and fresh cut wax apple fruit that stored in cold temperature. It also has been reported that the decrease of L* value was also related to internal browning symptoms during cold storage in pineapple cv. Queen (Quyen et al., 2013). Rosnah et al. (2009) stated that the parameter L*, a* and b* corrulated with the level of ripeness in pineapple. The value of a* represents the green colour of skin. The increased of a* and b* value indicates higher maturity index of pineapple fruits (Rosnah et al., 2009). This also supported by Wills et al. (2007) study where the increased in b* due to the degradation of chlorophyll, conversion of starch to sugars, dearease in pH and increase in acidity.

In the present study, no effect of LED wavelength on flesh firmness of pineapple throughout the experimental period (Figure 7). Meng et al. (2009) claimed that the reduction of fruit firmness might be due to pectin modification. Meanwhile Hu et al. (2012) reported that the degradation of the cell wall can be related to the action of several cell wall proteins and enzymes. PG activity in the pineapple degrades the pectin in the cell wall matrix which decrease in cell wall coherence leading to softening (Hu et al., 2012). PG also can de-esterified homogalacturonic acid as its substrate so that PME can catalyze the demethylation of the C6 carboxylic acid group in galacturonosyl residues. It also has been stated that blackheart in some fruits stored at low temperature causes by association of PG and PME activities (Hu et al., 2012). This could be the main reason why the value of firmness of pineapple flesh deacreased throughout storage period.

Soluble solids concentration (SSC) is one of the important quality factor for many fruits in determining fruit maturity (Hajar et al., 2012). In this present study, pineapple treated fruits showed same values of SSC without regards to treaments and had an increasing trend throughout the storage period (Figure 8). Similarly, Rosnah et al. (2009) claimed that SSC in pineapple cv. Josapine increased at low temperature storage stored for 16 days. In contrast, SSC in pineapple cv. N36 (Abdullah et al., 1996) and Sarawak (Abdullah et al., 1986) tended to declined during one week storage at 10°C. These sporadic outcomes warrant further investigation. However, Rosnah et al. (1996) claimed that the changes in SSC correlate with the changes of skin colour. This was in agreement with the report of

Mohamed and Ahmad Khir (1993) where SSC in pineapple cv. Mauritius corresponded with maturity or skin colour (a* and b* values). The increase in SSS in this study could be due to the ripening process in progress.

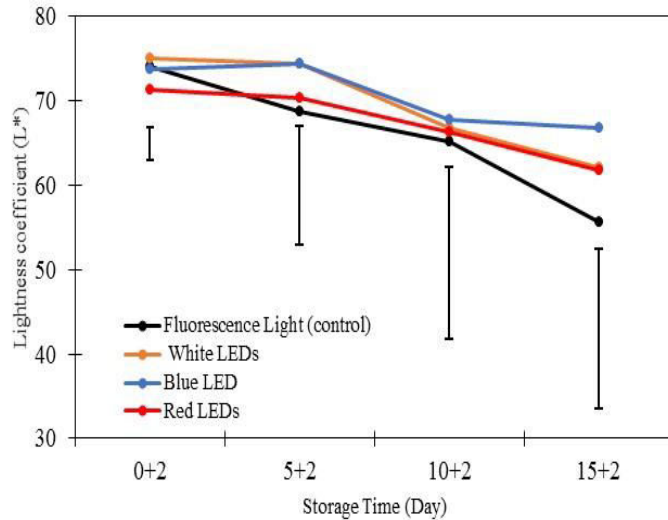

Figure 3: Effects of different lights wavelength exposure on lightness coefficient of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=3.868, Day 5+2=13.988, Day 10+2=20.471 and Day 15+2=18.909).

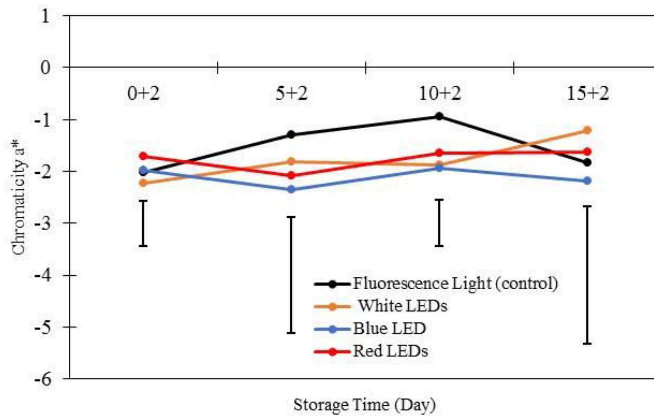

Figure 4: Effects of different lights wavelength exposure on chromaticity a* of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=0.869, Day 5+2=2.227, Day 10+2=0.897 and Day 15+2=2.656).

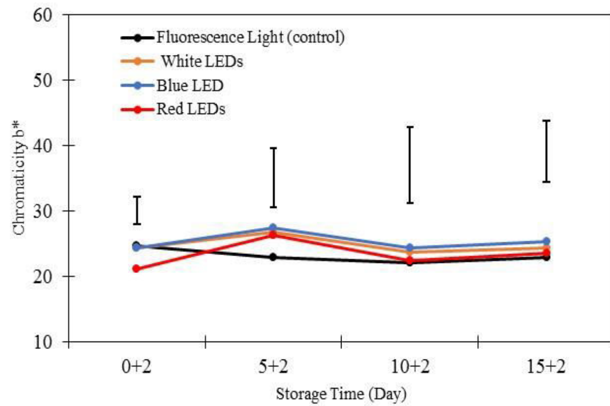

Figure 5: Effects of different lights wavelength exposure on chromaticity b* of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=4.221, Day 5+2=8.999, Day 10+2=11.666 and Day 15+2=9.3315).

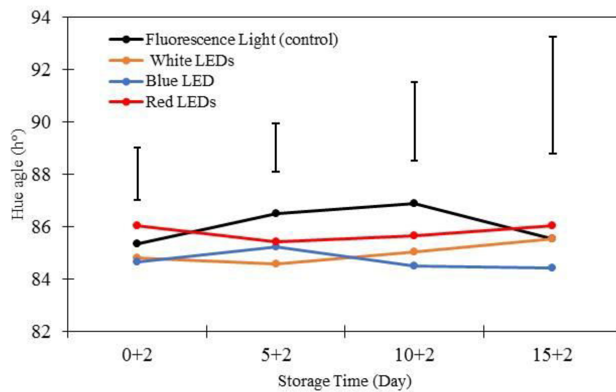

Figure 6: Effects of different lights wavelength exposure on hue angle of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=1.977, Day 5+2=1.831, Day 10+2=2.999 and Day 15+2=4.479).

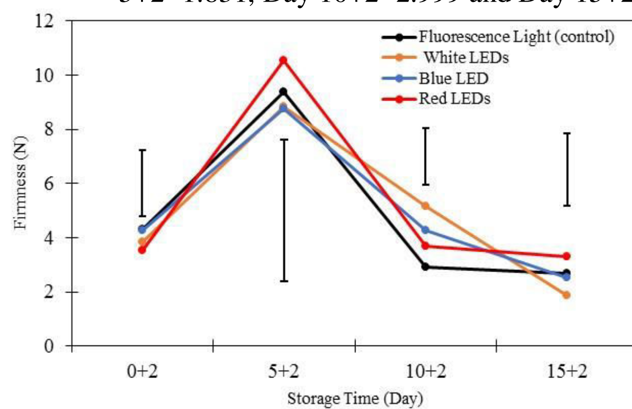

Figure 7: Effects of different lights wavelength exposure on flesh firmness of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=2.448, Day 5+2=5.220, Day 10+2=2.071 and Day 15+2=2.667).

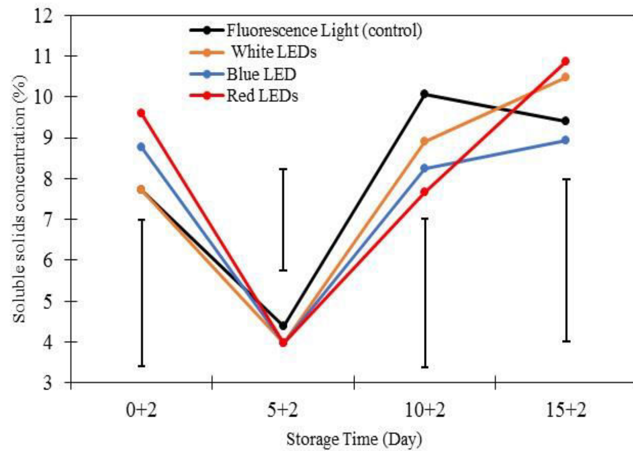

Figure 8: Effects of different lights wavelength exposure on soluble solids concentration of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=3.558, Day 5+2=2.477, Day 10+2=3.655 and Day 15+2=3.980).

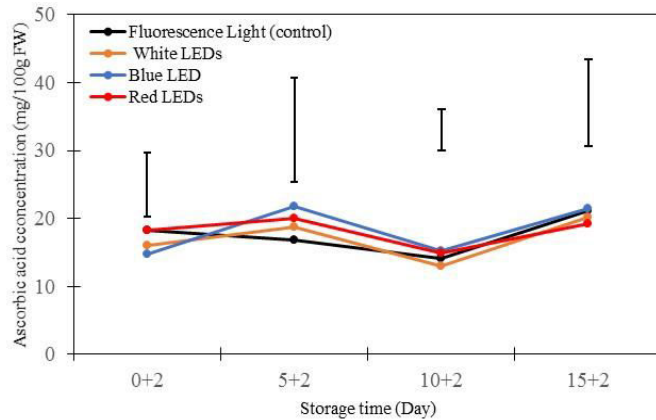

Figure 9: Effects of different lights wavelength exposure on ascorbic acid concentration of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=0.954, Day 5+2=1.531, Day 10+2=0.606 and Day 15+2=1.273).

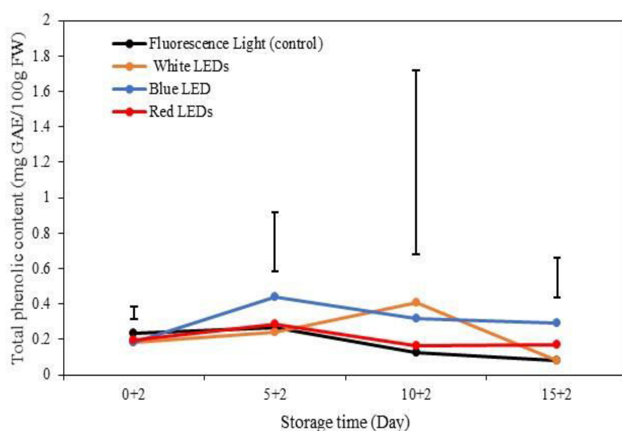

Figure 10: Effects of different lights wavelength exposure on total phenolic content of pineapple Morris stored at 5 days interval in (10+3°C) and following 2 days at ambient temperature (25+3°C). Vertical bars represent Tukey's HSD at 5% level. (Tukey's HSD value on Day 0+2=0.074, Day 5+2=0.328, Day 10+2=1.043 and Day 15+2=0.227).

According to Lee and Kader (2000), low content of ascorbic acid in pineapple causes higher susceptibility to CI when stored temperatures below 7°C. The ion leakage is the main factor of membrane damage and loss of membrane integrity due to respiration rate (Shadmani et al., 2015). In general, the increase of ion leakage in pineapples (most of pineapple cultivars) will increase the membrane damage corresponded to longer storage periods. The lowest score of chilling injury indicates highest value of ascorbic acid content in pineapple. As natural antioxidants in fresh commodities, ascorbic acid able to decrease or delay internal browning symptoms by the conversion of quinone to phenols (Raimbault et al., 2011). Moreover, ascorbic acid also can scavenge reactive oxygen species (ROS) which then will maintain the membrane structure and function (Lamb and Dixon, 1997). In present study, although no apparent effect was observed, pineapple treated with blue LEDs tended to show higher value of ascorbic acid until the end of experimental periods (Figure 9). This indicated that blue LEDs, possibly enhance the antioxidant capability to scavange ROS. Cao et al. (2009) stated that membrane dysfunction corresponded with chilling stress causes by internal browning in fruit.

The total phenolic content (TPC) in fruits and vegetables is widely distributed and contributes to antioxidant intake and is known to have a health protective action in humans (Hossain and Rahman, 2011). Ascorbic acid, total phenolic content and the activities of PPO play important roles in the normal defence mechanisms of plants under abiotic and biotic stresses including during enzymatic browning reactions (Supapvanich and Promyou, 2013). Nilprapruck and Yodmingkhwan (2009) and Boonyaritthongchai (2017) stated that internal browning in pineapple causes by the association with an increase in polyphenol oxidase (PPO) activity, accumulation of total phenolic compounds and membrane breakdown

In this study, pineapple treated with fluorescence and LEDs tended to show fluctuated trend on the values of TPC throughout the storage period (Figure 10). Stewart et al. (2001) stated that internal browning symptoms in pineapple fruit from chilling stress were correlated with the oxidation of polyphenols by PPO activity. The production of brown compounds occurred from the polymerization of quinone products from phenol oxidation. The brown compound then will bind with amino groups of cellular proteins. However, Leungwilai et al. (2018) reported that phenolic compounds cannot be used as an indicator of CI or internal browning during 10°C storage in pineapple. Generally, the occurrences of internal browning in fruit flesh due to the induction of browning enzymes activity such as POD which responsible in conversion of phenolic compounds to melatonin (Stewart et al., 2001). In this study, the results showed no despicable change on the value of TPC during 10°C storage stored for 15+2 days eventhough CI and internal browning were detected throughout the experimental periods. The results supported by Soares et al. (2005) study which stated that during chilling induced higher phenolic acid synthesis. However, some phenolic compounds that newly produced were converted to the polymers which responsible to internal browning associated with CI. Therefore, the TPC values were the same and maintain until the end of storage.

Conclusion

Blue LED delays CI incidence by maintaining the mobility of metabolic substances from one tissue to another in Morris pineapple stored in chiller at 10°C. In addition, blue LED maintains the postharvest quality of pineapple fruits. For future study, it is recommended to conduct sensory evaluation test to observe the acceptability of consumer. This test is to ensure that the fruits are still acceptable by consumer even though no appearance changes occur. Besides that, it is recommended to conduct different exposure times of LEDs on pineapple to further determine its effect on delaying chilling injury incidence.

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Effects of Acidic Soil on Root Growth of Potential Slope Plants

Fahmi, S., Normaniza, O.* and Rosazlin, A.
Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
**E-mail: normaniza@um.edu.my*

Introduction

The slope soils in Malaysia are mostly infertile and have a negative impact on plant productivity for it is acidic in nature. Acidic soil obstructs root elongation and retards root growth, thus restricting access to water and nutrients. This condition results in poor plant growth and reduced slope coverage. The inhibited root profile in acidic slope hampers root coherence which further complicates the prevention of soil due to (Harter, 2002). Acidic slope condition reduces vegetation coverage which subsequently leads to high erosion potential due to unstable slope (Saifuddin et al., 2016). Thus, the use of vegetation has become an alternative approach for slope stabilization against erosion besides minimizing the occurrence of landslides. The process of evapotranspiration facilitates reduction of pore pressure while increasing the shear strength of soil, thus increasing its resistance (Normaniza and Barakbah, 2011). In this study, there are three potential slope plants examined namely *Melastoma malabathricum, Hibiscus rosa-sinensis* and *Syzygium campanulatum*. For the *M. malabathricum* and *H. rosa-sinensis*, they were chosen based on their potential to eliminate Al ion from the soil and to adsorb contaminant such as zinc ion, cadmium and lead, respectively (Rohailah, 2011; Vankar et al., 2012; Bhaduri and Fulekar, 2015). Whilst, *S. campanulatum* has high resistance towards harsh environment by increasing its biomass (Arunbabu et al., 2015). Therefore, it is crucial to identify the most suitable plant that has the ability to rehabilitate the acidic condition of the slope. Hence, the objective of this study is to identify the effect of acidic soil on root profiles of potential slope plant species.

Materials and Methods

Treatment and experimental design

This experiment was carried out in a glasshouse located at Rimba Ilmu, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia (3° 7' 52.1076" N, 101° 39' 25.218" E). Three different species of plants with varying degree of tolerance to acidic soil condition were chosen namely *M. malabathricum*, *H. rosa-sinensis* and *S. campanulatum*. These plants were planted at three different levels of soil pH, 3-4(T1), 4-5(T2) and 6-7(C), as control. Each plant was planted in a polybag with the size of 36 cm x 20 cm x 16.5 cm and a soil volume of 8910 cm^3. The soil in T1 and T2 were pre-treated with aluminium sulphate ($Al_2(SO_4)_3$). A soil incubation experiment in the laboratory was executed to determine the actual amount of aluminium sulphate ($Al_2(SO_4)_3$) needed to reach the targeted soil pH level for the treatment applied in the glasshouse experiment. A 100 g air-dried and 2 mm ground soil was placed in a plastic cup and mixed with five incremental rates (0.1, 0.2, 0.3, 0.4 and 0.5 g) of aluminium sulphate ($Al_2(SO_4)_3$). The soil was then moistened with distilled water, with a field capacity of 60% and placed under a polyethylene cover containing a hole. After two weeks, soil pH has reached the targeted pH (Baquy et al., 2017). Every treatment was replicated five times and arranged in a Completely Randomized Design (CRD) design. All the plants were watered twice per day with 1.2 L/day to keep the soil moisture below the field capacity. The whole experiment was conducted for 12 weeks. The Photosynthetically Active Radiation (PAR), relative humidity (RH) and atmospheric temperature of the glasshouse ranged at 300–2000 µE mol m^{-2} s^{-1}, 65-90% and 25-28°C, respectively.

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Determination of biomass

After three months of observation, the fresh shoot and root weight of plants were determined. All the roots and shoots were then oven dried at 80ºC for 48h and the dry weight were obtained by using an electrical weighing balance.

Root profiles

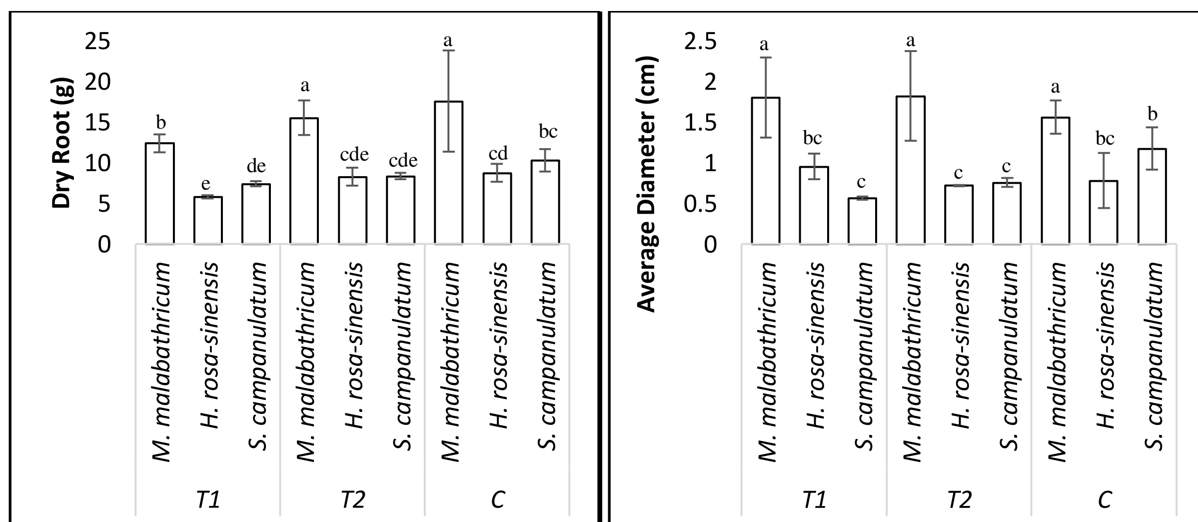
Determination of the root morphological parameters was done by using a root image analyzer (WinRHIZO (Pro v. 2008a., Regent Instruments Inc., Canada). The roots were placed on acrylic trays and rearranged to reduce root overlapping and crossing to get a clear scan image of the root. The scans of the root samples were analyzed by the software to obtain the data of total root length, root volume and diameter of root. The root diameter was categorized into the following classes: very fine (<0.1 mm), fine (0.1–0.2 mm), medium (0.2–0.5 mm), and coarse roots (>0.5 mm) at 10-cm intervals.

Statistical analysis

All the data were analyzed by using the two-way ANOVA procedure in the SPSS software followed by correlation analysis among parameters.

Results and Discussion

The results showed that the root biomass of *M. malabathricum* was the highest among the species in all treatments, proving that it is the most tolerant to acidic soil condition as compared to the other two plant species. Similarly, *M. malabathtricum* showed the highest in both root diameter and root volume, followed by *S. campanulatum* and *H. rosa-sinensis* across all treatments. Previous study had reported that thickening of root was found in roots that have been jeopardized to high acidity, which allows for greater pressure to be exerted on the soil (Croser et al., 2000; Bengough et al., 2005).



**Different letters showed significant difference at p≤0.05, n=5 by Duncan's New Multiple Range Test (DMRT).*

Figure 1: Dry root biomass of the species studied in each treatment.

Figure 2: Average root diameter of the species studied in each treatment.

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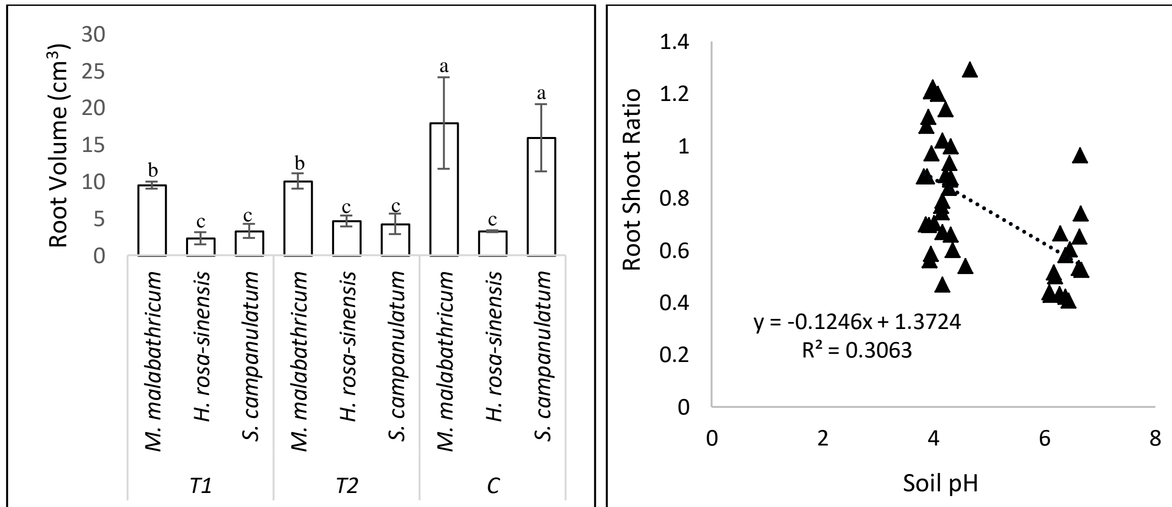

**Different letters showed significant difference at p≤0.05, n=5 by Duncan's New Multiple Range Test (DMRT).*

Figure 3: Root volume of the species studied in each treatment.

Figure 4: Correlation analysis between soil pH and root shoot ratio (r=0.55).

Furthermore, high root volume was found in high root diameter, indicating that it may increase soil root matrix, which eventually may increase effectiveness in absorbing moisture, thus improving saturation matric. As a result, eradication of disproportionate moisture would force to drying process of the soil (Saifuddin et al., 2013). This finding may lead to the promising characteristic of soil reinforcement of *M. malabathricum* via both mechanical and hydrological in acidic condition.

Apart from that, it is observed that the soil pH was inversely correlated with the root to shoot ratio (Figure 4). The result indicates that there is a greater root contribution to plants than shoots when the plants were grown in a lower pH condition. The alteration of root morphology may partly tolerate low pH by water conservation (Kidd and Proctor, 2001).

Furthermore, the root architecture of the three selected potential plants were identified (Figure 5). *M. malabathricum* showed dense and shallow roots (M-type) which is deemed suitable in controlling soil erosion (Reubens et al., 2007) while *H. rosa-sinensis* was identified as more promising than the other root system because it has homogenous reinforcement effect on both lateral and tap roots which developed vertically and horizontally. Thus, the VH-type root architecture displayed by hibiscus is most effective in stabilizing the slope and wind resistance (Reubens et al., 2007). Meanwhile, the root architecture of *S. campanulatum* (Figure 5) was observed as oblique root (R-type), which may sustain a longer intransigence of soil catastrophe (Li et al., 2016). Irrespective of the differences in root profile parameters, the root architecture of the slope plant species was not much affected by soil acidity. Moreover, unlike the norm, where acidic soil retards the growth and development of plants, our observation implies the selected plant species, except *H. rosa-sinensis*, as having tolerance mechanism towards acidic condition for they did not suffer from root growth inhibition. The results also suggest that *M. malabathricum* and *S. campanulatum* could be grown on slope with severe acidic condition whilst *H. rosa-sinensis* on slope with soil pH of more than 4.

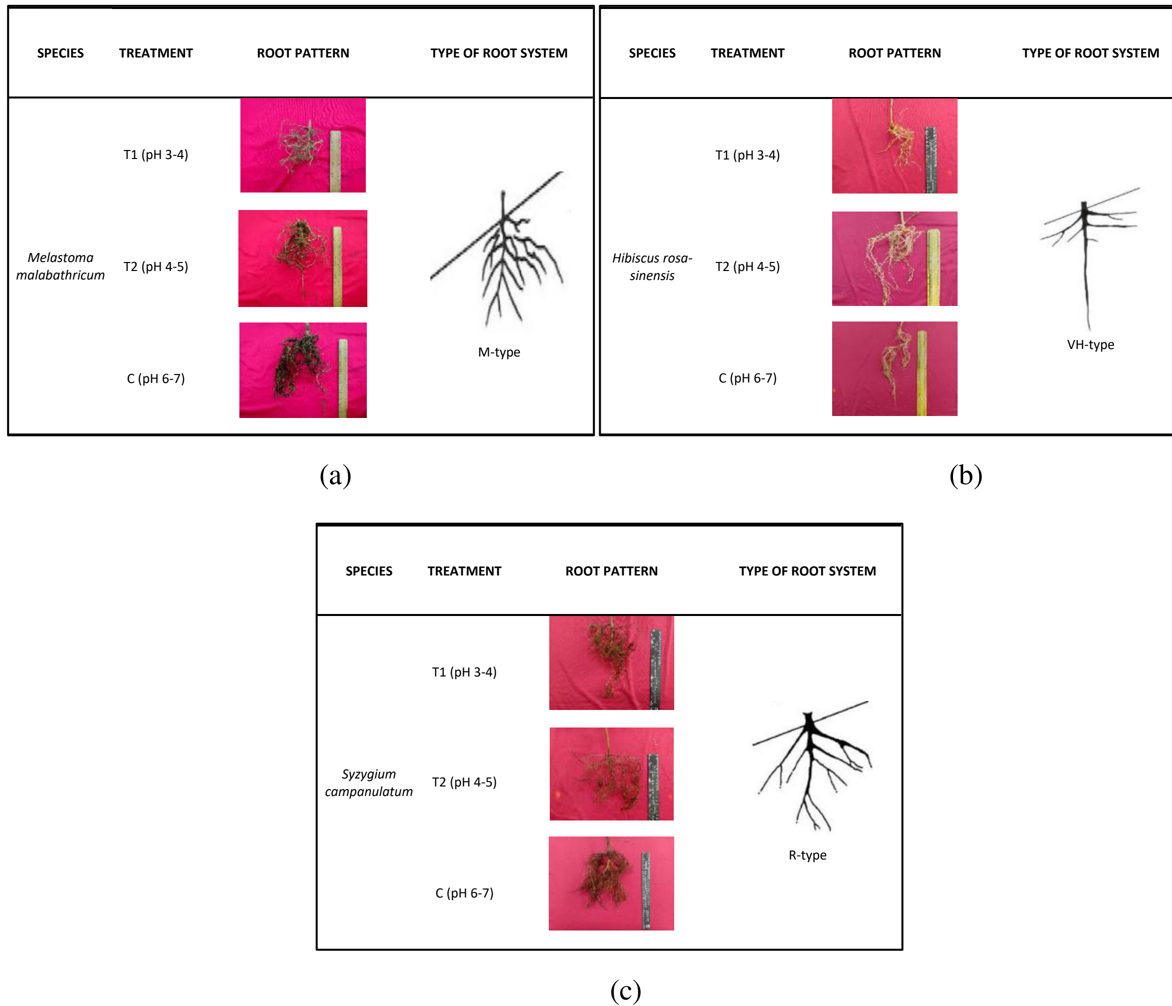

Figure 5: Root architecture and profiles of selected slope plants; (a) *M. malabathricum*, (b) *H. rosa-sinensis* and (c) *S. campanulatum* in three soil pH treatments.

Conclusion

In general, soil acidic condition always leads to an adverse impact on the plant growth especially on the root growth. In this study, however, *M. malabathricum* showed an inverse effect by having the highest root growth in terms of root biomass and root average diameter in lower soil pH conditions (T1 and T2). Correlation analysis also showed that the root to shoot ratio is observed higher at lower soil pH, implying the tolerance mechanism of the species studied towards acidity. The soil architectures of *M. malabathricum* and *S. campanulatum* were also not much affected by the acidic condition, indicating that selected plants have tolerance mechanism by maintaining if not increasing the root growth in a lower soil pH. The results also indicate that the soil pH ranged of 4-5 is considered as the most suitable condition for establishing an extensive root profile, a prominent criterion for slope plants grown on acidic slope condition. In conclusion, these initial findings could serve database of plant profiles which can grow and withstand the acidic condition of the slope in slope rehabilitation programme.

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Chapter 3

Post-harvest Technology and Quality Control

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The Effects of Water Management on Plant Physiological Performances, Plant Growth and Yield in Rice (*Oryza sativa* L.)

Mohd Aziz, R.[1,*], Fauzi, J.[1], Hariz, M.A.R.[1], Zabawi, M.A.G.[1], Norlida, M.H.[2], Fazlyzan, A.[1], Norfarhah, A.R.[3], Shaidatul, A.A.T.[1], Nizam, S.A.B.[1], Subahir, S.[1], Malek, A.K.[1], Liyana, N.Z.[1], Ghazali, M.R.[1], Alif, O.M.M.[1], Azmie, M.N.A.J.H.[2], Czahari, C.M.[2] and Alif, M.S.[2]
[1]Agrobiodiversity and Environment Research Centre, [2]Soil Science, Water and Fertilizer Research Centre, [3]Technology Commercialization and Business Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: azizrd@mardi.gov.my*

Introduction

Reducing water input and methane (CH_4) while maintaining grain yield is essential for sustainable rice production. Climate change has brought an alarming situation in the scarcity of fresh water for irrigation due to the present global water crisis, climate variability, drought, increasing demands of water from the industrial sectors and contamination of water resources. In Malaysia, the largest fresh water withdrawal of more than 75% is for irrigation in the agriculture sector and is mainly confined to irrigated rice production (Sariam and Anuar, 2010). Rice is a heavy consumer of water, but its water use efficiency is relatively low. It is estimated about 3,000 L of water is used to produce 1 kg of rice and that the water productivity index (WPI) of rice is 0.3 kg grain/m^3 water. Fresh water is becoming increasingly scarce resources (Rijsberman, 2006), which has posed a serious threat to the productivity and sustainability of irrigated paddy systems in many countries (Peng et al., 2009). The present global water crisis, climate variability, drought, increasing demands of water from the industrial sectors and contamination of water resources made water more scarce for irrigation (Dawe, 2005; Sariam and Anuar, 2010).

Irrigated rice is normally grown in a flooded environment during most of its growing period; thus, growing rice requires a large amount of water. Flooding conditions create anaerobic conditions and a high level of organic substrates in the soil, which increase the activity of methanogenic bacteria that produce CH_4 (Buendia et al., 1997). The Third National Communication and Second Biennial Update Report to the United Nations Framework Convention on Climate Change (UNFCCC) reported that in 2014, rice cultivations in Malaysia produced 88.08 Gg of methane (CH_4) that contributed to 21.84% of greenhouse gases (GHG) emissions from the agriculture sector. Paddy water management and water-saving irrigation are promising options for CH_4 mitigation (Tyagi et al., 2010). The previous study on Tanjung Karang paddy field showed that maintaining saturated soil conditions throughout or at certain stages of rice cultivations decreased 20-40% of CH_4 emissions as compared to continuous flooding (unpublished data). Therefore, this study was conducted to examine the effectiveness of different water management on conserving water, plant growth, plant physiological performance, mitigating GHG and maintaining yields in rice production.

Materials and Methods

A randomized complete block experimental design was set-up in a greenhouse in MARDI Serdang from October 2017-February 2018. The treatments consist of six different water management with four replications (Table 1). The six different water management are continuous flooding (T1), continuous saturated soil condition (T2), flooding from transplanting to panicle initiation (PI) and saturated until harvest (T3), saturated from transplanting to PI and flooding until harvest (T4), flooding from transplanting to grain filling and saturated until harvest (T5) and saturated from transplanting to grain filling and flooding until harvest (T6). The plants of MARDI Siraj 297 rice variety were grown in 1.5 m x 1.5 m x 45 cm square troughs containing soil from Tanjung Karang paddy field. The plants were fertilized with 120:70:80 kg/ha of N, P_2O_5 and K_2O. Non-destructive growth analysis and leaf physiological performance were conducted at four phases of growth (tillering,

active tillering, flowering and ripening). Plant growth parameters determined were plant height, tiller number, leaf width and stem width. Relative leaf chlorophyll content of stevia was determined using a SPAD, portable chlorophyll meter (SPAD-502, Konica-Minolta, Japan). The measurements of net photosynthetic rates, stomatal conductance and transpiration rate were taken using a portable photosynthesis system (LI6400XT, LICOR Inc., Nebrasca, USA). The chlorophyll fluorescence measurements were made using a portable Plant Efficiency Analyzer (PEA) (FMS 2, Hansatech Instruments Ltd, U.K.). The Fv/Fm ratio was used to determine the leaf chlorophyll fluorescence responses.

The grain yield was obtained using crop cutting test (CCT) of 1 m by 1 m. The harvested grains were dried, winnowed and weighed. The weight was then converted to per unit area crop yield based on 14% grain moisture content and presented as grain yield (t/ha). Data obtained were analyzed for statistical analysis using a one-way Analysis of Variance (ANOVA) to test the significant effect of all variables investigated. Means separation was performed using the least significant difference (LSD) method at 5% (P = 0.05) by the statistical package of SAS 9.3 Institute Inc. USA.

Table 1: Water management treatment for rice production.

	Transplant to PI (40 DAT)	PI to grain filling (70 DAT)	Grain filling to harvest (110 DAT)
T1	Flooding		
T2	Saturated		
T3	Flooding	Saturated	
T4	Saturated	Flooding	
T5	Flooding		Saturated
T6	Saturated		Flooding

Results and Discussion

Plant growth parameter and relative chlorophyll content

Plant growth parameter and relative chlorophyll content of rice of all water management treatment showed no significant difference during tillering, active tillering, flowering and ripening stages. These results indicated that saturated soil conditions did not affect the plant growth of rice as compared to flooded conditions. Volumetric water content and metric water potential of flooding and saturated conditions were not significantly different, indicated that saturated soil conditions provided adequate soil moisture content for the plant growth similar to flooded conditions. Although there was no observable standing water in the field, rice can take up adequate water from the subsurface soil around the root zone (Lempayan et al., 2015).

Table 2: Plant growth performance and relative chlorophyll content (SPAD reading) as affected by water management treatment.

Treat-ment	Tillering stage					Active tillering stage				
	Height (cm)	Tiller No.	Leaf Width (cm)	Stem Width (cm)	SPAD	Height (cm)	Tiller No.	Leaf Width (cm)	Stem Width (cm)	SPAD
T1	68.0^{a}	19.9^{b}	1.0^{a}	1.0^{a}	42.7^{a}	93.7^{b}	39.1^{a}	1.3^{a}	1.4^{a}	46.4^{a}
T2	71.8^{a}	26.0^{a}	1.0^{a}	1.0^{a}	41.2^{a}	98.0^{ab}	45.6^{a}	1.3^{a}	1.4^{a}	46.9^{a}
T3	70.2^{a}	20.0^{b}	1.0^{a}	1.0^{a}	43.2^{a}	95.0^{ab}	39.7^{a}	1.3^{a}	1.3^{a}	46.4^{a}
T4	70.8^{a}	21.2^{ab}	1.0^{a}	1.9^{a}	42.1^{a}	96.8^{ab}	41.1^{a}	1.3^{a}	1.3^{a}	46.8^{a}
T5	72.1^{a}	23.4^{ab}	0.9^{a}	0.9^{a}	41.7^{a}	99.6^{a}	44.6^{a}	1.3^{a}	1.4^{a}	46.7^{a}
T6	68.4^{a}	18.8^{b}	0.9^{a}	0.9^{a}	44.9^{a}	94.6^{ab}	39.0^{a}	1.3^{a}	1.3^{a}	46.8^{a}

Treat-ment	Flowering stage					Ripening stage				
	Height (cm)	Tiller No.	Leaf Width (cm)	Stem Width (cm)	SPAD	Height (cm)	Tiller No.	Leaf Width (cm)	Stem Width (cm)	SPAD
T1	123.9[a]	52.7[a]	2.2[a]	1.6[a]	52.2[a]	130.1[a]	47.6[a]	2.1[a]	1.4[a]	49.4[a]
T2	122.2[a]	50.8[a]	2.2[a]	1.5[a]	48.8[a]	133.5[a]	52.7[a]	2.2[a]	1.5[a]	48.4[a]
T3	121.7[a]	48.7[a]	2.2[a]	1.5[a]	48.3[a]	128.9[a]	50.8[a]	2.2[a]	1.5[a]	49.5[a]
T4	120.8[a]	47.9[a]	2.2[a]	1.5[a]	47.6[a]	132.8[a]	48.7[a]	2.2[a]	1.5[a]	46.4[b]
T5	119.8[a]	47.7[a]	2.1[a]	1.5[a]	47.6[a]	132.8[a]	50.3[a]	2.2[a]	1.5[a]	48.9[a]
T6	119.4[a]	47.6[a]	2.1[a]	1.5[a]	47.5[a]	130.5[a]	47.4[a]	2.3[a]	1.5[a]	48.7[a]

**Means followed by the same letter within column are not significantly different by Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.*

Leaf physiological responses

The study on leaf photosynthesis rate, stomatal conductance and transpiration rate are essential for a basic understanding of leaf physiology and plant productivity (Schaper and Chacko, 1993). Most of the leaf physiological performance was not significantly different between all water management (Table 3). Volumetric water content (60-65%) and metric water potential (0-5 kPa) of flooding and saturated soil conditions were not significantly different, indicated that both soil conditions provided adequate soil moisture content to support leaf physiological activities of photosynthesis, stomatal conductance and transpiration. Chlorophyll fluorescence (Fv/Fm ratio) between different water management also showed no significant difference at all plant stages. Chlorophyll fluorescence provides detailed information on the saturation characteristics of electron transport, as well as the overall photosynthetic performance of a plant (Ralph and Gademan, 2005). In this study, Fv/Fm ratios of flooding and saturated soil conditions of all water management were mostly between 0.75-0.80 and high Fv/Fm indicated that plants were at higher plant photosynthetic performance and not under stress factors such as drought or limited water condition.

Table 3: Leaf physiological responses and chlorophyll fluorescence (Fv/Fm ratio) as affected by water management treatment.

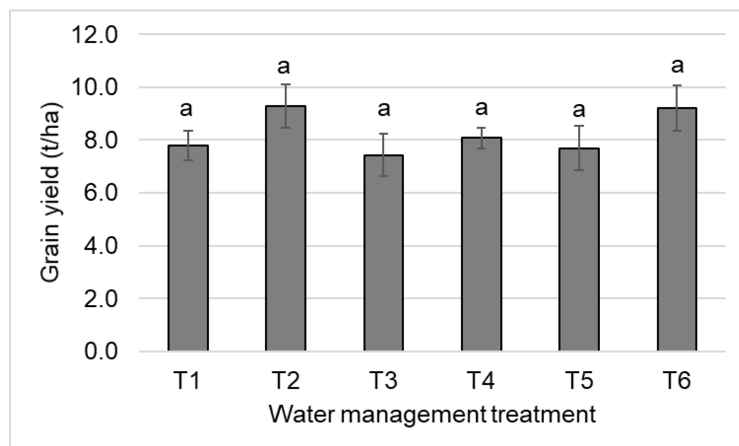
Treat-ment	Tillering stage				Active Tillering stage			
	Photo-sythetic rate (µmol/ m^2/s)	Stomatal conduc-tance (mol/ m^2/s)	Leaf trans-piration rate (mmol/ m^2/s)	Chloro-phyll fluores-cence (Fv/Fm)	Photo-sythetic rate (µmol/ m^2/s)	Stomatal conduc-tance (mol/ m^2/s)	Leaf trans-piration rate (mmol/ m^2/s)	Chloro-phyll fluores-cence (Fv/Fm)
T1	29.47[a]	1.30[ab]	6.16[a]	0.75[a]	28.30[a]	0.81[a]	8.09[a]	0.76[a]
T2	29.94[a]	1.18[ab]	6.15[a]	0.73[a]	28.38[a]	0.81[a]	8.19[a]	0.78[a]
T3	29.97[a]	1.13[ab]	6.15[a]	0.76[a]	29.64[a]	0.77[a]	7.98[a]	0.78[a]
T4	26.01[b]	0.91[b]	5.70[b]	0.75[a]	29.48[a]	0.83[a]	8.33[a]	0.76[a]
T5	28.99[a]	1.00[b]	6.26[a]	0.75[a]	28.89[a]	0.82[a]	8.42[a]	0.78[a]
T6	30.47[a]	1.5[a]	6.33[a]	0.76[a]	30.14[a]	0.86[a]	8.44[a]	0.77[a]

Treatment	Flowering stage				Ripening stage			
	Photosythetic rate (µmol/m²/s)	Stomatal conductance (mol/m²/s)	Leaf transpiration rate (mmol/m²/s)	Chlorophyll fluorescence (Fv/Fm)	Photosythetic rate (µmol/m²/s)	Stomatal conductance (mol/m²/s)	Leaf transpiration rate (mmol/m²/s)	Chlorophyll fluorescence (Fv/Fm)
T1	15.54^{a}	1.15^{a}	14.14^{a}	0.81^{a}	14.23^{b}	0.58^{b}	6.27^{b}	0.77_{a}
T2	13.58^{a}	0.98^{a}	14.10^{a}	0.81^{a}	16.26^{a}	0.76^{a}	7.35^{a}	0.73^{a}
T3	13.11^{a}	0.95^{a}	13.87^{a}	0.80^{a}	14.34^{b}	0.77^{a}	7.15^{a}	0.73^{a}
T4	13.06^{a}	1.13^{a}	14.14^{a}	0.79^{a}	13.92^{b}	0.88^{a}	7.77^{a}	0.74^{a}
T5	13.39^{a}	0.87^{a}	13.63^{a}	0.80^{a}	16.27^{a}	0.81^{a}	7.83^{a}	0.75^{a}
T6	13.33^{a}	1.03^{a}	14.18^{a}	0.78^{a}	17.59^{a}	0.89^{a}	8.00^{a}	0.73^{a}

**Means followed by the same letter within column are not significantly different by Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.*

Grain yield

The grain yield of rice under different water management system was between 7.4-9.2 t/ha and showed no significant difference between treatment (Figure 1). These results indicated that conserving water by maintaining soil water conditions at saturation did not cause any reduction in grain yield. Water management at saturated soil condition was shown to sustain similar moisture content in the soil, thus supporting leaf physiological and plant growth performances that resulted in maintaining a high grain yield of rice. Maintaining soil at saturated condition could also be an effective technique to conserve water and reducing CH_4 emission. Moreover, it has the potential to prevent the reduction of grain yield by which the other adaptation strategy, such as Alternate Wetting and Drying (AWD) technique, was reported to cause a decrease in grain yield (Sariam and Anuar, 2010; Xu et al., 2015).



Means followed by the same letter are not significantly different by Fisher's Least Significant Difference (LSD) test at $p \leq 0.05$.

Figure 1: Grain yield by different water management treatment.

Conclusion

Plant physiological performance and growth parameters of rice were mostly not significantly different between all water management at all plant stages. These results indicated that flooded condition was not a requirement for rice plant at any stage or throughout the growing period for the plant's optimal physiological and growth performance. Water management at saturated soil condition was shown to sustain similar moisture content in the soil, thus supporting leaf physiological and plant growth performances that resulted in maintaining high grain yield of rice production. Rice cultivation by maintaining soil at saturated conditions could be an effective adaptation technique for simultaneously saving water and mitigating GHG without reducing rice yields.

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Terung Rapuh (*Solanum melongena* L.): From Traditional to New Potential Vegetable in Postharvest Aspect

Nur Syafini, G.[1,*], Azhar, M.N.[1], Nurul Khdijah, R.[1], Razali, M.[1], Umikalsum, M.B.[2] and Rozlaily, Z.[3]
[1]Horticulture Research Centre, [2]Biodiversity and Environment Research Centre, [3]Industrial Crop Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: syafini@mardi.gov.my*

Introduction

Eggplant (*Solanum melongena* L.) is a fruity tropical vegetable and consists of various shapes, sizes, and colours such as purple, green, white or striped, depending on cultivars (Analia et al., 2007). Eggplant is an essential vegetable in the world market, especially in Asia and the Mediterranean. In Malaysia, some traditional eggplant cultivars, such as terung rapuh, terung telunjuk and terung asam, have a potential for commercialisation, and yet, they are commonly planted in a small-scale. Among the cultivars, terung rapuh is gaining popularity due to high nutrient value and rich in phenolic compounds (Hanson et al., 2006). It is typically eaten as an *ulam*, or added in curry dishes. The high phenolics properties indicate the degree of potential antioxidant, antibacterial and immunostimulant. However, the information on postharvest handling of the traditional vegetables, especially terung rapuh in Malaysia, is limited. According to Cantwell and Suslow (2013), the quality of commercial eggplant is based on maturity, fruit freshness, free from injury or defect, skin colour and green petals. Proper postharvest handling practices can preserve the fruit quality, prolong the shelf life and reducing losses due to dehydration and diseases (Abdullah, H., 1999). Hence, this study aims to investigate the right harvesting maturity, optimum storage temperature, and proper techniques to maintain the freshness of terung rapuh during the storage period.

Materials and Methods

Determination of maturity harvesting

Terung rapuh was planted at the experimental plot in Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor. The flowers at full bloom stage were tagged, and the assessment of maturity harvesting was conducted by collecting data on fruit's growth and colour at an interval of four days until day 35. After 17 to 35 days of tagging, terung rapuh was harvested, and the samples were placed at ambient condition, 28±1°C. Rates of respiration and ethylene production, as well as colour of the pericarp, were measured and recorded.

Determination of optimum storage temperature

Terung rapuh was harvested between day 21-25, sorted and packed in low-density polyethylene (LDPE) plastic bag with 0.04 mm thickness. Then, the vegetable was stored at three different storage temperatures (4, 7 and 10°C) for 28 days, and exposed to ambient temperature 28±1°C to allow for the development of chilling injury. The postharvest quality assessment was evaluated visually, and the criteria used were retention of original colour, freshness and severity. The colour was measured using a chromameter (Model CR-400 Minolta, Japan). Each colour value of lightness (L*), chroma (C*), and hue angle (h°) was expressed as the means of three measurements. The texture of terung rapuh was measured using a texture analyzer (Model 1140 Instron Universal Testing Machine) with a 2 mm diameter size probe. Soluble solids content (SSC) was determined with a digital refractometer (Model DBX-55, Atago Co., Ltd, Japan). Titratable acidity (TTA) was determined by titrating 20 mL of extraction with 0.1 mol^{-1} NaOH to pH 8.2 (Shaw et al. 1987). Ascorbic acid content was determined by extraction of 10 g of sample with the addition of 100 mL of 3% metaphosphoric acid. Then, 10 mL

of extraction was titrated immediately with a standard dye solution to the first permanent pink endpoint. All the samples were analyzed in weekly intervals.

Determination of suitable packaging

Four different packagings were used to determine the best packaging. There were LDPE 0.04 mm (as a control), polypropylene (PP) with two thicknesses (0.04 mm and 0.08 mm), and film wrapping (0.008 mm thickness). Terung rapuh were then stored at 10°C for 28 days. Postharvest quality assessment includes physical and chemical analysis at weekly intervals.

Statistical analysis

The experiment was designed using a completely randomized design with three replications. The obtained data were analyzed using analysis of variance (ANOVA), and mean comparison was conducted on the data collected using the Statistical Analysis System Version 9.4. The mean was separated by Duncan Multiple Range at the 5% level of significant treatment effects within the analysis of variance. Unless otherwise specified, all significant differences in this paper were $p \leq 0.05$.

Results and Discussion

Determination of maturity harvesting

Fruit growth data were taken as early as day 7 until day 35. At day 13 to 17, the fruit colour was dark purple with hue angle value ranged between 274.34-286.33 and specified as the immature stage (Figure 1). At day 21-25, the fruit became mature with pale purple colour and had the optimum fruit size. The optimum growth rate of fruit began between day 23-28, with optimum respiration based on CO_2 production (Figure 2). However, at day 28, fruit colour changed to pale purple to creamy with yellow trace at bottom and seeds becoming harder and more browning. It was shown that the fruits started to overripe and fruit colour changed to entirely yellow (86.25 hues) at day 35. According to Sargent (1998), the characteristics of a matured eggplant are the flesh becomes softer and spongy, the skin develops areas of an unusual colour, and the seeds become larger and harder. Overmature eggplants are pithy and bitter and feel soft to finger pressure.

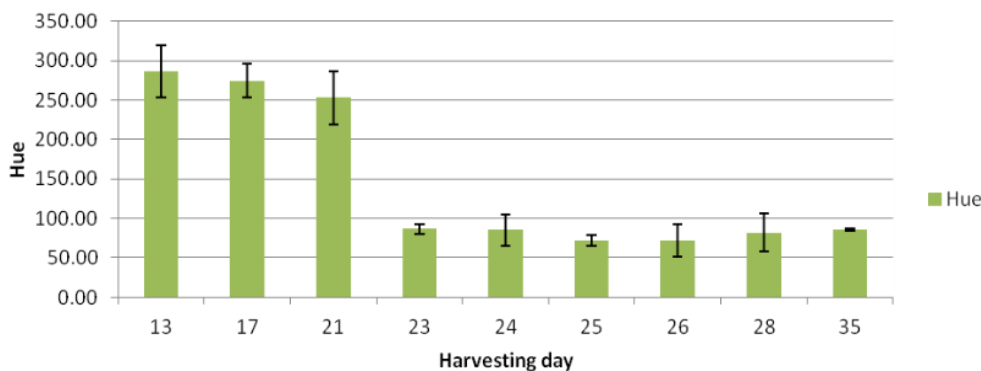

Figure 1: The changes of hue value of terung rapuh during fruit development.

(A) (B)

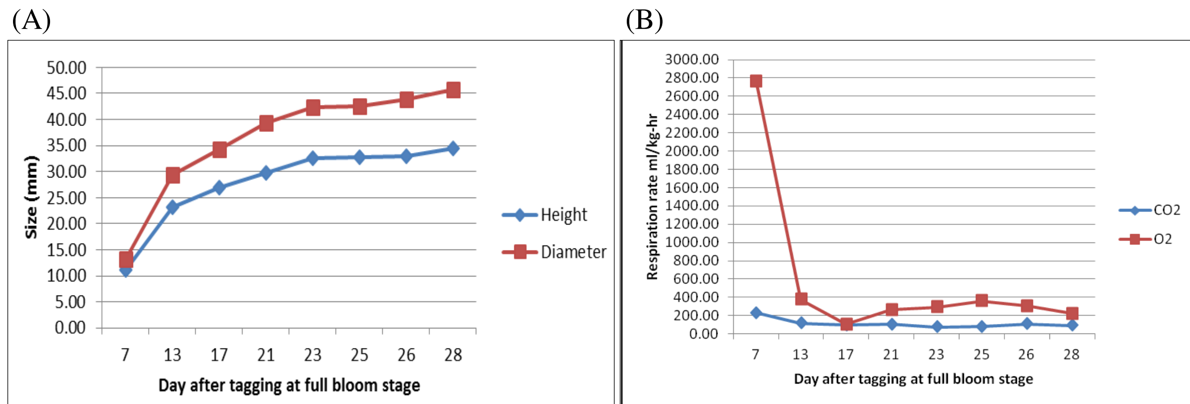

Figure 2: Growth rate (A) and respiration rate (B) of terung rapuh after harvest.

Determination of optimum temperature storage

According to Table 1, the results showed that terung rapuh stored at 10°C maintained the quality and extended the storage life up to 21 days compared to 14 days at the lower temperature (4°C and 7°C). The shelf life of the fruit at ambient temperature was four to five days. The fruit texture maintained the firmness at 10°C compared to other temperatures which have shown shriveling due to water loss and caused chilling injury and decay. At temperature below 10°C, the eggplants suffered physiological disorders, manifested mainly by the appearance of surface injuries, such as pitting and scald, seed darkening and flesh browning (Salunkhe and Desai, 1984). Similarly, Analia et al. (2007) found that the eggplant quality and shelf-life were degraded by the development of skin and tissue browning when stored at chilling temperatures.

There were significant differences in SSC, pH, TTA, sugar/acid ratio and texture between each temperature ($p>0.05$). At 7°C and 10°C, the fruits maintained firm as compared to 4°C. However, ascorbic acid content did not significantly affect by the storage temperatures (Table 1).

Table 1: Changes in soluble solids content (SSC), pH total titratable acidity (TTA), sugar/acid ratio, ascorbic acid content (AAC) and firmness of terung rapuh stored at different temperature (4°C, 7°C and 10°C).

Main factors	SSC (°Brix)	pH	TTA (% citric acid)	Sugar/ acid ratio	AAC (mg/100g)	Firmness (N)
Temperature (T)						
4°C	6.25[b]	5.94[b]	0.16[b]	40.81[a]	3.02	15.58[b]
7°C	6.66[a]	6.07[a]	0.16[b]	44.68[a]	2.97	17.22[a]
10°C	6.60[b]	5.90[b]	0.17[a]	38.95[b]	2.91	17.36[a]
F-Test Significant	*	*	*	*	NS	*
Storage period (D)						
0	5.93[c]	5.96[b]	0.17[a]	35.28[c]	3.20[a]	15.98
7	6.46[b]	6.06[a]	0.17[a]	38.10[bc]	3.14[a]	17.61
14	7.21[a]	5.97[b]	0.15[b]	50.85[a]	3.09[a]	17.11
21	6.42[b]	5.89[b]	0.16[b]	41.70[b]	2.43[b]	16.17
F-Test Significant	**	*	*	**	*	NS
Interaction T*D	*	*	*	*	NS	NS

Means separation within columns and main effect by Duncan's Multiple Range test at $p \leq 0.05$.
*NS, *, ** Non significant or significant or highly significant at $p \leq 0.05$, respectively.*

Determination of suitable packaging

The fruits packed in LDPE 0.04 mm and film wrapping can maintain the freshness, reduced browning and marketable up to 21 days at 10°C compared to PP 0.04 mm and PP 0.08 mm packaging, which

only lasted for seven days. Based on Table 2, LDPE 0.04 mm gave a high value of SSC, whereby film wrapping gave a high value of ascorbic acid content during storage. Packing with PP 0.04 mm and 0.08 mm showed minimal weight loss between 0.28-0.43% during the storage period compared to LDPE 0.04 mm and film wrapping (range 0.51-0.79% of weight loss). However, the quality appearance of terung rapuh in LDPE 0.04 mm and film wrapping were better to maintain the fruit freshness and reduced seed browning caused by chilling injury compared to both PP packaging.

Table 2: Changes in soluble solids content (SSC), pH, total titratable acidity (TTA), sugar/acid ratio, ascorbic acid content (AAC), firmness, and weight loss of terung rapuh with different type of packaging stored at 10°C.

Main factors	SSC (°Brix)	pH	TTA (% citric acid)	Sugar/ acid ratio	AAC (mg/100g)	Firmness (N)	Weight Loss (%)
Packaging							
LDPE 0.04 mm (control)	6.85^{a}	5.88^{b}	0.17	40.22	2.81^{c}	17.55	0.51^{b}
PP 0.08 mm	6.49^{b}	5.92^{b}	0.17	37.65	2.54^{d}	16.48	0.43^{c}
PP 0.04 mm	6.37^{b}	6.06^{a}	0.17	37.88	3.05^{b}	17.89	0.28^{d}
Film Wrapping	6.33^{b}	5.97^{c}	0.18	36.59	3.34^{a}	17.90	0.79^{a}
F-Test Significant	*	**	NS	NS	**	NS	**

Means separation within columns and main effect by Duncan's Multiple Range test at $p \leq 0.05$.
*NS, *, ** Non significant or significant or highly significant at $p \leq 0.05$, respectively.*

Conclusion

Harvesting maturity within day 21 to day 25 is considered to be at its maximum of visual and eating quality. The freshness and storage life of terung rapuh can be prolonged up to 21 to 28 days by storing at 10°C using suitable packaging materials (LDPE 0.04 mm and film wrapping).

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The Effect of Different Types of Plant Growth Regulators in Developing Parthenocarpic Melon Manis Terengganu and Improving its Postharvest Quality

Siti Aishah, Y. and Wan Zaliha, W.S.*
Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.
**E-mail: wzaliha@umt.edu.my*

Introduction

In Malaysia, watermelon (*Citrullus lanatus*), rockmelon (*Cucumis melo* var. Cantalupensis) and honeydew (*Cucumis melo* var. Inodorus) are widely cultivated and easily obtained. Among these three types of melon, rockmelon is sweeter and has more compact pulp than watermelon. Melons not only boost our health esteem, but can also become an important part of our healthy diet with its refreshing characteristics (Rasmuna and Nik, 2016). Recently, a new developed variety of Inoduros melon has been introduced exclusively for Terengganu known as Melon Manis Terengganu (MMT) and has been developed by the Green World Genetics (GWG) Sdn. Bhd., Selangor. Based on the report of Utusan Malaysia (2016), it has been claimed to have high nutritional value and can be further processed into various products such as melon ice cream, jelly, juice and jam. According to Sinar Harian (2015), 666 metric tonnes of MMT can be produced from 222,000 melon plants which are worth about RM2 million per harvest and it can reach RM6 million in a year. The demand for melon in Malaysia is relatively high. Melons are naturally low in fat and sodium, have no cholesterol and provide many important nutrients such as potassium and rich source of beta-carotene and vitamin C (Gene, 1997). However, melon contains a large number of seeds and it can be a nuisance. With today lifestyle, consumers prefer fruit without seeds as removing the seeds is time consuming and troublesome. With seedless fruit, customers can enjoy the fruit without the hassle of removing large number and cumbersome seeds. Seedless fruits also have better organoleptic attributes as compared to seeded fruits. In order to produce seedless MMT, spray application of GA_3, auxins (IAA, IBA and NAA), and their combinations could be the possible alternative to be conducted. The objective of this study viz. i) to determine the effect of different plant growth regulators in developing seedless MMT fruit and improving its postharvest quality and ii) to determine the best plant growth regulators in developing seedless MMT.

Materials and Methods

Planting materials, experimental location and experimental design

The experiment was conducted in the Greenhouse, Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu. The MMT seeds were purchased from Department of Agriculture, Kuala Terengganu. A total of 56 MMT seeds were sown in plug trays containing growing media of peat moss. Ten-day old seedlings were transferred to polybags sized 16 x 16 cm containing 3 kg of cocopeat. The fertilization of melon was the fertilizing standard operating procedures provided by Department of Agriculture. Fertilizers used in this study were type A [$Ca(NO_3)_2$ and iron] and type B [KNO_3, KH_2PO_4, $MgSO_4$, $MnSO_4$, $CuSO_4$, $ZnSO_4$, boric acid, ammonium molybdate or sodium molybdate]. The irrigation water was scheduled for 5 minutes, twice a day [7.30 a.m.–8.30 a.m. and 5.45 p.m.–6.45 p.m.]. At 20 to 30 days after sowing, the main stem was twisted along the trellis to allow the plant to grow upright. On day 36 after sowing, MMT flowers were exposed to hand pollination process. The manual pollination was done by picking the male flowers, removing the petals and placing the male pollens onto the female flower stigma. Hand pollination was done at 0930 solar power as it is the specific time for the flower to bloom (Shahid et al., 2011). Only one fruit was left to grow per plant. Fully matured fruits were harvested on day 70 to 75. The assigned treatments were applied on 36 day-after-sowing (DAS), once the female flower bloomed. The experiment was

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arranged in a randomized complete block design (RCBD) with seven (7) treatments viz i) Control without PGRs sprayed, ii) IAA at 800 ppm, iii) NAA at 800 ppm, iv) IBA at 800 ppm, v) 400 ppm IAA + 400 ppm GA_3, vi) 400 ppm NAA + 400 ppm GA_3 and vii) 400 ppm IBA + 400 ppm GA_3. Each treatment was replicated four (4) times with two (2) experimental unit each.

Parameter evaluation

Parameter evaluations includeed fresh weight, number of seeds, diameter of fruit, soluble solid concentration, flesh firmness, and total phenolic content and sensory evaluation.

Statistical analysis

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

Results and Discussion

In developing parthenocarpic fruits, many types of plant growth regulators (PGRs) with various combinations have been investigated. Indeed, they have been discovered and proven to have the ability of inducing seedless pepper, eggplant, dates, peach and loquat (Shaheen et al., 1988; Coneva and Cline, 2006; Mesejo et al., 2010; Tiwari, 2011). In teasle gourd which belongs to Cucurbitaceae family, parthenocarpic is induced by means of gibberelic acid (GA_3) (Rasul et al., 2008). However, the spray application of GA_3 only resulted in parthenocarpic teasle gourd when applied on the day of full bloom of female flower (Rasul et al., 2008). The other success of PGRs usage is the elimination of endocarp in stone fruits such as apples (Watanabe et al., 2008) and prevention of seed development for inducing seedless fruit production in grapes by GA_3 (Crane, 1964). Apart from that, PGR applications have also effectively increased the fruit set and abscission of Mandarin (Talon et al., 1990). Furthermore, treatments with PGRs can also be used in inducing a wide variety response including manipulation of fruit size, fruit shape and maturation (Zhang and Whitting, 2013).

Similarly in this present study, 400 mg/L of IAA combined with 400 mg/L GA_3 had developed seedless fruits with less number of seeds (Figures 1 and 2). The possible reason might be due to the response of IAA and GA_3 in discarding the occurance of zygote that will develop into seeds during double fertilization (Russell, 1992). Wittwer et al. (1957) claimed that gibberellin, which is also known as second type of growth substance, can stimulate parthenocarpic fruit set. Rasul et al. (2008) claimed that the usage of GA_3 at the concentration of up to 200 mg/L had induced the production of seedless teasle gourd while Tiwari (2011) claimed that GA_3 application enhanced the parthenocarpic fruit set in chilli pepper.

Interestingly, fruits without seeds (400 mg/L IAA + 400 mg/L GA_3) had similar fresh weight and flesh thickness with other PGR treatments and control fruits (Figures 3 and 4). According to De Jong et al. (2009), apart from the variation in cell elongation and cell division activity, there were differences in the morphology of fruits obtained after auxin and GA_3 application. Auxin and GA_3 applications have induced different changes in morphology, histology and sugar metabolism during the fruit development as reported by Tiwari (2011). In the present study, the fruit diameter exhibited relatively the same diameter. This might be attributed to the PGR spray application that played specific role in cell elongation and cell division (De Jong et al., 2009). The roles of IAA and GA_3 are specific in inducing seedless or aborting the formation of seed but not increasing the mass or size of fruits (Pandolfini et al., 2007). In contrast, Serrani et al. (2008) and Vivian-Smith and Koltunov (1999) claimed that the auxin-induced tomato had bigger size than the GA_3 induced fruit. However, in order to obtain the better results as in the present study, the spray application of PGRs should be applied continuously and more frequently to the target cell.

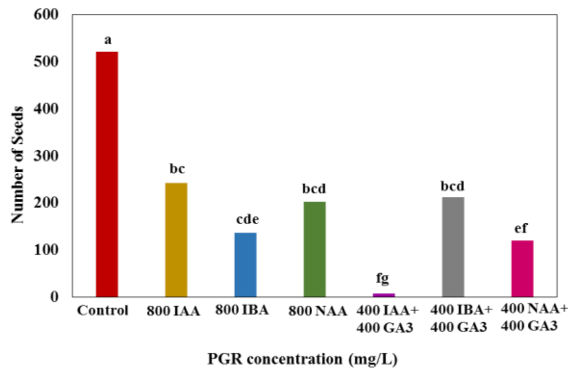

Figure 1: Effect of different types of plant growth regulators in developing parthenocarpic Melon Manis Terengganu (MMT) on number of seeds of MMT. Means with different letters are significantly different at 5% level according to HSD test.

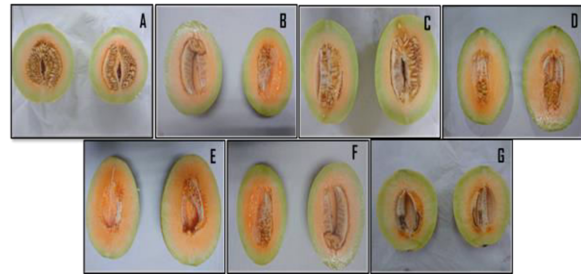

Figure 2: Number of MMT seeds on 75 DAT. A: Control, B: 800 mg/L IAA, C: 800 mg/L IBA, D: 800 mg/L NAA, E: 400 mg/L IAA + 400 mg/L GA_3, F: 400 mg/L IBA + 400 mg/L GA_3, G: 400 mg/L NAA + 400 mg/L GA_3

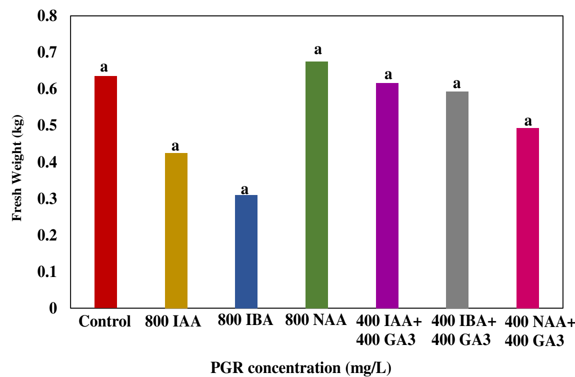

Figure 3: Effect of different types of plant growth regulators in developing parthenocarpic Melon Manis Terengganu (MMT) on fresh weight of MMT. Means with different letters are significantly different at 5% level according to HSD test.

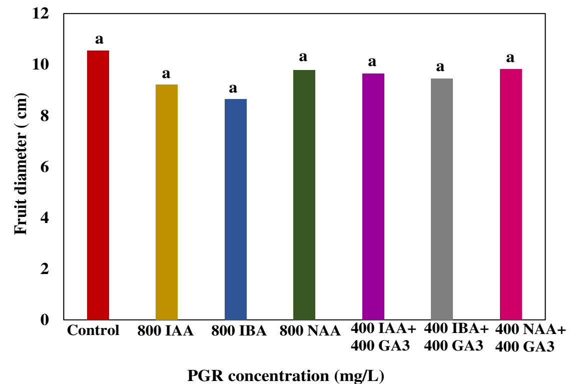

Figure 4: Effect of different types of plant growth regulators in developing parthenocarpic Melon Manis Terengganu (MMT) on fruit diameter of MMT. Means with different letters are significantly different at 5% level according to HSD test.

All of the postharvest quality parameters such as flesh firmness, soluble solids concentration (SSC) and total phenolic content (TPC) were not significantly influenced by the application of different types of PGRs and their combinations. (Table 1). The values of SSC in all treatments have ranged from 11.60% to 12.91% which were in agreement with Aisyah Athirah et al. (2017). Previous reports claimed that fruits treated with auxin and GA_3 were firmer than non-treated fruits for some fruits such as tomato (Serrani et al., 2008) and sweet cherry (Proebsting and Mills, 1964). In the present study, even though no difference was observed on flesh firmness, PGRs treated fruits tend to show firmer flesh as compared to control fruit. Similarly, Wan Zaliha and Norsyuhada (2015) reported that the application of PGRs did not significantly affect the preharvest and postharvest quality of roselle.

Table 1: Effect of different types of plant growth regulators in developing parthenocarpic Melon Manis Terengganu (MMT) on flesh firmness, SSC and TPC of MMT.

Treatment	Flesh firmness (N)	SSC (% Brix)	TPC (mg GAE/100g FW)
Control	14.05[a]	11.60[a]	29.04[a]
800 mg/L IAA	15.81[a]	11.37[a]	41.13[a]
800 mg/L IBA	18.14[a]	12.58[a]	37.12[a]
800 mg/L NAA	18.83[a]	12.83[a]	37.27[a]
400 IAA + 400 mg/L GA_3	16.68[a]	12.91[a]	39.10[a]
400 IBA + 400 mg/L GA_3	17.29[a]	12.65[a]	38.74[a]
400 NAA + 400 mg/L GA_3	18.35[a]	12.02[a]	34.59[a]

Means with the same letter within column are significantly different at the 5% level according to HSD test.

For TPC, all PGRs treated fruits had comparable amount to control fruit which is contradict to North et al. (2012) where the fruits increased their TPC in response to GA_3. In the present study, the TPC of MMT flesh ranged between 30 and 40 mg GAE/100g FW.

Organoleptic attributes and nutritive value of fruit play a vital role in consumer purchasing decision and also influence further consumption. Taste, aroma, texture and appearance are the most important quality attributes measured. For sensory evaluation, the physical and chemical parameters are considered to reflect the taste (SSC and TA), aroma (volatile), texture, and colour (L*, a*, b*) of the fruit sample. Generally, all of the thirty panelists liked the aroma, texture, colour and sweetness (data not included). In this study, the sensory test was done a few hours after harvesting when the fruits are still fresh. Thus, all panellists agreed at score 6 regardless of the PGRs application (Figure 5). According to Lester (2006), consumer highlighted that flavour, sweetness and texture were the key factors in assessing the 'overall acceptance'. Thus, the panellist preference for overall acceptance level was based on the colour, taste, aroma, sweetness and sourness of the fruits. Due to the relationship of many parameters in determining the fruit characteristics, measuring only one parameter is insufficient to relate an objective assessment of the overall fruit flavour quality (Vallone et al., 2013).

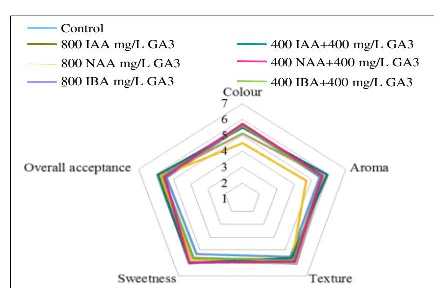

Figure 5: Effects of different types of plant growth regulators on sensory attributes of parthenocarpic MMT fruit.

Conclusion

In conclusion, the combination of 400 mg/L of IAA and 400 mg/L GA_3 had the ability to produce seedless MMT fruit without significant reduction in other quality parameters. For further study, it is recommended to apply other types of PGRs such as cytokinin and also non-synthetic PGRs from plant extract. In addition, the experiment can be conducted in a large scale in the field.

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Chapter 4

Pest and Disease Management

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Mapping the Incidence of *Ceratocystis* spp. in Planted *Acacia mangium* Stands in Sarawak

Jessica Diana, L.*, Jayneeca, L. and Affendi, S.
6th Floor, Jabatan Hutan Sarawak, Wisma Sumber Alam, Stadium Road, Petra Jaya, 93660 Kuching, Sarawak, Malaysia.
**E-mail: jessica_diana9361@yahoo.com*

Introduction

The monitoring of *Ceratocystis* spp. on *Acacia mangium* plantation forest health is very important to ensure productivity and future sustainability of forests yields. Nowadays, *Ceratocystis* spp. has increasingly been reported as the cause of wilting and death of *A. mangium* plantations in Sarawak. This fungal pathogen also may cause the browning of canopy leaves in a few weeks to months. Therefore, hyperspectral remote sensing technology has been identified as a critically important contributor to management efforts intended to control the incidence and spread of *Ceratocystis* spp. in the forest plantations. Airborne hyperspectral imaging can be used to detect early stages of diseased trees rapidly over a large area based on their unique spectral signatures. Moreover, these spectral signatures can help us gain a better understanding of the physical, physiology and chemical processes in plants due to disease attack and to detect the resulting biotic stress. This paper provides an overview of airborne hyperspectral imaging in tracking the incidences of *Ceratocystis* spp. on 4 years old *A. mangium* stands. It relates to the combination of ground-truth with airborne hyperspectral to develop a special spectral signature for leaf canopy of *A. mangium* caused by *Ceratocystis* spp. The output of this study would be utilised by the management in controlling the incidence and spread of *Ceratocystis* spp. within the planted forest stands in Sarawak.

Materials and Methods

Test site

The test site was located in the *A. mangium* plantation in Kuala Baram, Miri, Sarawak, Malaysia (4° 31’ 21’’ N and 114° 9’ 14’’ E) in Figure 1. The size of the area is estimated to be around 4.7 ha. The planting density of the site 11 1110 *A. mangium* trees per hectare and age of trees was 4 years old. The site was selected due to drastic *Ceratocystis* spp. infections.

Ground-truth data collection

The ground data collection involved fieldworks to record disease severity for *Ceratocystis* spp. symptoms for *A. mangium* trees. This assessment of disease severity relies on visual judgement. The stems were inspected for symptoms of discoloration, wound, crack, gummosis and insect holes; the canopy was inspected for chlorosis, wilting and dieback. Each *A. mangium* tree was assigned a score in five-level rating scale symptom: 0 for healthy (not sick) trees, 1 for a light attack, 2 for a moderate one, 3 for severe, 4 for very severe (near-fatal) and 5 for dead tree.

Airborne hyperspectral

The airborne hyperspectral images were acquired by using Advanced Imaging Spectrometer for Applications (AISA) sensor in March 2019 and will be continued until the end of year 2019. The sensor was mounted on light aircraft nomad. AISA airborne hyperspectral imaging spectrometer is capable of collecting data within spectral range of 401-982 nm, 286 band spectral and 500 m ground spatial resolution at 762 m of flight altitude.

Image processing

The raw hyperspectral images were processed by using ENVI software environment called CaliGeo.

Results and Discussion

Figure 1 and Figure 2 showed that, 75% of *A. mangium* stands in the test site were affected (light blue tones) by *Ceratocystis* spp. In terms of severity, it could be observed in Figure 3 that trees that are located in the left portion of the plot were severely affected by the *Ceratocystis* spp. infection where dead trees are found in the areas with canopy gaps. The pattern of infection could also be observed running diagonally across the top right to the bottom left of the plot. Trees that were not infected by this disease could be observed as red tones as found in the bottom right and top left of the image. According to Coops et al. (2003), leaf reflectance in the visible and near-infrared portions of electromagnetic spectrum can provide an early indication of plant stress or the onset of disease. With narrow and contiguous spectral bands, hyperspectral remote sensing has the capability to identify the early signs of disease or stress in forest plantation before visual symptoms are apparent (Pontius et al., 2005).

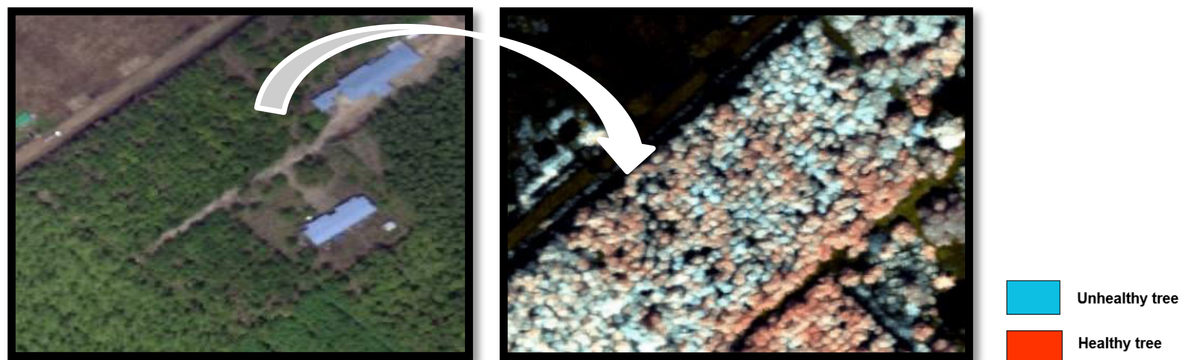

Figure 1: True colour image. Figure 2: Airborne hyperspectral image.

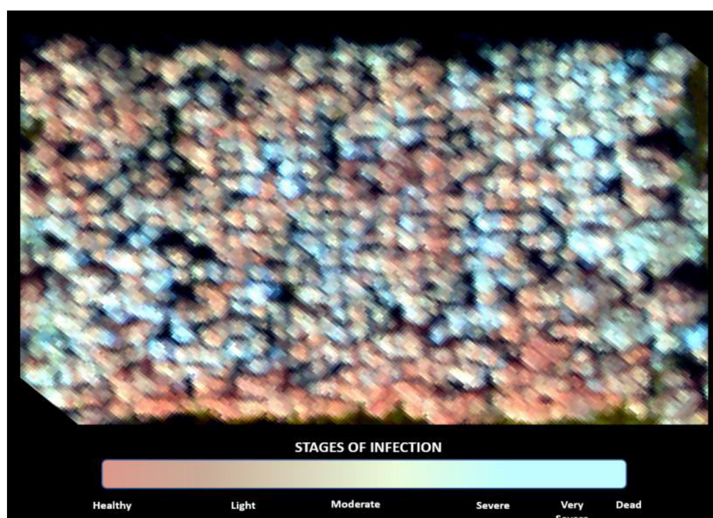

Figure 3: Processed hyperspectral image describing the various stages of infections of *Ceratocystis* spp. on the *A. mangium* stand.

Hyperspectral images offer high diagnostic capability for early disease detection. The spectral bands with high absorption are more sensitive to several leaf pigment including chlorophyll *a*, chlorophyll *b*, violaxanthin, β- carotene, neoxanthin and carotenoids. Pathogenesis in plants directly affects biochemical concentrations (Kamlesh et al., 2018).

This result will provide the opportunity to study *Ceratocystis* spp. infections over large areas so that the outbreaks can be related to other environmental factors using spatial modelling techniques in a geographic information system (GIS) environment. Environmental factors such as rainfall, altitude and host species will give more intuition on the build-up and decline of *Ceratocystis* spp. infections thus enabling future outbreaks to be modelled.

Conclusions

The ability to detect *Ceratocystis* spp. infections in *A. mangium* plantations using hyperspectral remote sensing would be beneficial to several aspects of forest plantation management including timber harvest and salvage operations. The early detection of *Ceratocystis* spp. infections would provide forest plantation managers with rapid assessments of current damage so that stands of high mortality can be salvaged.

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Identification and Assessment of Major Diseases of Multi-species Flowering Annuals

Nur Adliza, B.[1,*], Farah Farhanah, H.[1], Hanim, A.[2], Muhammad Aliff Fitri, A.[1] and Norhasbulloh, A.[1]
[1]Horticulture Research Centre, [2]Industrial Crops Research Centre, Malaysian Agricultural Research and Development Institute Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: nuradliza@mardi.gov.my*

Introduction

Multi-species flowering annuals is comprising of several species of ornamental flowers in a plant community resulting in an attractive array of flower colours. Malaysian Agricultural Research and Development Institute (MARDI) have formulated a mix of annual flower seeds and launch the technology in 2016 during the Royal Floria Putrajaya. Planting of annual multi-species in the garden is a new approach newly explored in Malaysia. This planting concept is still new and facing challenges in terms of species selection, planting techniques and maintenance to suit the Malaysian climates (Hanim, 2015; 2018). Current research that has been conducted at MARDI through direct seeding sowing technique produces a multi-species community that have many attributes. These kinds of communities are cheaper and easier to manage and provide more vegetation diversity per unit area (more than 50 plants/m²) as compared to planting nursery grown plants (10-25 plants/m², plant materials; RM40-100/m²) (Hanim et al., 2011). In addition, such communities can provide a strong impact on the public aesthetic values through flowers which bloom at different times and period. Potentially, these kinds of communities display early, intermediate and late flowering species. Ongoing research on multispecies have been carried out in MARDI since the 10th Malaysia Plan and up to the present Malaysia Plan (RMK11) to identify the natural dynamics of mixing several multi-species flowering annuals in tropical landscape (Hanim, 2018). From there, we have identified that diseases can be one of the challenges in planting multispecies. Therefore, this study was carried out to identify major diseases of multi-species flowering annuals and to evaluate the disease incidences and severity according to plant genus.

Materials and Methods

Detection and identification of seedborne fungi

Multi-species seeds (Table 1) were surface disinfected with 0.1% mercuric chloride before assayed. The standard blotter method by the International Seed Testing Association (ISTA, 2003) was used for assessing the seedborne fungi. One hundred seeds were taken randomly and 25 seeds per plate were placed in a 9 cm diameter petri plate containing three well-moistened blotters. Four replicates were maintained and incubated at room temperature for 7 days. Seedborne fungi were identified based on morphological characteristics of the colony and sporulation structures using reference manual by Watanabe (2010) and Mathur and Kongsdal (2003).

Disease assessment

This study was conducted at the MARDI, Ornamental Complex, Serdang, Malaysia. A total of nine species of flowering annuals with six genus were selected for this study (Table 1). All sources of the seeds were collected locally from MARDI seed production plots. Seeds which were not available in MARDI were obtained from commercial nursery. Such seeds included *Cosmos* spp. and *Gomphrena globosa*. The selected flowering annual plants were directly sown in an experimental plot measuring 200 cm in length x 200 cm in width x 20 cm in height. Three seeds of flowering annual plants were sown in each of planting holes with a distance of 20 cm x 20 cm between species for 10 rows. The

experimental design was a completely randomized design. Once the seedlings were approximately 5 cm tall, the thinning process was conducted by retaining one plant per planting hole. A week after sowing, the seedlings were fertilized with a compound fertilizer (15:15:15 $N:P_2O_5:K_2O$) at approximately 15 g/m^2. The plants were watered once daily.

Table 1: Multi-species of flowering annuals used for disease assessment.

Species	Family	Plant type/Habitat	Flower color
Zinnia elegans	Asteraceae	Annual/Dry-wet	Red Coral
Cosmos bipinnatus	Asteraceae	Annual/Dry-wet	White Candistripe Pink
Cosmos caudatus	Asteraceae	Annual/Dry-wet	Pink
Tagetes erecta	Asteraceae	Annual/Dry-wet	Yellow
Tithonia rotundifolia	Asteraceae	Annua/Wet	Orange
Gomphrena globosa	Amaranthaceae	Annual/Dry-wet	White purple Pink
Gomphrena haagena	Amaranthaceae	Annual/Dry-wet	Red
Celosia plumosa	Amaranthaceae	Annual/Dry-wet	Red
Sanvitalia procumbens	Asteraceae	Annual/Dry-wet	Yellow

Disease incidence and severity

Multi-species flowering annual were assessed at weekly interval based on the plant genus. Evaluation was done by measuring the percentage of disease incidence (DI). Disease incidence is the number of infected flowers relative to the number of flowers assessed using following formula (modified from Waller, 1992):

$$\text{Disease Incidence (\%)} = \frac{\text{Numbers of flowers infected}}{\text{Total number of flowers assessed}} \times 100$$

The severity of the infection was also rated in terms of disease severity (DS). DS refers to the disease infected total area or volume of plant tissue (Kranz, 1988). DS was calculated using the formula derived from Liu (1995), as follows:

$$\text{Disease Severity} = \sum \{(P \times Q)\} / (M \times N)] \times 100$$

where, P = severity score, Q = number of infected plants observed, N = maximum rating scale number and M = total number of plants observed. Disease symptoms that appeared on plant were rated by using a modified grading scale rating from Banerjee and Kalloo (1987) (Table 2).

Table 2: Disease scale rating used in disease assessment of multispecies flowering annual.

Disease scale rating	Symptoms
0	No disease symptom
1	1-5% of the canopy showing disease symptoms
2	6-25% of the canopy showing disease symptoms
3	26-50% of the canopy showing disease symptoms
4	51-75 of the canopy showing disease symptoms
5	76-100% of the canopy showing disease symptoms

Isolation of pathogens

Infected flower parts were washed under running tap water and dipped for 10 minutes in 10% Clorox. The tissue were then re-washed with sterile distilled water for three times. The samples were then air dried and plated onto Potato Dextrose Agar (DIFCO, USA) and incubated at 27±1°C for 7 days or

until visible sign of mycelial growth from the sample. The isolates were then purified and maintained on their respective agar for identification.

Identification of pathogens

All isolates were identified and characterized by morphological and molecular techniques. All isolates were identified using ITS region of rRDNA (Qiagen GmbH, Germany). The ITS nucleotide sequences for each isolates were then compared to those in the public domain database NCBI (National Centre for Biotechnology Information, www.ncbi.nih.gov) using Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN).

Statistical analysis

Statistical analysis was performed using SAS (Version 9) software. All data were subjected to analysis of variance (ANOVA) where means comparison were determined using Least Significant Difference (LSD) at 99% confidence level.

Results and Discussion

The present study had revealed nine seedborne fungi detected in multi-species flowering annuals. *Lasidiplodia theobromae, Aspergillus flavus, Choanephora* sp., *Curvularia lunata, Bipolaris* sp., *Aspergillus parasiticus, Fusarium* sp., *Aspergillus niger* and *Alternaria eichhoriae* were identified. The isolated fungi proved that the possible diseases that affect the seeds and emerging seedlings were seedborne. The infected seeds would transmitted the pathogens to plants, thus it acts as a primary source of inoculums for disease infestation development. Hence early identification and listing of seedborne pathogens will allow for control development and management strategies in order to avoid crop losses and to prevent the spread of plant diseases to new areas (Chigoziri and Ekefan, 2013). The findings were in line with the disease assessment done at the experimental plot where the results showed that major diseases of multi-species flowering annual were *Alternaria* leaf spot, leaf and flower spot and *Choanephora* leaf blight (Table 3). *Zinnia elegans* showed the highest disease incidence and severity for Alternaria leaf spot, *Choanephora* leaf blight and leaf spot disease. *Gomphrena* spp. showed 1.63% and 0.3% of disease incidence and severity respectively for leaf spot disease. There was no incidence of diseases recorded for other plant genus and species.

Table 3: Assessment of major diseases of multispecies flowering annuals and its causal pathogens.

Plants/Causal pathogens	Disease incidence (%)			Disease severity (%)		
	Alternaria leaf spot	leaf blight	Leaf /Flower spot	*Alternaria* leaf spot	leaf blight	Leaf/ Flower spot
*Zinnia elegans**	16.65^a	0.3^a	15.23^a	23.33^a	1.0^a	7.0^a
Gomphrena spp. **	0^b	0^b	1.63^b	0^b	0^b	0.3^b
Celosia plumosa	0^b	0^b	0^b	0^b	0^b	0^b
Cosmos spp. ***	0^b	0^b	0^b	0^b	0^b	0^b
Tegetes erecta	0^b	0^b	0^b	0^b	0^b	0^b
Tithonia roduntifolia	0^b	0^b	0^b	0^b	0^b	0^b
Sanvitalia procumbes	0^b	0^b	0^b	0^b	0^b	0^b
Causal pathogens	*Alternaria eichhorniae*	*Choanephora infundibulifera*	*Colletotrichum gleosporoides*	*Alternaria eichhorniae*	*Choanephora infundibuilfera*	*Colletotrichum gleosporoides*

Means followed by the same letter within column are not significantly different (p< 0.05) by LSD
*Zinnia: *Zinnia elegans, Zinnia elegans 'Dreamlands'*
**Gomphrena: *Gomphrena globosa, Gomphrena globose ' Fireworks', Gomphrena globosa 'QIS Red'*
***Cosmos: *Cosmos bipinnatus' White dwarf', Cosmos bipinnatus 'Candistripe', Cosmos haagena 'Dazzlers'*

Conclusion

From the results, it showed that disease is not a major problem in planting multi-species of annual ornamental flowers. However, *Z. elegans* was prone to a seedborne disease namely leaf spot that caused by *A. eichhorniae* and *C. infundibulifera.* Therefore, the use of disease-free seeds is crucial in managing multi-species flowering annuals diseases in addition to seed treatment in preventing disease occurrences.

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Effect of Eriophyid Mite Infestation on Coconut Fruits in Bagan Datuk

Nor Ahya, M.[1,*], Tajul Arifin, A.Y.[1]., Ahmad, N.[2] , Sentoor Kumeran, G.[2] and Mazidah, M.[3]
[1]Pest and Disease Management Program, Industrial Crops Research Center, Malaysian Agricultural Research and Development Institute Bagan Datuk, 36307 Sungai Sumun, Perak, Malaysia.
[2]Plant Breeding Program, Industrial Crops Research Center, Malaysian Agricultural Research and Development Institute Bagan Datuk, 36307 Sungai Sumun, Perak, Malaysia.
[3]Pest and Disease Management Program, Industrial Crops Research Center, Malaysian Agricultural Research and Development Institute, Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: ahya@mardi.gov.my*

Introduction

Coconut eriophyid mite is an invasive coconut pest in many African, South American and Asian countries including major coconut producing countries such as Sri Lanka, India and Philippines (EPPO, 2017). The pest infests primarily on young coconut fruits of various stages and coconut seedlings. These mite colonies establish under the perianth of the young fruits which later cause necrosis on lower surface of the perianth. During outbreaks, it can cause serious fruit scarring and abortion especially in developing fruits. According to Haq (2011), infestation by the coconut eriophyid mites can reduce yield up to 60%.

In Malaysia, this pest has never been the main concern as its damage was insignificant. Recently, several farmers (Priatno, personal communication, February 16, 2017) had claimed that their buyers refused to buy the extensively scarred fruits which lead to huge loss. Therefore, this initial study was conducted to investigate the infestation level of this pest on selected coconut varieties to understand the current scenario of this mite infestations locally.

Materials and Methods

Three coconut varieties namely Aromatic dwarf, Nias and Catigan were used in this study. Palms aged 5-7 years old were selected and 20 palms from each variety were tagged randomly. Two types of young fruits aged 3-4 months (small) and 7-8 months (large) were sampled from each palm. The fruits were collected and kept in labelled plastic bags and brought back to the laboratory. At the laboratory, two types of parameters were recorded which were (i) damaged and total surface areas (Figure 1) of each fruit and (ii) damaged and total circumference of the fruit's perianths (Figure 2) following Galvao et al. (2008) and Sousa et al. (2017). Symptoms of infestation were also observed and recorded.

Figure 1: Damaged area (brown) of the fruit's surface.

Figure 2: Circumference of the infested fruit's Perianth.

Experiment was arranged following the Complete Randomized Design (CRD). Data of damage area surface and circumference of the infested fruit's perianth were analysed using 2-factorials Analysis of Variance (ANOVA) and multiple mean comparisons were done using Least Significant Difference (LSD) at confidence level of 95%.

Results and Discussion

Results from Table 1 show that size of fruits did not affect the damage level on the perianth (P=0.69) as compared to the effects of coconut varieties which differs significantly (P<0.05). On the contrary, there were differences on damage area affected by both fruit size and varieties (Table 2).

Even though the overall percentage of damage area is low (<30%), Table 3 and 4 reveals that eriophyid mites caused more damage to Aromatic dwarf fruits as compared to Nias and Catigan fruits. This could be attributed by the aroma emitted which attracts coconut pests including mites to the palms. Several studies have reported high infestations of coconut pests on Aromatic dwarf such as red palm weevil (Dilipkumar et al., 2014), coconut black-headed caterpillar and hispine beetles (Krisanapook, 2015).

Bigger fruit size recorded more damaged area than the smaller fruits even though the circumference of damaged perianth for both fruits size are the same. Sousa et al. (2017) mentioned that the damaged area (scarring) of the fruits represent past damage caused by the mites but does not reflect current infestation level. Scarring occurs due to the expansion of necrotic tissues of the fruits surface under the perianth during fruit's development. Thus, the occurrence of larger damage areas on bigger fruits in this study coincided with the above statement. However, information on mites' population should be included to confirm the infestation level.

Table 1: Circumference of damaged perianth affected by different fruits size and varieties.

Source	DF	F-value	Pr > F
Fruit sizes	1	0.16	0.6889
Varieties	2	30.21	<.0001*
Fruit sizes x Varieties	2	14.01	<.0001*

Asterisks indicate significant at α=0.05.

Table 2: Fruit's damaged area affected by different fruits size and varieties.

Source	DF	F Value	Pr > F
Fruit sizes	1	62.92	<.0001*
Varieties	2	40.65	<.0001*
Fruit sizes x Varieties	2	19.80	<.0001*

Asterisks indicate significant at α=0.05.

Table 3 (a-b): Effect of coconut varieties and fruits size on percentage of damage area.

(a) Varieties	*Means percentage damaged area± S.E (%)
Aromatic dwarf	4.43±0.43[a]
Nias	2.64±0.62[b]
Catigan	1.35±0.12[c]

(b) Fruit sizes	*Means percentage damaged area± S.E (%)
Small	1.70±0.14[b]
Large	3.92±0.37[a]

**Means in column with different letters indicate significant at α=0.05.*

Table 4 (a-b): Effect of coconut varieties and fruits size on percentage of damage perianth.

(a)Varieties	*Means percentage damaged circumference of perianth (%)
Aromatic dwarf	29.22±2.59[a]
Nias	8.09±1.34[c]
Catigan	14.85±3.79[b]

(b) Fruit sizes	*Means percentage damaged circumference of perianth (%)
Small	16.93±2.46[a]
Large	17.84±1.66[a]

**Means in column with different letters indicate significant at α=0.05.*

Conclusions

In conclusion, Aromatic dwarf coconut is more susceptible to eriophyid mite infestations as compared to Nias and Catigan dwarf. However, it is suggested that this study should be conducted in a longer time scale while considering the mite population to understand further the relationship and interaction between damage levels and pest incidence.

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Evaluation of Disease Severity of *Colletotrichum gloeosporioides* on Selected Variants of *Mangifera indica* L. var. 'Chok Anan'

Nor Dalila, N.D.[*], Muhamad Afiq, T.A., Nurul Fahima, M.A. and Nur Aisyah Anis, A.K.
Horticulture Research Centre, MARDI Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia.
**E-mail: daleyla@mardi.gov.my*

Introduction

The mango plant is widely cultivated in tropical and subtropical regions of the world. Currently, India is the world's largest producer of the crop followed by China and Thailand (FAOSTAT, 2010). The mango plant is abundantly grown in Malaysia with a planted area of 6,048.29 hectares at estimated value of RM149,475.20 in 2017 (Department of Agriculture, Malaysia, 2019). 'Chok Anan' is one of popular mango variety in Malaysia. It has fruit of oval shape, medium size (250-350 g), golden-yellow skin, sweet taste (TSS: 14-16%) and slightly fibrous flesh. Previously MARDI had studied variation among 'Chok Anan' accessions in MUDA Agriculture Development Authority (MADA) mango collection farm, at Tobiar, Kedah. Planting materials from selected plants with identified variation were propagated and planted at MARDI Sintok, following Randomized Complete Block Design (RCBD). The plot was also planted with MA 224 as check variety. MA 224 is the original Chokanan variety registered by Department of Agriculture. The study plot was established in June 2013.

There are more than 100 mango varieties registered in Malaysia but only a small number have been recommended for planting. This is due to the need to consider the yield, fruit quality, and also the plants weakness towards anthracnose disease. Anthracnose is recognized as the most important pre- and postharvest fungal disease of mango worldwide caused by *Colletotrichum gloeosporioides* Penz (Sundravadana et al., 2007). The disease affects both leaves, twigs, petioles, panicles and fruits (Nelson, 2008). The postharvest phase is the most damaging and economically significant phase of the disease.

There is a need to screen these variants to acquire such variants that are suitable and can be recommended for large scale planting. Hence, the objective of this study is to evaluate thirteen selected 'Chok Anan' variants for susceptibility towards anthracnose disease caused by *C. gloeosporioides.*

Materials and Methods

The pathogen, *C. gloeosporioides,* causing anthracnose on mango fruits, was isolated from infected mango fruits from orchards of the Malaysian Agricultural Research and Development Institute (MARDI) Sintok, Kedah. Four samples of diseased tissues were washed and dipped for 10 minutes in 10% (v/v) Clorox. Consequently, the tissue was re-washed for three times with sterile distilled water and subsequently dried. Then it was placed on potato dextrose agar (PDA). The isolates were identified using morphological characteristics and molecular procedure (PCR) (Figure 1).

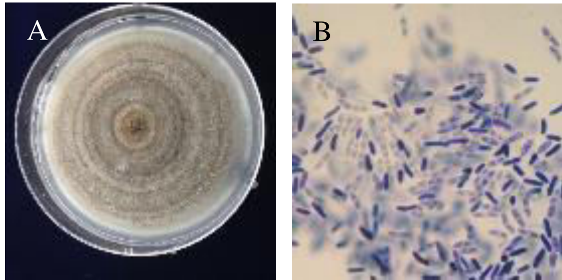

Figure 1: *C. gloeosporioides* (A) Culture plate on PDA and (B) Spore under microscope observation (40x).

The thirteen 'Chok Anan' variants are grown in Malaysian Agricultural Research and Development Institute (MARDI) Sintok, Kedah. They were harvested in early July 2018 and were subjected to the study at fully matured stage. All of the mango fruit samples were surface disinfected with 10% Clorox for 2 minutes and rinsed three times using sterile distilled water. Once dry, the samples were then inoculated with spore suspension of *C. gloeosporioides* by pinprick method (Kumari et al., 2017). This was done by dipping a sterile inoculation needle into 10^6 spore/mL spore suspension and poking the fruit 1 cm deep at the centre of the mango fruit (Figure 2). Spore suspension of *C. gloeosporioides* was obtained from a 10 days old plate. It was flooded with 20 mL sterile water and slightly shaken to suspend the conidia. Conidial suspensions were adjusted to 10^6 conidia/mL with the use of a haemocytometer. Inoculated fruits were incubated in a moist container for 12 days under room condition.

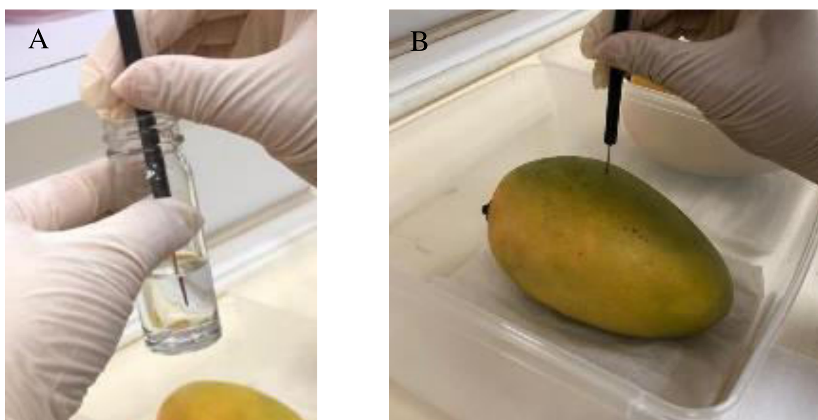

Figure 2: Pinprick method; (A) Sterile inoculation needle is dipped in spore suspension of 10^6 conidia/mL and (B) Spore is inoculated 1 cm deep in the centre of the mango sample.

Observations were recorded daily for 12 days after inoculation. Anthracnose disease symptoms are mainly sunken round spots. After some time, the spots would grow and unite. Eventually the whole fruit sample will rot. Disease severity was recorded by taking the percentage of the overall mango fruit surface affected by the anthracnose disease. Disease severity rating scale used for this study was from Lakshmi et al. (2011) (Table 1).

Table 1: Disease severity rating scale used for the assessment of disease severity.

Rating	Visual observation	Response
0	No infection	Resistant
1	Up to 5% of fruit surface area covered	Tolerant
2	6-10% of fruit area affected	Moderately tolerant
3	between 11 and 20% of fruit area covered	Moderately susceptible
4	21-50% of fruit area affected	Susceptible
5	more than 50% of the fruit surface area covered	Highly susceptible

The experiment consisted of thirteen treatments with four replications. The design was a completely randomized design (CRD). Disease occurrence was analysed by using ANOVA (SAS 9.3). Differences within the means were compared by using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Occurrence of disease were seen 2 days after inoculation was done for all of the treatments with different degrees of severity. Within the two-day period, conidium of *C. gloeosporioides* has germinated on the fruit surface and produced germ tubes or saprophytic hypha whose tips will swell and form appressoria (Dinh and Sangchote, 2002). Table 2 showed significant differences between the 'Chok Anan' variants throughout the 12 days of disease observation. Mango MA 224 was tested as the check variety. Here, MA 224 and CV03 were seen to have the lowest occurring lesion development of 42.07% and 39.4% respectively. They were grouped together, having no significant difference compared with each other. CV04, on the other hand, showed the highest occurrence of disease at 54.57 % lesion symptom. Its rating at index 5 showed that this variant is highly susceptible towards anthracnose disease at 12 days after inoculation. All of the 'Chok Anan' variants tested showed different or varying responses towards the artificial inoculation of anthracnose disease where no variety was found to be resistant against the disease (Figure 3).

Table 2: Duncan's Multiple Range Test of comparison among the thirteen 'Chok Anan' variant susceptibility towards *Colletotrichum gloeosporioides* at *in vivo* stage of 12 days after inoculation.

Chok Anan Mango variants	Disease lesion (%)	Severity index/Rating	Response
MA224	42.071[e]	4	Susceptible
CV01	49.929[bcd]	4	Susceptible
CV02	52.571[abc]	5	Highly susceptible
CV03	39.036[e]	4	Susceptible
CV04	54.571[a]	5	Highly susceptible
CV06	48.107[cd]	4	Susceptible
CV07	51.714[abcd]	5	Highly susceptible
CV08	52.214[abc]	5	Highly susceptible
CV09	50.071[abcd]	5	Highly susceptible
CV12	48.107[cd]	5	Highly susceptible
CV13	51.643[abcd]	5	Highly susceptible
CV15	52.893[ab]	5	Highly susceptible
CV16	47.393[d]	4	Susceptible

Figure 3: Disease progression of 13 ‘Chok Anan’ variants.

Conclusions

The various 'Chok Anan' variants showed varying responses in relation to the infection of anthracnose disease. There were no variants found to be resistant against the disease. Nevertheless, it is concluded that CV03 is seen to have the least disease development, similar to control cultivar of MA 224. However, the highest lesion development was from CV04.

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Occurrence of Papaya Dieback Disease on Grafted Eksotika Papaya Plants

Mohd Azhar, H.[1,*], Sarip, J.[2], Razali, M.[1], Noor Faimah, G.[1] and Muhammad Zulfa, M.R.[1]
[1]Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Director General Office, MARDI Johor Bahru, Lot 6313, Jalan Taruka, 80350 Johor Bahru, Johor, Malaysia.
**E-mail: mazhar@mardi.gov.my*

Introduction

Papaya (*Carica papaya* L.) is also known as the fruit of the angels. Like other types of tropical fruit, papaya also contains high vitamins A and C, minerals, flavonoids and antioxidants (Ayoola and Adeyeye, 2010). The production of papaya is particularly attractive for fruit crop diversification in Malaysia due to its yield potential, high demand on local market and potential for export. However, Malaysian papaya industry was shocked by a threatening and lethal disease known as papaya dieback disease (PDD), which has led to a decline in local papaya production. Local supplies and papaya export value have decreased from RM120 million in 2003 to RM30 million in 2012 and continued to reduce to RM3 million by 2016. Two cultivars commonly grown in Malaysia, which are Sekaki and Eksotika, are very susceptible to this disease.

PDD is currently known as the most threatening disease of papaya in Malaysia is caused by *Erwinia mallotivora* bacteria (Mat Amin et al., 2011). This disease is believed to be spread by wind and the entry into host is usually through injury points. Known also by other names like bacterial crown rot and bacterial canker, this disease has been reported mostly in West Indies, Virgin Islands, Venezuela, Java, Taiwan and the Mariana Islands (Ollitrault et al., 2005). In Malaysia, this disease was first detected at Johor in 2003 (Mat Amin et al., 2011), which was then spread three years later to five other states namely Melaka, Negeri Sembilan, Pahang, Kedah and Perlis. This had caused the destruction of 800 ha of papaya cultivated land throughout the country and contributed to the loss of USD58 million of export value (Maktar et al., 2008). The common symptoms of PDD include greasy, water-soaked lesions and spots on petioles, leaves, trunks and fruits (Wan et al., 2017). The detail of the symptoms has been explained by Nishijima (1994) and Mat Amin et al. (2011).

Today, farmers are very reluctant to invest in papaya plantation due to the incidence of the dieback disease. Many approaches have been employed to combat the disease demonstrating limited success. Chemical control has been observed inefficient (Frossard, 1985), whereas genetic control was seen more promising as some local populations have shown high level of tolerance, particularly in Guadeloupe, Venezuela, Granada and Trinidad (Webb, 1983; de Lapeyre and Lyannaz, 1992). The development of new varieties through conventional and molecular breeding becomes more challenging due to the lack of resistant accessions until Viorica was discovered in 2012 (Sarip et al., 2012). Viorica is a papaya variety that is highly tolerance to PDD, which was developed by MARDI's researcher on 2012 through selection and purification step using composite controlled pollination (Sarip et al., 2013). Viorica has low eating interest that does not reach consumers taste and market value. Nevertheless, this variety is suitable to be used as a parent in breeding activities towards developing new superior papaya hybrid (Sarip and Noraisah, 2018).

There is a possibility to produce elite grafted papaya seedling using Viorica as a high tolerant rootstock. The idea of using resistant rootstock is due to the metabolite translocation and interaction between scion and rootstock as proposed by Aloni et al. (2010). A study of grafted grapevine onto crown-gall resistant rootstock conducted by Sule and Burr in 1998 show bigger crown gall formed on grapevine with susceptible rootstock rather than resistant rootstock. In watermelon (Yetisir et al., 2003), the fusarium wilt resistant rootstock was used to influence disease resistance, fruit yield and quality. The same conclusion also made by Nisini et al. (2002) in their muskmelon study. The objective of this study was to produce elite Eksotika papaya seedlings with high tolerance to PDD using Viorica as a rootstock. Preliminary studies show that PDD's resistance factor can be transferred

from Viorica to the elite scion (Sarip et al., 2016). Therefore, the assessment on a larger scale should be carried out to further reinforce the previous breakthrough.

Materials and Methods

Selection of rootstocks and scions

Selection of rootstocks based on the resistant level from glass house and hot spot screening (Sarip et al., 2012). Viorica has been observed to possess the characteristics of moderate fruit quality and high tolerance against PDD. Eksotika variety was selected as the elite scions based on the superior agronomic characters (Simoh and Sarip, 2015).

Preparation of grafted seedlings

Three grafting combinations of scion and rootstock were used in this study i.e. Eksotika onto Eksotika (E/E), Eksotika onto Viorica (E/V) and Viorica onto Viorica (V/V). The part of the plant that would be grafted was sterilised using sterilised cotton with 50% ethanol solution. The scions were cut in 'V' shape using a grafting knife. The rootstocks plants were cut in vertical order. Then, the scions were inserted into the rootstocks. The grafted part was protected and tightened with elastic parafilm. The grafted seedlings were covered and assembled under transparent plastic for 14 days (Sarip et al., 2017). After 3 weeks, 2 months old of 90 uniform and healthy grafted seedlings from each treatment were planted at MARDI Serdang hot-spot area using Randomized Complete Block Design (RCBD) comprising of 3 replications. The grafting combination was screened against PDD to determine the ability of resistance rootstock to induce resistance towards the susceptible elite scion.

Assessment of field disease occurrence

Numbers of healthy and symptomatic selections were recorded from each sample plants. Disease incidence (%) was valued based on symptoms recorded from each treatment of grafted papaya. The disease incidence (DI) was determined by the following formula (Cooke, 2006):

$$\text{DI (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \text{ x } 100$$

Disease severity (%) was assessed using the incidence score (Table 1) data recorded from each plant sample. The disease severity (DSI) was computed by applying the equation as proposed by Horsfall and Barrat (1945):

$$\text{DS (\%)} = \frac{\sum(\text{a xb})}{(\text{N})(\text{Z})} \text{ x } 100$$

$\sum$ (a x b) = Sum of the symptomatic plant and their corresponding score
N = Total number of sampled plants
Z = Highest score

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Table 1: Bacterial dieback score description.

Score	Description of symptom
0	No symptom (free from disease).
1	Water-soaked spot on petioles and stems.
2	Yellowing on leaves. Few black spots on leaves and fruits.
3	Wilting of the leaf followed by petiole and stem (flag leaf symptom). Brown lesion on fruits and canker on stems.
4	Brown lesion spread to crown and leading to dieback. Only a few leaves/leafstalks on papaya trees.
5	Stems and all plants part become rotten as well as death of papaya trees (plant death).

Disease score (mean) was also assessed using the incidence score data recorded from each plant sample. The disease score was measured by the following formula and the translation of disease score is listed in Table 2:

$$\text{Disease score} = \frac{\sum(\text{a xb})}{\text{N}}$$

Table 2: Bacterial dieback disease score translation.

Disease score	Translation
0	Resistance
0.1 - 1.0	Highly tolerance
1.1 - 2.0	Tolerance
2.1 - 3.0	Susceptible
3.1 - 5.0	Highly susceptible

Data collection and statistical analysis

Evaluation of field disease incidence was conducted at the papaya dieback disease hot-spot area at MARDI Serdang. Data on dieback disease attack was recorded every month on each grafted plant from 5 months until 14 months old. Statistical analysis was determined by one-way Analysis of Variance (ANOVA) using Statistic Analysis Software 9.4 programme (SAS 9.4). Analysis was carried out using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Normally, papaya tree will start flowering when the tree reach 5 months of age and fruits can be harvested as early as 9 months old after fruit set. The present study found that overall mean disease incidence of dieback on grafted papaya plants during 9 and 14 months old after planting were 42.3% and 100.0%, respectively (Figure 1). During 9 months old, around half of plants generated from E/V and E/E grafting were infected by dieback disease. However, only quarter of all V/V grafted plants were infected by the disease during 9 months old. The disease incidence value was reached 100% in each treatment after the age of grafted plants turn to 14 months old.

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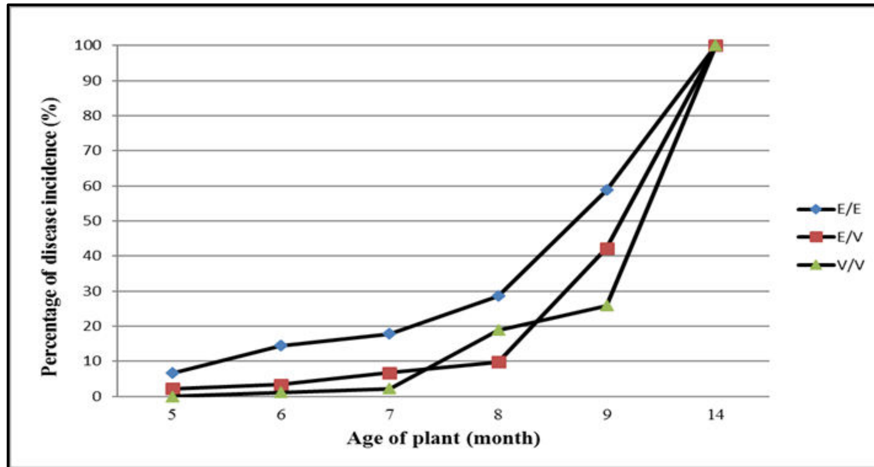

Figure 1: Disease incidence of papaya dieback on 5-14 months old grafted papaya plants.

The results of the disease severity and disease score of papaya dieback on grafted papaya plants were presented in Table 3. Disease severity was relatively low when compared with disease incidence. Overall mean disease severity of papaya dieback on 9 and 14 months old grafted papaya plants were 24.8% and 64.0%, respectively. One-way ANOVA with DMRT test highlighted that, the highest disease severity was observed on E/E grafted plants (86.7%) during 14 months old and the lowest severity was observed on V/V grafted plants (8.7%) during 9 months old. According to statistical analysis, the severity of dieback disease on 9 months old grafted plants was not significantly difference within each treatment. Meanwhile, the severity of dieback disease in V/V treatment was significantly difference compared to two other treatments on 14 months old grafted plants. Percentage of disease severity of 14 months old grafted plants of all treatments were higher compared to 9 months old grafted plants.

Table 3: Disease severity and disease score of papaya dieback on 9 and 14 months old grafted papaya plants.

Treatment	Disease severity (%)		Disease score (mean)		Disease score translation	
	9 months	14 months	9 months	14 months	9 months	14 months
E/V	22.0	71.0^{a}	0.86	2.78	Highly tolerance	Susceptible
V/V	8.7	34.3^{b}	0.35	1.36	Highly tolerance	Tolerance
E/E	43.7	86.7^{a}	1.66	3.44	Tolerance	Highly susceptible
Mean	24.8	64.0	-	-	-	-
CV	74.6	22.7	-	-	-	-
F Value	2.74^{ns}	10.27*	-	-	-	-
Pr > F	0.1781	0.0266	-	-	-	-

**Means with the same letter(s) are not significantly different by DMRT at $p≤0.05$.*

The disease score was determined the tolerance level of grafted papaya plants against papaya dieback disease. The disease score for 9 months old grafted plant combinations indicates that the V/V (0.35) and E/V (0.86) possessed the highly tolerance level compared to E/E (1.66) that only showed tolerance level against dieback disease. Meanwhile the tolerance level of 14 months old grafted plants were different among the three grafting combinations which were V/V (tolerance), E/V (susceptible) and E/E (highly susceptible).

Conclusions

In this study, the occurrence of papaya dieback disease on grafted papaya plants showed that the disease was mostly found in Eksotika/Eksotika (E/E) combination with disease severity of 86.7% and

disease score of 3.44 on 14 months old grafted papaya plants. While the disease severity and disease score of Eksotika/Viorica (E/V) grafting was 71.0% and 2.78, respectively. Whereas the lowest disease occurrence was recorded on Viorica/Viorica (V/V) grafted plants with disease severity of 34.3% and tolerance scale of 1.36. Based on the disease score, the 14 months old grafted papaya plants showed that E/E has highly susceptible, E/V has susceptible and V/V has tolerance level character against papaya dieback disease incident. Viorica, a resistant rootstock to papaya dieback disease has influenced the occurrence level of Eksotika scion to dieback attack but still not enough to turn it into a disease tolerance papaya plant.

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Chapter 5

Plant Production

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Thawing Techniques of Frozen Whole Fruit Durian (*Durio zibethinus* cv. Musang King)

Siti Aisyah, A.*, Razali, M., Mohd Kamal, M.T., Shamsudin, O., Norsyafiqah, R., Nur Azlin, R., Nur Syafini, G., Nurul Khdijah, R., Siti Ilyani, A., Nur Hidayah, S. and Siti Khuzaimah, T.
Horticulture Research Centre MARDI, Malaysian Agricultural Research and Development Institute, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail:ctaisyah@mardi.gov.my*

Introduction

Durian (*Durio zibethinus* Murr.) is a native and highly priced fruit in Southeast Asia due to its seasonality, unique smell, taste and texture. Nowadays, D197 Durian (Musang King) is one of the popular varieties due its thick flesh, small core and good taste (Nur Azlin et al., 2018). Musang King whole durian fruit exported to China requires a phytosanitary frozen treatment to sterilize potential insect pests and microbes. Quarantine disinfestations treatment is very important in order to prevent the establishment of pest associated with a commodity to be imported into a country or region where it does not occur or where its presence is restricted. Freezing is well known for keeping the quality of foods for a longer period. However there are still quality concerns associated with this technique, depending on the types and characteristics of the commodity. Freezing is recognized as the postharvest technique that best preserves fruit flavor (Skrede, 1996). On the other hand, thawing process is also important as it may further affect the food quality after freezing. In reference to the study with strawberry being froze at -20°C and thawed at 4°C, which is commonly practiced, it actually had caused most pronounced pigment and ascorbic acid losses. Less information was found on thawing techniques of frozen whole durian. However, freeze-thaw method for frozen pulp studied by Jackie Lou et al. (2016) had found that no significant variations were noted in the pH, soluble solid content, organic acids and texture of the durian pulp after freezing and thawing. Therefore, in this study, different methods of thawing were studied and the best method will be recommended to consumers as an alternative to consume frozen whole durian fruit.

Materials and Methods

Frozen whole durian fruits that were treated with cryofreezing at -80 to -110°C were purchased from the Far East Import Export, Nilai and brought to the Post Harvest Complex, MARDI for evaluation. Fruits were stored at -18°C to maintain their frozen state. Three treatments were used in this study; fresh drop fruit served as control (T1), thawing the frozen fruits in the microwave oven (Panasonic Model NN-CD997S 1000 watt) on medium high capacity for 20 minutes (T2) and normal thawing by placing the frozen fruits in ambient temperature plus fan exposure for 4 hours (T3). After thawing, the quality of fruits was evaluated by chemical and sensory evaluations. Three replicates from each treatment were used to analyse its flesh colour, soluble solids concentration (SSC), pH and titratable acidity (TA). A sensory evaluation of the fresh and freeze-thawed durian was conducted by 10 panellists. This evaluation compared the texture of the reference samples using a scale from 1 to 7 which represented the freshness, colour, texture, taste, odour and overall acceptability of the fresh and freeze-thawed durian with 1=very much dislike, 2=moderately dislike, 3=slightly dislike, 4=neither like or dislike, 5=like slightly, 6=like moderately, and 7=like very much. The experimental design was a completely randomized design with three replications. The obtained data was analyzed using analysis of variance (ANOVA) and mean comparison was tested by the Duncan Multiple Range at the 5% level of significant.

Results and Discussion

Chemical properties

Result has shown that, duration time of treatments given was enough to thaw the whole fruit including the pulp. The symptom of frozen injuries was not appeared during this period. The chemical results in Table 1 such as pH, SSC, colour of pulp (L and C) did not show any significant difference among all the treatments. But there was a significant difference among treatment on titratable acidity (TTA) and h value. This result is in line with finding by Marin et al. (1992), where they examined the chemical and biochemical changes in mango after air blast freezing at -40°C and during storage at -18°C for a 4 months period. In their study, they found that the titratable acidity of the mango slices decreased due to the freezing process, however the freezing treatment did not affect moisture content or soluble solids content. Thawing under ambient temperature gave no significant different with fresh fruits means where the colour of pulp remain similar to fresh drop fruit. Acidity of durian frozen thawed in microwave oven and ambient decreased as compared with fresh drop durian.

Table 1: The effect of different thawing techniques on soluble solids content (SSC), titratable acidity (TTA), pH, colour of pulp (L, C and h) in frozen durian whole fruit.

Treatment	SSC (°Brix)	TTA (mg/100g)	pH	Pulp colour		
				L	C	Hue (h°)
Control (Fresh drop fruit)	41.77[a]	0.11[a]	6.78[a]	68.00[a]	51.84[a]	89.64[ab]
Microwave 20 min (Medium high capacity)	39.95[a]	0.07[b]	7.06[a]	67.90[a]	49.44[a]	88.26[b]
Ambient+Fan (4 hrs exposure)	40.53[a]	0.06[b]	7.11[a]	72.02[a]	49.08[a]	91.44[a]

Means followed by the same letter in the column are not significantly different by DMRTat p≤0.05.

Sensory evaluation

Sensory analysis in Table 2 showed no significant difference on all parameters between control fruit and thawing method using microwave oven at medium high capacity for 20 minutes. However, for treatment thawing in ambient temperature plus fan for 4 hours, has resulted in the difference between control fruit in terms of texture, taste, and overall parameters. This shows that panels tend to choose the durian thawed in microwave oven as compared to thawing in ambient+fan exposure. Aroma of durian between control fruit and fruits that thawed in microwave oven has no significant difference means, hence the panels could not differentiate between fresh durian and frozen durian.

Table 2: The effect of different thawing techniques on sensory evaluation (freshness, colour, texture, taste, odour and overall acceptability) in frozen durian whole fruit.

Treatment	Freshness	Colour	Texture	Taste	Odour	Overall
Control (Fresh drop fruit)	4.41[a]	4.45[a]	4.54[a]	4.58[a]	4.41[a]	4.58[a]
Microwave 20 min (Medium high capacity)	4.5[a]	4.71[a]	4.37[ab]	4.37[ab]	4.41[a]	4.42[ab]
Ambient+Fan (4 hrs exposure)	4.13[a]	4.39[a]	3.95[b]	4.08[b]	3.5[b]	4.06[b]

Means followed by the same letter in the column are not significantly different by DMRT at p≤0.05.

Conclusion

Thawing method by using microwave oven medium high capacity for 20 minutes is the best method of thawing whole durian fruit without changing the original taste of fresh durian Musang King. This can be the faster method to consume whole frozen durian. Thawing method at ambient+fan for 4 hours exposure can be an alternative method for those consumers whose not having microwave oven.

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Evaluation of Cutting Technique on Seedless Mangosteen (*Garcinia mangostana*)

Mohd Ridzuan, M.D.[1,*], Hartinee, A.[1] and Ab Kahar, S.[2]
[1]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 06050 Bukit Kayu Hitam, Kedah, Malaysia.
[2]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia.
**E-mail: ridzone@mardi.gov.my*

Introduction

Apomixis feature in mangosteen makes the introduction of new varieties through hybridization methods impossible. To date, the Department of Agriculture (Jabatan Pertanian Semenanjung Malaysia, 1999) has registered two mangosteen clone 'GA1' (regular mangosteen) and 'GA2' (mesta). MARDI, through research projects, has found a new clone that is seedless mangosteen (MTB). MTB has characteristics intermediate between regular mangosteen and mesta, with medium to large-sized fruit (about 90 g-135 g), round, dark brown skin color, white flesh, sweet and sour taste, smooth texture with slightly juicy and seedless. Currently, this clone is propagated by conventional grafting (cleft) onto the mangosteen seedlings (about 1-1.5 years old). The technique has quite a high success rate. However, other methods of propagation such as by cutting should be investigated. Mostly mangosteen is propagated using seeds but since this variety is seedless, therefore another method of propagation should be evaluated. This experiment evaluated the cutting technique to propagate the MTB.

Materials and Methods

The experiment was conducted in the nursery at MARDI Sintok, Kedah with 50% shade level. The cutting was propagated under close capillary particle system (CCPS). According to Awang et al. (2009), the CCPS was constructed using polystyrene box and covered with polyethylene sheet. A water reservoir was created and maintained at 5 cm at the bottom of the box filled with light expanded clay aggregates (LECA) and water would then flow up through capillary into perlite as media (8 cm deep). At 5 cm on the sidewall of the box, a hole was created as an overflow to maintain the good water level in the box. The box was watered once a week. This is a 2x3 factorial experiment arranged in a completely randomized design with two factors, two number of nodes and three varieties of mangosteen. The number of nodes was two nodes and a single node, the varieties were mangosteen, mesta, and MTB. The treatments were described in Table 1. The mature shoots were taken as the source of cutting. All leaves were removed from the cutting. The cutting was treated with Mencozeb® before planting into CCPS. The treatment was replicated four times. Data on the percentage of mortality were taken at 33 days after planting. Experimental data were analyzed using ANOVA (SAS software version 9.4) and means were compared using Tukey's HSD Test.

Results and Discussion

From Table 2, mortality percentages were significantly different in varieties and number of nodes. Significant interaction in the mortality percentage between varieties and number nodes were recorded. From Figure1, the mortality rate is highly dependable on the variety used. For MTB, the mortality percentage was significantly reduced to 100% at 33 days after planting. These results can prolong the lifetime of the cutting to allow for rooting. Sandrang et al. (2015) also recorded using two nodes cutting of mesta through micro-cutting technique are better than a single node.

Table 1: Description of the treatments.

Treatments
T1 – 1 node mesta
T2 – 1 node MTB
T3 – 1 node mangosteen
T4 – 2 nodes mesta
T5 – 2 nodes MTB
T6 – 2 nodes mangosteen

Table 2: Main and interaction effect of three mangosteen varieties and 2 type of nodes at 33 days after planting.

Factor	% mortality
Varierties	
MTB	56.25[a]
Mesta	18.75[b]
Mangosteen	43.75[a]
Number of nodes	
one node	62.50[a]
two nodes	16.67[b]
Node	**
Variety	*
Node*Variety	*

*** significant at 1% probability level, * significant at 5% probability level. Means in each column with the different letters within each factor indicate significant differences at P≤0.005% level according to Tukey's HSD.*

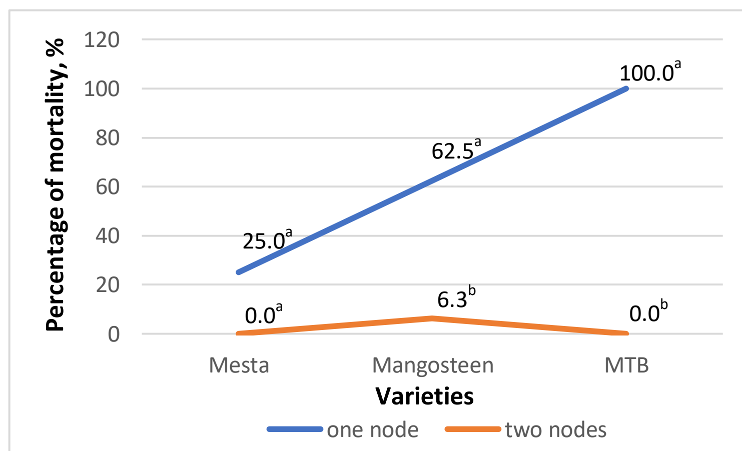

Figure 1: Interaction between number of nodes and varieties of mangosteen at 33 days after planting.

Conclusions

For propagation of MTB (seedless mangosteen) using cutting is recommended to use two nodes compared to single node due to low cutting mortality percentage at 33 days after planting.

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Vegetative Propagation Technique of Black Velvet Aroids (*Alocasia reginula*) Potentially for Commercial Nursery Production

Hafeifi, M.B.[1,*], Hanim, A.[1], Puteri, A.M.A.[1], Masnira, M.Y.[1], Nadhirah, I.M.[2], Khairil, M.A.[1], Adib, M.[1] and Yusmizan, M.[1]
[1]Pusat Penyelidikan Hortikultur, Ibu Pejabat MARDI, 43400, Serdang, Selangor, Malaysia.
[2]Pusat Penyelidikan Sains Tanaman dan Tanah, Ibu Pejabat MARDI, 43400, Serdang, Selangor, Malaysia.
**E-mail: hafeifi@mardi.gov.my*

Introduction

A great concern has grown amongst the people on searching for a pleasant environment athome, working place and indoor buildings. Indoor houseplants were introduced in the 1990s to create warmth and scenic beauty of nature to the environment (Davison, 2002). Foliage potted plants have been identified as suitable to perform as interior decoration plants that have potential to be used as miniature displays, small potted and cut foliage display and architectural display on living wall (Jenny and Val, 1998). Increasing awareness of a clean and safe environment, generating demands and creates a new market on producing high-value indoor plants. Floriculture industry in Malaysia has bloomed from RM417 million to almost RM514 million from 2011-2015 (Rozana and Noorlidawati, 2016). Introducing a new market on potted foliage will generate new income for the local nursery producers.

Aroids species were endemic to South East Asia, especially in rainforest region of Borneo Island. Aroids family consist of *Alocasia* genus. Some of *Alocasia* genus were found out to have medicinal value such as antitumor, antidiabetic, antidiarrhea and antibacterial properties (Romeo, 2017). Thus, the Malaysian Agricultural Research and Development Institute (MARDI) had identified one species from Aroids family, *Alocasia reginula* to be introduced and used as indoor plants. This species is native of Borneo, which was found a few years back in terrestrial forest (Boyce, 2004) and known as Black Velvet Alocasia or 'Tabin' from local Borneo peoplebecause of the black colour of the leaves visually and the smooth surface (Figure 1). 1 cm^2 *A. reginula* leaf area can absorb 7.34 parts per billion volatile organic compound in indoor environment (Hamdan, 2019). *A. reginula* can grow 30-45 cm tall. The leaves are cordate, black-green lamina and silver-white vein, with a length of 11-22 cm and width of 8-12 cm on mature plants (Figure 2). The flowers are white oblong shapes; grown at the centre of the leaf stalk (Figure 3). The plant is a slow-growing plant and can stand in an indoor office or house condition for almost 90 days (Hanim, 2014). The plant only produces the productive bulb after one year of planting. Past study shown Alocasia species such as *Alocasia amazonica*, *Alocasia cuprea*, *Alocasia robusta*, *Alocasia longiloba* and *Alocasia chaii*can be propagated using micropropagation and tissue culture protocol (Arvind, 2013). Information on agronomic practices on *A. reginula* is still limited, especially for conventional vegetative propagation techniques. Therefore, a study on vegetative propagation techniques for *A. reginula* using corm plant parts and suitable growth substratehad been conducted to identify the productive techniques for optimum regenerative growth.

Figure 1: *Alocasia reginula* plant structure.

Figure 2: *Alocasia reginula* motherplants.

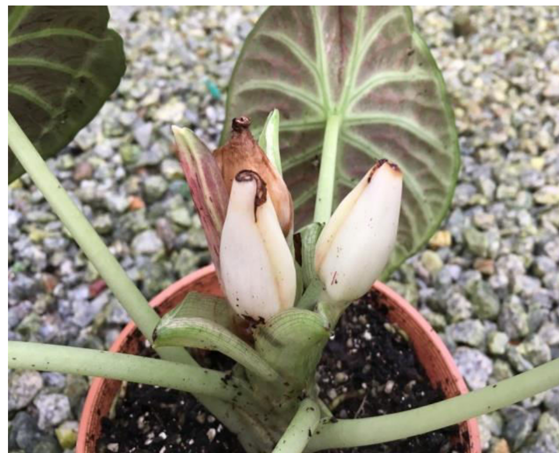

Figure 3: *Alocasia reginula* flower.

Materials and Methods

The experiments were carried out on 50% shaded condition at Flower and Orchid Complex, Malaysian Agricultural Research and Development Institute (MARDI). The experimental was laid out in a Randomized Complete Block Design (RCBD; factorial design). Two types of substrates, vermiculite (M1) and vermiculite mix with perlite (1:1 by volume) (M2) was used as the first treatment factor in this study and two types of horizontal corm cutting techniques, top of corm cutting (1-2 cm) (K1) and bottom of corm cutting (1-2 cm) (K2) was used as the second treatment factor. Four cuttings samples were used for each treatment (Figure 4). The treatments combinations are M1K1, M1K2, M2K1 and M2K2. The study of this species was established using six months old mother plants (Figure 2), taken from floriculture nursery, MARDI with a diameter of corm at an average of 2.2 cm and 3.1 cm. Parameters from the study were the day of shoot appearance, day of root appearance, the number of rooted cuttings, shoot numbers, the number of leaves, leaf length and root fresh weight. All parameters were collected from day one until day 30 after planting. The irrigation was manually applied to the plants once a day with 300 mL of plain water per block. The data collected were analysed by analysis of variance (ANOVA) using SAS software version 9.4. All significant values were analysed using the Least Significant Difference (LSD) at a significance level of $P<0.05$.

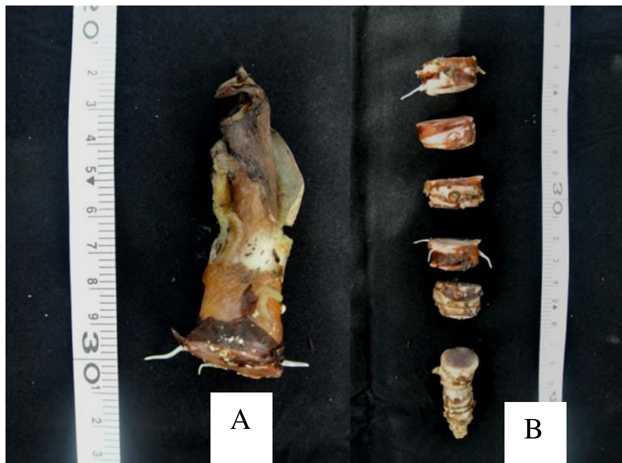

Figure 4: *Alocasia reginula* top of corm (A) and *Alocasia reginula* bottom of the corm (B).

Results and Discussion

The roots were established at 6-8 days after planting on both top and bottom of the corm. Regenerative of shoots of all treatments were shown at 9-15 days while the roots were abundantly regenerated at 10-15 days after planting. All tested treatments showed varying result for both propagation substrates and cutting treatments factor (Table 1). No significant difference was shown on the percentage of rooted cutting, number of roots on rooted cuttings, number of shoots and root fresh weight. Both treatment factors, type of substrates and horizontal corm cutting technique showed no interaction on all parameters taken except for leaf length of the regenerative cuttings. Treatments factor for substrates showed M2 was significantly different on shoot appearance compare to M1.

The shoot for M2 was generated earlier (10.75 days) compare to M1 (14.13 days). No significant difference was shown on root appearance for M1 and M2 treatments factor. However, for treatments factor K1 and K2, there was a significant difference in both shoot and roots appearance. K1 showed earlier shoot (9.5 days) and root (6.25 days) appearance compare to K2 shoot (15.38 days) and root (8.00 days) appearance. There was significantly different on leaf length for substrate treatment factor. Leaf length for M1 (1.9 cm) was significantly longer than M2 (1.36 cm). However, there was no significant difference on leaf length for treatment factor for horizontal corm cuttings. The best propagation parts were K1- the top of the corm horizontal cutting while M2-vermiculite shows the best propagation substrates to regenerates *A. reginula* vegetatively (Figure 5).

Alocasia reginula tend to generate new bud of corm on the perlite and vermiculite substrates. The new bud of corm will form a new shoot that becomes leaves, and on the corm surface, new primary roots were developed. Thus, *A. reginula* corm part planted in 50-60% of total porosity aggregate mineral substrates such as perlite and vermiculite is the best way to regenerate plant from the vegetative part.

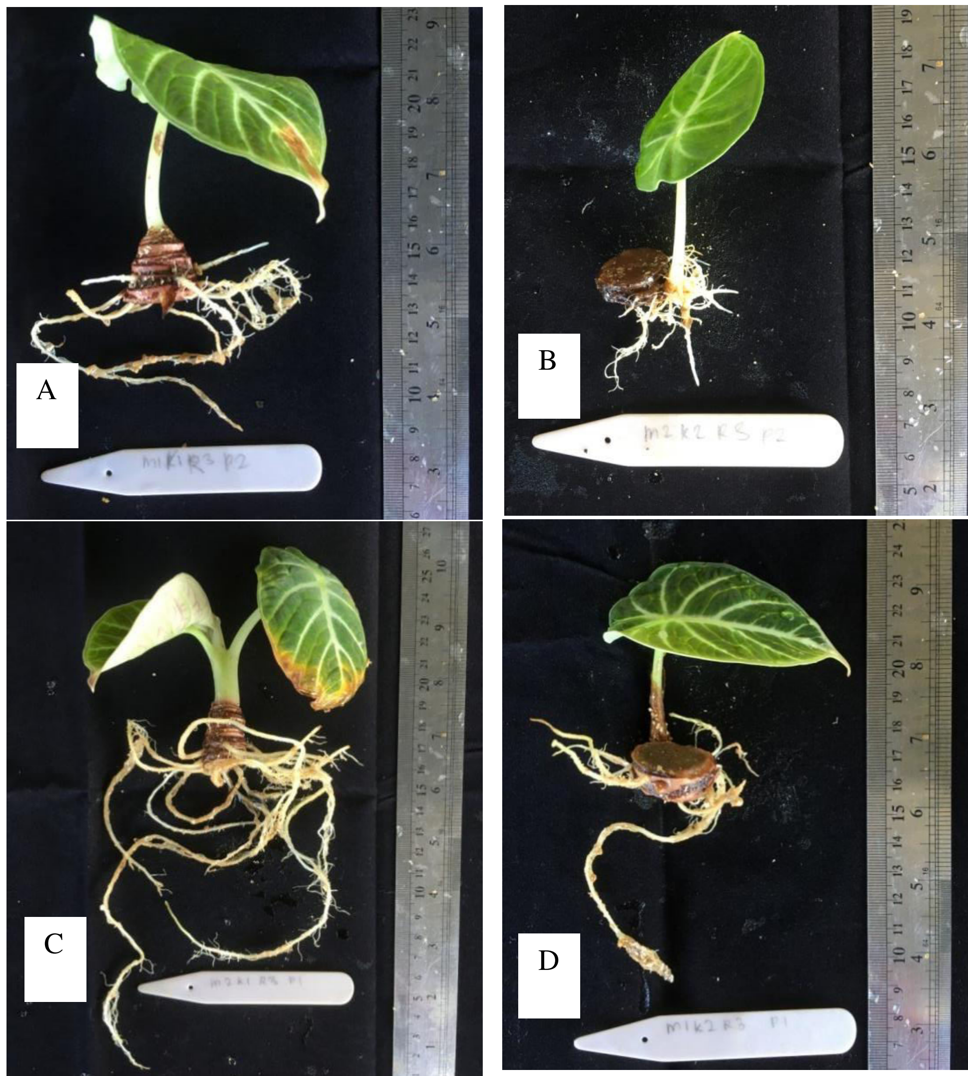

Figure 5: *Alocasia reginula* rooted cuttings. Treatments M2K1 (C) shows the best performance for regenerative growth on *Alocasia reginula*. However, other treatments (A, B and D) can be used as alternative vegetative propagation techniques.

Table 1: *Alocasia reginula* cuttings regenerative performance in different substrates.

Treatments		Shoot appearance (day)	Root appearance (day)	No. of rooted cuttings	Rooted cutting (%)	No. of roots on rooted cuttings	Shoot no.	No. of new leaves	Leaf length (cm)	Root fresh weight (g)
Substrate	M1	14.13^{a}	7.19^{a}	2.44^{a}	61.00^{a}	5.81^{a}	1.31^{a}	1.00^{a}	1.9^{a}	0.99^{a}
	M2	10.75^{b}	7.06^{a}	2.38^{a}	59.50^{a}	4.75^{a}	1.31^{a}	1.00^{a}	1.36^{b}	0.94^{a}
Cuttings	K1	9.5^{b}	6.25^{b}	2.38^{a}	84.50^{a}	5.63^{a}	1.31^{a}	1.00^{a}	1.68^{a}	1.03^{a}
	K2	15.38^{a}	8.00^{a}	3.44^{a}	86.00^{a}	4.94^{a}	1.31^{a}	1.00^{a}	1.58^{a}	0.90^{a}
Substrate* cuttings		ns	ns	ns	ns	ns	ns	ns	*	ns

(Notes: *Means with the same letter were not significantly different among the type of substrates and cuttings (p<0.05) using LSD).*

Conclusions

Horizontal corm cuttings for *A. reginula* can be used as practical vegetative propagation part. From this study, the top of the horizontal cutting corms part was the best part for propagating *A. reginula*. However, the bottom of the horizontal cutting corms part can be used to increase the number of propagated regenerative plants. The corm of *A. reginula* was better to generate new shoot and roots in vermiculite or both vermiculite and perlite combination substrates conditions. Thus, vermiculite and perlite can be used to propagate *A. reginula* in nursery conditions. This procedure provides new knowledge to propagate *A. reginula* using a suitable substrate and corm cutting technique for commercial ornamental nursery entrepreneur to produce mass production with low input capital and cost-effective. The established procedure will further compare with another method using micro propagation techniques.

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Traditional Eggplant as Future Crop for Food Security and Improving Livelihood

Mohd Zulkhairi, A.[1,*], Umikalsum, M.B.[1], Razali, M.[2,*], Nur Syafini, G.[2], Aimi Athirah, A.[3], Rosliza, J.[1], Rozlaily, Z.[4], Siti Aisyah, M.N.[1], Nur Daliana, Y.[1], Rosali, H.[1] and Noralienatul Azlia, A.A.[1]
[1]Agrobiodiversity and Environmental Research Centre, [2]Horticulture Research Centre, [3]Economic, Market Intelligence and Agribusiness Research Centre, [4]Industrial Crop Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: zulkhairi@mardi.gov.my*

Introduction

Solanum melongena belongs to the family of Solanaceae. *Solanum melongena* or locally known as terung in Malay was listed in the Annex 1 in the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). This highlighted the importance of this crop as one of the future crops for food security. Two (2) varieties of eggplant (*S. melongena*) available in the Malaysia are terung telunjuk and terung rapuh. These underutilize eggplant normally consume as *ulam* and also added mostly in curry dish. Terung rapuh has a rounded shape and can grow up to 1 meter in height. The fruit is much more smaller compare to normal round eggplant in the market with 3 cm in diameter size. The fruit is purplish in colour (Figure 1). Meanwhile, terung telunjuk can grow up to 1 meter and has a long shape fruit (12 cm) (Figure 2). The colour of the fruit upon ripening is green in colour. USDA data showed the nutritional background of *S. melongena* is rich in dietary fibres and also carbohydrate. On top of that, *S. melongena* also contain several beneficial minerals needed for the body such as calcium and magnesium. In addition, vitamins such as vitamin A, B6 and folate also were found presence in *S. melongena*.

Figure 1: Terung rapuh (*Solanum melongena* variety).

Figure 2: Terung telunjuk (*Solanum melongena* variety).

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Phytochemical studies on medicinal value from several varieties of *S. melongena* have been carried out such as anticancer, antidiabetic and antiviral (Kuo et al., 2000; Li et al., 2017; Sinani and Eltayeb, 2017; Gu et al., 2018). Besides that, a study found that fruit powder from *S. melongena* collected from Japan showed antihypertensive effect as the powder can significantly lowered acute and chronic blood pressure levels at very low doses (Yamaguchi et al., 2019). Nisha et al. (2019) reported that four different varieties of *S. melongena* exhibit scavenging activities against free radical with IC_{50} value ranging from 126.5–228.24 ug as compared with standard of ascorbic acid (IC_{50} 183.90 ug). However, up to this date, there is no study on these two varieties of *S. melongena* (terung rapuh and terung telunjuk) for its phytochemicals content. Hence, this study was carried out to determine the phytochemical content on these selected underutilize eggplants.

Materials and Methods

Plant materials

Terung rapuh and telunjuk were cultivated at Malaysian Agricultural Research and Development Institute (MARDI) germplasm located in Serdang, Selangor. The two varieties samples were harvested according to their ripening maturity stage and suitable for human consumption. Terung rapuh was harvested at day 23 of its maturity stage while terung telunjuk was harvested at day 28 of its maturity. The fruit samples were cut into small pieces and dried using freeze dryer (Labconco FreeZone and Virtis Benchtop SLC). The samples were ground into fine powder using a mechanical grinder (IKA Werke MF 10 basic, Germany) and the samples were kept in the chiller at -80ºC until needed for further analysis.

Free radical scavenging assay

All the crude extract was tested for its free radical scavenging assay as described earlier with minor modifications (Molyneux, 2003). The test was carried out using 96 well plates. Five mg of extract was prepared in one mL of 100 % methanol as stock solution. The stock solution was diluted accordingly into desired concentration for the working solution. The final volume obtained (7 µL) was mixed with 280 µL methanolic solution of (2,2-diphenyl-1-picrylhydrazyl) DPPH (Sigma, USA). The plate was covered with aluminium foil to avoid exposure with the sunlight and kept in the dark place for 30 minutes. Analysis was carried out using spectrophotometer at 517 nm. The results were expressed as inhibition concentration (IC_{50}) value (mg/mL) which is the inhibitory concentration at which DPPH radicals were scavenged by 50%. The ability of the sample to scavenge DPPH radical was determined from:

$$\text{DPPH scavenging effect} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Total phenolic content (TPC)

Total phenolic content of the crude extract was determined by the Folin–Ciocalteu method with some modifications (Singleton and Rossi 1965). Briefly, 50 µL of the crude extract were mixed with 100 µL of Folin Ciocalteau's phenol reagent (Merck, Germany). After 3 min, 100 µL of 10% sodium carbonate (Na_2CO_3) (Sigma Aldrich, USA) was added to the reaction mixture and allowed to stand in the dark for 60 min. The absorbance was measured at 725 nm and the total phenolic content was obtained from a calibration curve using gallic acid (0-10 µg/mL) as a standard reference. Estimation of the phenolic content was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg gallic acid per 100 g samples (mg GA/100g).

Statistical analysis

All the statistical analysis were carried out using MINITAB software (Version 17), and results are expressed as means ± standard deviation.

Results and Discussion

Figure 3 showed the DPPH free radical scavenging assay of terung telunjuk and terung rapuh extracts. The DPPH values are show as an inhibition concentration at 50% (IC_{50}) in mg/mL. Inhibition concentration (IC_{50}) define as concentration of extract needed to scavenge or inhibit free radical by 50%. The lower IC_{50} value indicates stronger the antioxidant activity. Based from the graph in Figure 3, terung telunjuk has stronger free radical scavenging activity with IC_{50} 3.54±1.3 mg/mL compared to terung rapuh with IC_{50} 12.47±0.84 mg/mL. IC_{50} value lower than 10 mg/mL is indicative of the effectiveness on its antioxidant activity (Lee et al., 2007). These two extracts were significantly difference (α=0.05) between each other.

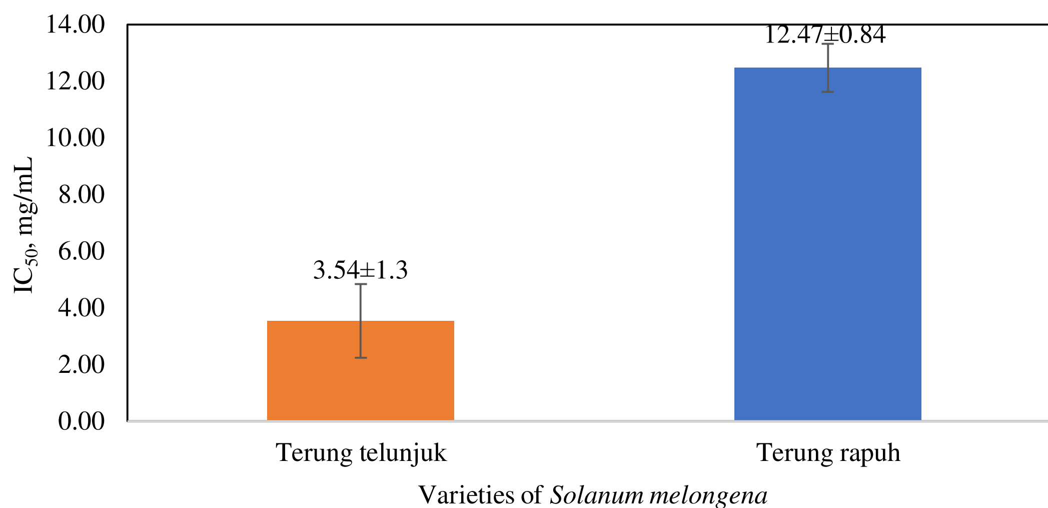

Figure 3: Free radical scavenging activity (DPPH) from two different varieties of *Solanum melongena*, terung telunjuk and terung rapuh. Each value is the mean ± standard deviation. Significance difference at value (α=0.05).

Meanwhile, Figure 4 showed the total phenolic content (TPC) in terung telunjuk and terung rapuh. The total phenolic content in terung telunjuk and terung rapuh showed no significance difference (α=0.05). However, terung rapuh has higher phenolic content compared to terung telunjuk with 11275±637.1 mg GA/100 g while terung telunjuk has 8713±796.3 mg GA/100 g respectively. Phenolic compounds are known to possess several bioactivities including antioxidant (Amirul Alam et al., 2018). The highest content of phenolic compounds in the terung rapuh does not affect the ability of the extract to scavenge the free radical as showed in Figure 3. This suggested other factor contribute to the antioxidant activity. Hence, further study needs to be conducted such as profiling the chemical compounds in these two underutilize eggplants.

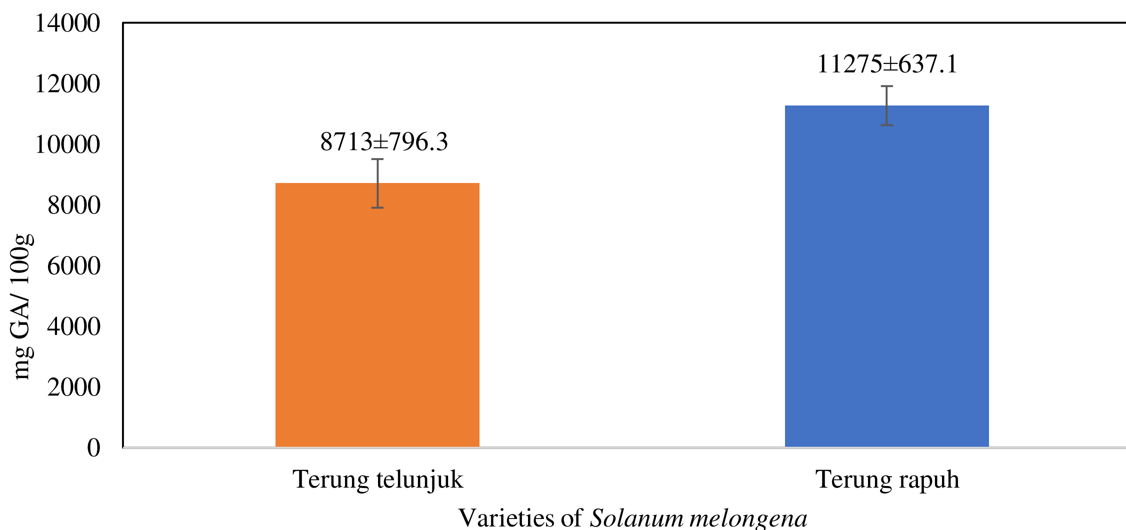

Figure 4: Total phenolic content from two different varieties of *Solanum melongena*, terung telunjuk and terung rapuh. Each value is the mean ± standard deviation. Significance difference at value (α=0.05).

Conclusion

Two varieties of *S. melongena* (terung rapuh and terung telunjuk) showed promising antioxidant activities. Terung telunjuk exhibit stronger free radical scavenging activity compare to terung rapuh (IC_{50}<5 mg/mL). However, the phenolic content in terung telunjuk is lower compare to terung rapuh with no signifance difference. Hence, the phenolic content in these eggplants does not contribute to the free radical scavenging activity. Hence, profiling on its chemical compounds in these two varieties is suggested as this information would be helpful to determine the cause of its antioxidant behaviour. Nevertheless, these two varieties of eggplants are potential to be cultivated commercially as future crop for food security and also to improve livelihood.

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Evaluation Rice-duck Integrated Farming System on Rice Growth and Yield

Noorsuhaila, A.B.[1,*], Wan Abdullah, W.Y.[2], Shajarutulwardah, M.Y.[4], Norziana, Z.Z.[2], Noraini, S.[3], Badrulhadza, A.[1], Chong, T.V.[4], Mohd Hadi Akbar, B.[5] and Arina Shairah, A.S.[6]
[1]Industrial Crop Research Centre, [2]Soil Science, Water and Fertilizer Research Centre, [3]Livestock Science Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[4]Paddy and Rice Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), PO Box No. 203, 13200 Seberang Perai, Pulau Pinang, Malaysia.
[5]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[6]Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
**E-mail: noorsuhaila@mardi.gov.my*

Introduction

Rice (*Oryza sativa* L.) is a staple food for Malaysia. Malaysia consumed 2.7 million tonnes (mt) of rice in year 2016. The self-sufficient level (SSL) of rice is 70% and the balance is importing from neighbouring country to meet local demand for Malaysia consumption (Yogambigai et al., 2015). Demand for rice is expected to rise every year as the population continues to grow. Narrowing existing gap in rice production in Malaysia as rice yield is a priority to secure food availability for the increasing human population. Increase food crop yield by intensifying agriculture systems using high inputs chemicals lead to degradation of soil and environment damage (Khumairoh et al., 2012).

In order to increase agriculture production without affect to environment, sustainable agriculture approaches should be introduced such as integrated farming (Karimi et al., 2011). Integrated farming is a mix of agriculture involves plants and animals in a same piece of land. Rice-duck integrated farming is an effective way to reduce chemical input, pesticides and herbicides used in plot. Integrated farming between rice and duck can increase rice production compared to conventional sole rice cultivation (Long et al., 2013). In this system, manure can be used as fertilizer to increase yield and improve properties of soil (Majidian et al., 2006; Noorsuhaila, A.B. 2017). Present of ducks in field enhance 5 percent the yield obtained (Karkacier and Goktolga, 2005). Other studies in integrated farming of rice and duck showed increasing the yield and yield components of rice (Hossain et al, 2001; Furono, 2009). Besides that, ducks can prevent plant diseases and pests (Yu et al., 2004; Lopes et al., 2011) which is a powerful tool in integrated pest management (IPM) and weed control in the rice field (Liang et al., 2012). Research on the rise duck integrated farming system has not been fully studied in adapting Malaysia condition. Only a study done by Teo (2001) suggested that ducks were an effective biological control agent against the golden apple snail in rice field. Thus, a field experiment of rice cultivation was carried out to evaluate integrated farming of rice and duck on growth performance and yield of rice.

Materials and Methods

A field experiment was carried out at the Malaysian Agricultural Research and Development Institute (MARDI) Seberang Perai, Pulau Pinang, Malaysia during main and off season in 2017. The plot size was 0.5 ha and surrounded with plastic fences to prevent movement of duck into the control plot. The rice variety used was MR 263. The seeds were soaked in water for 24 hours, drained and germinated under moist conditions. The 14-day old rice seedlings were transplanted to the paddy field with planting distance of 30 x 17 cm with one seedling per hole. The treatments were arranged in a completely randomized design for the field experiment consisting of two treatments with seven replications. Each experimental plot size was 0.5 ha. Other cultural management practices such as

fertilization, pest and disease management and weed control were followed in accordance with the recommendations of MARDI manual. After 30 days of transplanting (DAT), 250 one year old ducklings were released into the plot. Each paddy field plot was surrounded by a 50 cm high nylon mesh fence to prevent from escaping. The ducks were fed with pellet once every day. The ducks were removed from paddy field at flowering stage. The water layer was kept at 6-8 cm while ducks were in the field and irrigation was stopped two weeks before harvest. Rice was harvested at 95-115 DAT appearance of 90% yellow coloured grain and flag leaf. Control plots were cultivated identically except without ducks as follow standard practise. The measured variables of plant growth performance were plant height, number of tiller per hill and chlorophyll content. Five plants from each replicate were destructively sampled at every growth stage and separated to roots and shoots. The samples were completely rinsed with distilled water. Shoot and root fresh weight were taken and they were oven dried for 48 hours at 72ºC until constant weight. Yield measurements including yield and yield components such as number of panicle, number of grain per panicle and 1000 seed weight were taken. The t-test was used to evaluate the differences in all parameters recorded between two control and rice duck farming. All data collected were subjected to analysis of variance (ANOVA) procedure using SAS 9.4 software.

Results and Discussion

The results of variance analysis indicate significant effect for the treatment, season and their interaction on plant height, number of tiller per hill and chlorophyll content as shown in Table 1. The results indicated that rice-duck farming significantly increased the plant height during main season compare to control plot which range between 48.2 cm to 91.3 cm (Table 2). Whereas the plant height in control plot during main season were slightly shorter than rice-duck farming plot which range between 28.4 cm to 80.0 cm. Hossan et al. (2004) reported that rice plant height in rice-duck cultivation system due to activity of duck in paddy field such as pecking on soil causes more oxygenating to root and could stimulate of rice growth. Variance analysis showed that a significant difference was observed between treatments on number of tillers per hill at 28 and 75 DAT. However, no significant difference was observed between seasons except at 60 DAT. Significant interaction was observed between treatment and season at 28 DAT. The rice-duck farming produced more tillers during main (15.63 tillers per hill) and off season (13.08 tillers per hill) at 28 DAT, whereas control plot produce less tillers number per hill during main (6.86 tillers per hill) and off season (12.83 tillers per hill). Mohammadi et al. (2013) reported that number of tillers per hill in all numbers of rice had an increasing progression by duck density.

Variance of analysis showed that no significant difference was observed between both treatments on chlorophyll content. For season, significant difference was observed at 28 and 60 DAT. Significant interaction between treatment and season were observed at 28 and 90 DAT. During main season, the highest value of chlorophyll content (30.00 SPAD units) was obtained from rice-duck farming plot, whereas control plot was 22.54 SPAD units at 28 DAT. However during off season the highest value of chlorophyll content were observed from control plot (34.38 SPAD units) compare to rice-duck farming plot (29.77 SPAD units). From Table 3 the results of variance analysis indicate significant different for treatment, season and their interaction between treatment and season on shoot dry weight, root dry weight and shoot to root ratio at each growth stages of rice plants. The results indicated that rice-duck farming system marked increase shoot dry weight and root dry weight as compared to the control plants which range from 3.76 g to 42.46 g and 3.54 to 29.87 g, respectively during main season (Table 4). Results of interaction between treatment and season showed that the highest values for shoot dry weight (42.83 g), root dry weight (53.86 g) and shoot to root ratio (3.89) recorded from rice-duck integration system. This finding was supported study from Mohammad (2013) reported that growth of rice root increase due to stimulation from greenhouse gases like methane from rice-duck integrated farming system. A study done by Holidi (2015) claimed that a significant improvement on soil quality after removal of chemical toxin in organic farms thus provides favourable conditions for root development.

Table 1: Mean squares for the height, number of tiller and chlorophyll content among different treatments.

Source of variation	df	Plant height (cm)				Number of tiller/hill				Chlorophyll content (SPAD unit)			
		28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat
Replication	6	14.72^{ns}	9.43^{ns}	16.60^{ns}	14.35^{ns}	1.40^{ns}	7.26^{ns}	8.82^{ns}	11.36^{ns}	4.76^{ns}	2.33^{ns}	4.25^{ns}	6.93^{ns}
Treatment	1	525.14**	533.75*	403.41*	220.08**	142.38**	21.17^{ns}	26.62*	9.04^{ns}	14.26^{ns}	10.31^{ns}	16.45^{ns}	0.79^{ns}
Season	1	98.59*	36.75*	938.21**	343.00**	20.57^{ns}	364.17**	5.94^{ns}	8.11^{ns}	235.83**	1515.75**	30.08^{ns}	0.07^{ns}
Treatment × Season	1	855.16**	529.40*	585.87**	225.72**	127.12**	0.14^{ns}	1.70^{ns}	36.78^{ns}	264.77**	0.25^{ns}	43.60^{ns}	78.34**
Error	18	12.48	65.86	54.84	25.23	6.02	6.41	4.84	5.56	7.73	5.52	10.87	8.12
CV (%)		8.79	10.37	8.43	5.63	20.28	13.93	14.57	13.12	9.53	7.43	8.25	7.49

**, ** Significant at the 0.05 and 0.01 probability levels, respectively, ns, non-significant, dat, days after transplant.*

Table 2: Effects of rice-duck farming on plant height, number of tiller per hill and chlorophyll content.

Treatment	Plant height (cm)				Number of tiller/hill				Chlorophyll content (SPAD unit)			
	28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat
Main season												
CK	28.4	73.0	73.7	80.0	6.86	6.10	13.91	19.14	22.54	39.49	39.42	39.59
DR	48.2	90.4	90.4	91.3	15.63	10.23	15.37	15.71	30.00	21.67	38.46	36.59
Off season												
CK	43.3	74.8	94.4	92.7	12.83	20.85	14.34	17.93	34.38	24.96	39.00	36.15
DR	40.9	74.9	92.8	92.6	13.08	8.93	16.79	19.08	29.77	20.11	43.03	39.83

CK, control; DR, rice-duck farming, dat, days after transplant.

Table 3: Mean squares for the root and shoot dry weight of rice among different treatments.

Source of variation	df	Shoot dry weight (g)				Root dry weight (g)				Shoot to root ratio			
		28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat
Replication	6	0.96^{ns}	27.52^{ns}	11.80^{ns}	33.47^{ns}	3.12^{ns}	193.35*	411.27**	106.73^{ns}	0.14^{ns}	0.22^{ns}	0.32**	1.24^{ns}
Treatment	1	8.58**	1152.01**	381.84*	344.40**	1.20^{ns}	1727.00**	140.40^{ns}	202.50^{ns}	0.06^{ns}	0.00^{ns}	0.39**	2.38^{ns}
Season	1	6.13*	1374.80**	6.80^{ns}	6475.20**	15.75^{ns}	5149.00**	2368.08**	45.52^{ns}	0.31^{ns}	2.79**	3.28**	109.57**
Treatment× Season	1	19.39**	1300.17**	230.77**	230.86*	38.89**	1564.52**	4218.92**	600.79*	0.29^{ns}	0.01^{ns}	0.18^{ns}	4.96*
Error	18	1.00	38.48	47.48	41.07	3.77	71.66	83.07	118.71	3.77	0.08	0.04	1.08
CV (%)		35.24	27.45	23.31	30.71	66.82	33.91	30.23	45.73	66.82	28.51	20.10	39.64

**, ** Significant at the 0.05 and 0.01 probability levels, respectively, ns, non-significant, dat, days after transplant.*

Table 4: Effects of rice-duck farming on shoot, root dry weight and shoot to root ratio of rice plant.

Treatment	Shoot dry weight (g)				Root dry weight (g)				Shoot to root			
	28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat	28 dat	60 dat	75 dat	90 dat
Main season												
CK	0.99	16.37	17.29	29.70	0.77	23.2	24.83	15.23	0.80	1.34	1.38	0.51
DR	3.76	42.83	42.83	42.46	3.54	53.86	53.86	29.87	0.91	1.31	1.31	0.77
Off season												
CK	3.59	15.96	34.46	5.03	4.63	11.03	30.99	27.04	1.21	0.68	0.86	5.31
DR	3.03	15.19	23.68	6.30	2.69	11.79	10.91	23.16	0.91	0.71	0.46	3.89

C, control; DR, rice-duck farming, dat, days after transplant.

From Table 5 the results of variance analysis indicate significant different for treatment on yield and 1000 grain weight. Meanwhile, significant difference on 1000 grain weight between seasons and interaction between treatment and season. Yield was significantly different between rice-duck farming and control plot (Table 5). From Table 6, rice-duck farming plot showed higher yield (3.16 tonne per hectare) for both season compare to control plot. Meanwhile, yield obtained from control plot were 2.24 and 2.46 tonne per hectare for main and off season, respectively. Rice duck farming system produced nearly a tonne more grain per hectare than control. This increase yield was due to the rice plants producing more spikelets per panicle and growing more grain. This increase in yield was similar with study done by Hassain et al. (2004) showed integrated rice duck farming system increase yield by 20% by controlling weeds and insect effectively. Rice-duck farming plot produced more number of panicle per meter square (m^2) (129.57) during off season (Table 6). In overall, number of panicle per m^2 obtained during main and off season were range from 105 to 109 and from 110 to 130, respectively. Rice-duck integrated farming gave the highest number of grains per panicle which were around 80 grains per panicle. Whereas number of grain per panicle during main and off seasons were range from 80 to 83 and from 75 to 83, respectively. This means that there were no difference between the quality of panicle between the two treatments. A study done by Ahmad et al. (2004) in Bagladesh claimed that duck caused an increase in number of panicle and number of grain per panicle which confirms results from our research. Overall, rice duck farming system might have great potential to improve grain yield. Raising ducks in paddy field increased the supply of nutrients for plant growth by excretion of animal material which greatly enhanced plant growth, and promoted total above ground biomass accumulation (Zhang et al., 2013).

Table 5: Mean squares for the yield and yield components of rice.

Source of variation	df	Yield (t/ha)	Number of panicle per m²	Number of grain per panicle	1000 grain weight (g)
Replication	6	0.31^{ns}	370.20^{ns}	206.86^{ns}	2.84^{ns}
Treatment	1	4.57^{**}	401.29^{ns}	178.27^{ns}	177.76^{**}
Season	1	0.09^{ns}	1056.57^{ns}	60.61^{ns}	582.08^{**}
Treatment × Season	1	0.08^{ns}	984.14^{ns}	51.05^{ns}	168.60^{**}
Error	18	0.55	278.47	301.08	5.51
CV (%)		26.92	14.67	21.64	11.03

**, ** Significant at the 0.05 and 0.01 probability levels, respectively, ns, non-significant, C, control; DR, rice-duck farming, MS, main season; OS, off season; dat, days after transplant.*

Table 6: Yield and yield components of rice.

Treatment	Yield (t/ha)	Number of panicle per m²	Number of grain per panicle	1000 grain weight (g)
Main season				
CK	2.24	109.71	80.49	21.69
DR	3.16	105.43	82.84	11.74
Off season				
CK	2.46	110.14	74.85	25.90
DR	3.16	129.57	82.60	25.77

C, control; DR, rice-duck farming, dat, days after transplant.

Conclusion

In conclusion, the results of this study indicated that rice duck farming promoted better growth performance and therefore increase yield of rice. Ducks might stimulated rice plant physiology as well as they moved around the paddy field. Rice-duck farming is environmentally friendly and economically profitable for small farmers. By this rice duck farming system the usage of pesticides and herbicides might be reduced and eventually promote sustainable agriculture.

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Effects of Two Different Rootstock Types on Grafting Success and Vegetative Parameters of *Baccaurea motleyana*

Ariff Merican, D.M.[1,*], Faizah Salvana, A.R.[2], Khadijah, A.[2], Muhammad Shafie, M.S.[3] and Mohd Shukri, M.A.[4]
[1]Seed, Planting Materials and Livestock Production Program, Technology Commercialization and Business Centre, [2]Industrial Crop Research Centre, [3]Agrobiodiversity and Environment Research Centre, [4]Horticulture Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: ariffmdm@mardi.gov.my*

Introduction

In Malaysia, there are 31 out of 42 known species of *Baccaurea* (Khadijah et al., 2014). Six species are popularly planted in home gardens and orchards (Khadijah et al., 2018). *Baccaurea motleyana* or locally known as rambai is a fruit tree species belonging to the Phyllanthaceae family and is commonly cultivated in the lowland areas of Malaysia. It is a seasonal fruit tree and the fruits are often eaten fresh. *Baccaurea* species has the potential to provide additional nutrition including due to its high antioxidant activities (Nurhazni et al., 2013; Khadijah et al., 2018). It was also reported that the peel of *B. motleyana* has antimicrobial properties (Khoo et al., 2016). Currently, the only known propagation method of rambai is through seeds. However, propagation through seeds causes genetic variations since they receive genes from male and female parents. Since two sets of genes are received in the seeds, many fruit trees planted from seeds are not true to type. Another important problem with propagation via seed for *Baccaurea* species is that there is 80 to 90% probability of seedlings becoming male plant (Khadijah et al., 2014). Plant sex identification (male or female) cannot be determined at seedling stage and flowering usually takes place 4 to 5 years after planting (Abdullah et al., 2005). Therefore, vegetative propagation is preferred due to its ability to produce plants that are the same as the parent. For fruit trees, grafting is the most preferred method to produce seedlings that have similar properties with their parent plant. MARDI currently has 19 accessions of rambai conserved at MARDI Jerangau, Negeri Sembilan. Out of the 19, two accessions were identified to have commercial potential due to their ability to bear sweeter fruits and are high yielding. Therefore, the objectives of this study were to identify the ability of rambai to be propagated through grafting, and to identify suitable rootstock for rambai using two different species of rootstock.

Materials and Methods

Rootstock preparation

Seeds of *B. polyneura* and *B. motleyana* were sown in the sand bed for 2 months. Seedlings were then transferred into 20.3 cm x 25.4 cm polybags with media mixture of 2 parts of topsoil, 1 part of cocopeat and 1 part of sand. Rootstocks were placed under shaded structures. Fertiliser was added monthly using NPK Blue fertiliser (12:12:17 + trace elements). Age of rootstocks used in this experiment was 6 months after transplant.

Scion preparation

Scion of rambai elite accession MDIR082 was collected from MARDI Jelebu. Each scion was cut at the length of 9 to 10 cm and wrapped using parafilm to avoid desiccation of the scion.

Grafting of rambai

Top wedge grafting method was used in this experiment (Figure 1). The rootstock of each *B. polyneura* and *B. motleyana* was cut at the top using a pair of secateurs where the girth of the

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rootstock at the region was almost equal to that of scion. A longitudinal cut of 1 to 2 cm in length was made at the terminal end of the rootstock. A scion of about the same thickness as the rootstock was selected. The lower end of the scion was sliced giving a wedge shape of about 1 to 2 cm by removing the bark and wood from the two opposite sides. The wedge-shaped scion was inserted into the V-shaped slit of the stock and secured firmly with a polyethylene grafting tape.

Aftercare of grafted seedlings

Grafted seedlings were placed under shaded structure and were watered twice daily. Prophylactic plant protection measures were adopted for the control of pest and disease. Any emerging sprouts below the graft union were removed regularly.

Observations recorded

Percentage of grafting success was taken two weeks after grafting. Scions with green shoots were considered as successful while brown and withered scions were recorded as unsuccessful grafting. Length of scion sprout (cm) was recorded weekly for 4 weeks for each successful graft. Length of scion sprout was measured from the point of sprout emergence to the tip of the sprout. Number of leaves was calculated every week until 4 weeks after successful grafting. Only leaves that were perfectly formed were counted.

This study was carried out at the planting material production nursery in Serdang, Selangor. Using a completely randomised design (CRD) with two treatments (two different species of rootstock), four replications and 30 samples each. The data obtained were subjected to the Analysis of Variance (ANOVA) and the means of differences among treatments were compared using Least Significant Design (LSD).

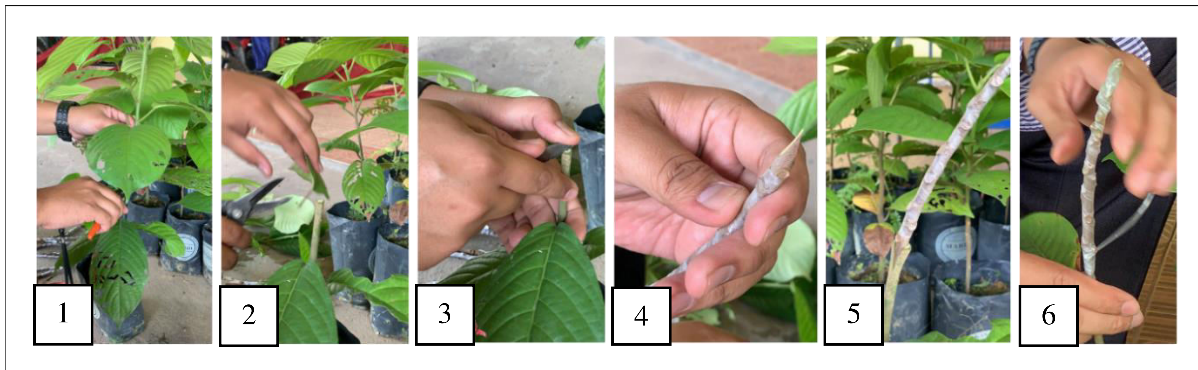

Figure 1: Top wedge grafting of rambai. The method involved cutting the top part of rootstock (1) and (2), longitudinal cut of 1 to 2 cm in length was made at the terminal end of rootstock (3), lower end of the scion was sliced giving a wedge (V) shape (4), the scion was wedged into the cut at the rootstock (5), and the union was secured firmly with polyethylene grafting tape (6).

Results and Discussion

Grafting success of B. motleyana

The results indicated that grafting success when *B. motleyana* scions were grafted on to *B. motleyana* rootstocks was 90% while scions grafted on *B. polyneura* was only 75% (Figure 2). The compatibility of scion and rootstock is very important to determine the success of a graft. Plants that are closely related, preferably within the same genus have a better chance of forming a union, while plants within the same family have a lower chance of success (Kumar, 2011).

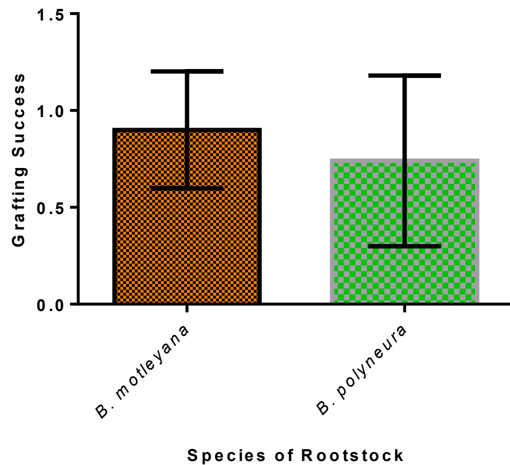

Figure 2: Grafting success between scions grafted on two different species of *Baccaurea.*

Length of scion sprout

The length of scion sprout did not show significant difference in the first two weeks. At week 4, scion sprout for scions grafted onto *B. motleyana* rootstocks grew up to 1.14 cm compared to 0.08 cm when grafted on to *B. polyneura* (Figure 3). The rapid growth of *B. motleyana* scions on top of rootstocks from the same species showed high compatibility of rootstock and scion. Grafting is a technique that involves the joining of vascular tissues between the scion and rootstock. The vascular cambium between both scion and rootstock should be pressed tightly together and oriented correctly in the direction of normal growth. Correct alignment and pressure help the tissues to join rapidly, allowing nutrients and water to transfer from the rootstock to the scion (Hartmann et al., 2014).

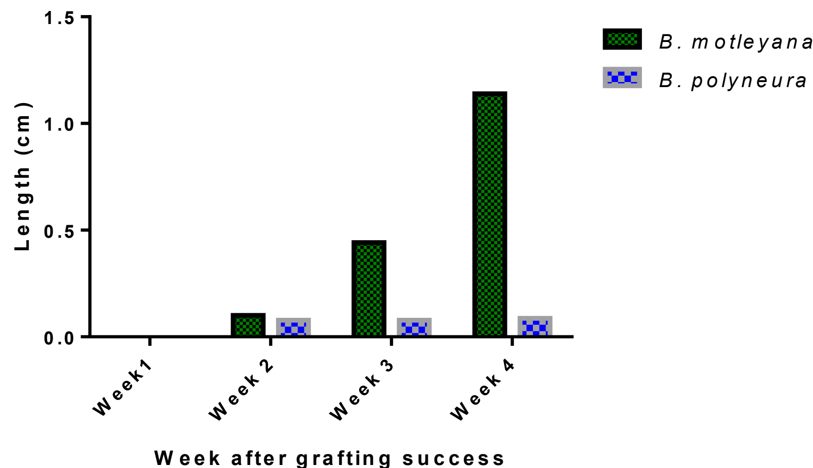

Figure 3: Length of scion sprout for *Baccaurea motleyana* and *B. polyneura* taken each week for four weeks after successful grafting.

Number of leaves

The leaves for scion grafted on top of *B. motleyana* started growing during the third week after successful grafting. The final leaf count on the fourth week was 3.675 leaves for *B. motleyana* rootstocks compared to 0 leaves of *B. polyneura* (Figure 4). The scion on top of *B. polyneura* maintained the green coloration and was still sprouting slowly without any development of true leaves.

Figure 4: Number of leaves for *Baccaurea motleyana* and *B. polyneura* taken each week until fourth week after successful grafting.

Conclusions

B. motleyena scions were more compatible with *B. motleyana* rootstocks. High percentage of success (90%), sprouting of scion and number of leaves indicated that top wedge grafting of *B. motleyana* using *B. motleyana* rootstocks is recommended for the propagation of *rambai*.

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Effect of Corm Cutting and Media Propagation on Functional Indoor Plant, *Tradescantia* sp.

Puteri, A.M.A.*, Masnira, M.Y., Hafeifi, M.B., Hanim, A., Adib, M. and Yusmizan, M.
Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: aminatulhawa@mardi.gov.my*

Introduction

Tradescantia, from the family Commelinaceae, is a genus of perennials that are grown for their flowers or ornamental foliage. Some of them are pendant, others grow more upright. The leaves are long, lance-shaped and from 10-20 cm long. The color of leaves can transform depending on the light intensity and moisture of the media. In the dark room and wet soil, the purple color of its leaves turn out to greenish green or greenish purple. Meanwhile, in hot and dry conditions, the leaves will be small, hard and have light purple color (Rukayah, 2015). The flowers can be white, pink and purple with small 3 petals. It is popular as ornamental houseplants. According to Placencia et al. (2019), *Tradescantia pallida* is more sensitive for gaseous pollutant exposure which makes them as an effective biomonitor for vehicular toxicity. Besides, it has superior removal efficiencies for four compounds including benzene, toluene, trichloroethylene and alpha pinene (Dong et al., 2009).

Tradescantia sp. as shown in Figure 2, can be propagated from seeds with a high germination rate, but the plants take a long time to reach flowering stage. Stem cutting is the most frequent method used for vegetative propagation of many plant species from herbaceous to woody plants. However, success of propagation via stem cuttings is usually affected by many factors including the status of the mother plant or cutting source, type of culture medium, type of cutting, rooting hormones and environmental conditions (light, temperature, air humidity and soil moisture) during propagation (Hassanein, 2013). There is very little information available concerning the vegetative propagation of this species from corm component. Therefore, propagation from corm cuttings should be considered. The aim of this study was to determine the effect of types of corm cutting and media for propagation of *Tradescantia* sp.

Materials and Methods

The experiment was conducted at the Flower and Orchid Complex, Horticulture Research Center with 50% shaded condition. There are two types of cutting (C1: Corm with leaf; C2: Corm) and two types of media [M1: Vermiculite; M2: Vermiculite:Perlite (1:1)] were used as treatment. The experiment was arranged in a factorial of 2 types of corms x 2 types of media in RCBD with four replications. Cuttings of 15.0-16.5 cm long, with six leaves, were trimmed to half leaves from top, without the apical meristems. Then, cutting were planted in media based on treatments. Data obtained included the survival rate, day of adventitious roots to appear, day of bud of corm to appear, rooting percentage, number of adventitious roots, number of bud and bud length. Irrigation was applied manually twice a day. The data was subjected to an analysis of variance (ANOVA) and differences among treatment groups were assessed using LSD using SAS software version 9.4. P values <0.05 were considered to indicate statistically significant results.

Results and Discussion

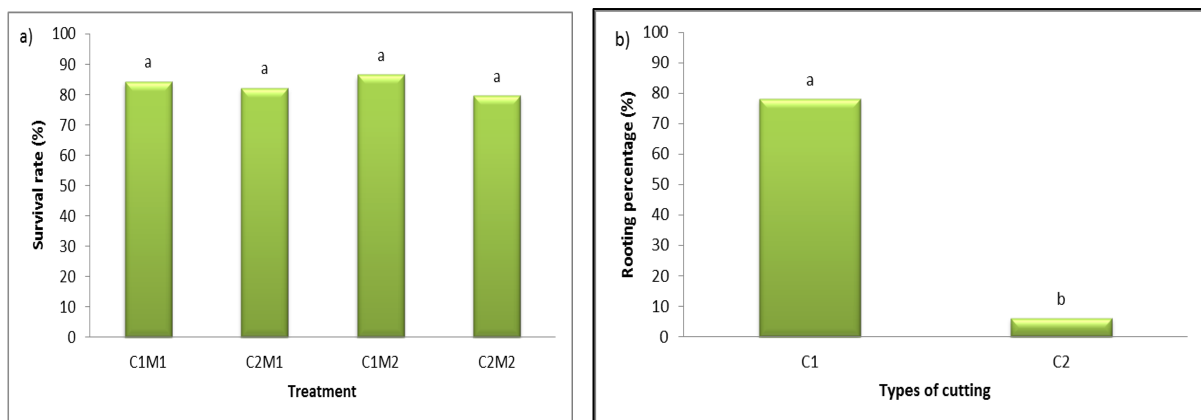
The result shows that the interaction between type of cutting and media was not significant. Hence, the main factor of type of cutting and media will be discussed further. Media did not significantly affect the survival percentage in *Tradescantia* sp. cutting. For all treatments, the survival percentage of the cuttings was over 82% (Figure 1a). The substrates alone (vermiculite) and the mixture of vermiculite and perlite (1:1) gives rooting percentage 46.7 % and 37.5 %, respectively. The effect of vermiculite

and perlite substrate was reported as the best survival percentage and rooting percentage of *Primulina tabucum* Hance, *Arbutus unedo* and cherry cuttings (Metaxas et al., 2008; Exadaktylou et al., 2009; Lu et al., 2012). For the types of *Tradescantia* sp. cuttings, the rooting percentage and the days of adventitious roots appear in cutting C1 (Figure 3) was significantly higher ($P<0.001$) than cutting C2. The root for C1 was generated earlier (5 days) compare to C2 (12 days). Moreover, the number of adventitious roots of cutting C1 was significantly higher ($P<0.01$) than C2. The number of root appear for C1 was higher (4) compare to C2 (1). As for the bud of corm formation (Figure 4) for *Tradescantia* sp. cuttings, the same trend shows for the days of bud to appear and length of bud whereby cutting C1 was significantly higher ($P<0.05$) than cutting C2. The bud for C1 was generated earlier (2 days) compare to C2 (12 days). However, there was no significant difference on the number of bud of corm for all treatments.

Table 1: Main and interaction effects of two types of cutting x two types of media on days of roots to appear, number of roots, days of bud of corm appear, number of bud of corm and bud length of *Tradescantia sp.*

	Days of roots to appear	Number of roots	Days of bud of corm to appear	Number of bud of corm	Bud length (cm)
Factor					
Type of Cutting (C)					
C1 : Corm with leaf	5^{a}	4^{a}	2^{a}	1^{a}	1.3^{a}
C2: Corm	21^{b}	1^{b}	12^{b}	1^{a}	0.1^{b}
Media (M)					
M1: Vermiculate	16^{a}	3^{a}	8^{a}	1^{a}	0.6^{a}
M2: Vermiculate:Perlite (1:1)	10^{a}	1^{a}	7^{a}	1^{a}	0.8^{a}
Significant level					
Cutting	***	**	*	ns	**
Media	ns	ns	ns	ns	ns
Interaction					
Cutting x Media	ns	ns	ns	ns	ns

*Means values in the same column followed by the same letter are not significantly different at *** $p<0.001$, ** $p<0.01$, * $p<0.05$ and ns = Not significant.*



Means with different letter are significantly different at $p<0.01$.

Figure 1: (a) Survival rate and (b) Rooting percentage of different types of cuttings of *Tradescantia* sp.

Figure 2: Mother plant of *Tradescantia* sp.

Figure 3: Corm with leaf of *Tradescantia* sp.

Figure 4: Bud of corm *Tradescantia* sp.

Conclusions

The result obtained from the study revealed that *Tradescantia* sp. will not root easily when propagated with corm cuttings alone. It would be advisable to use the corm with leaf for the propagation of *Tradescantia* sp. using substrates (vermiculite, perlite) to produce large amounts of planting materials.

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Chapter 6

Seed Technology and Quality Planting Materials

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Effect of Different Capsule Age on *in vitro* Seed Germination of *Paraphalaenopsis labukensis,* an Endangered Orchid in Sabah

Heira, V.N. and Nor Azizun, R.*
Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
**E-mail: azizun@ums.edu.my*

Introduction

The floral of Borneo is quite remarkable with a tremendous number of trees, pitchers, and unusual orchids species. Even Borneo was also known as Orchid Island (Chan et al., 1994). Orchidaceae is one of the largest families of flowering plants in the world. It was estimated that 2,500 to 3,000 of orchid species are recorded in Borneo (Chan et al., 1994). *Paraphalaenopsis labukensis* is an epiphytic orchid, with a vast distribution in Borneo, where it can only be found in Sabah specifically in Kuala Labuk and Mount Kinabalu. The flower has purplish-cinnamon and turns more golden yellow with age. The existence of terete leaves which could exceed more than 2 m in the wild is one of the unique characteristics of this species.

Human activities such as logging, farming, illegal collection and trade have led to the extinction of this species. This species is also difficult to propagate vegetatively and naturally grows slowly. Hence, *P. labukensis* was listed as one of the endangered orchid species in Appendices I of the Convention for International Trade in Endangered Species Wild Fauna and Flora (CITES) (Lamb, 1991). Therefore, there is a need to explore the propagation methods to avoid endangering its natural populations. Seed germination and development of orchids started when an embryo enlarged to form protocorm and further developed into new seeds (Jaime et al., 2005). However, in natural environment, seed germination of wild orchid is considered difficult because of a symbiosis relationship (Salifah et al., 2011). Hence, lots of propagation techniques were developed for various orchid species including hybrids through the *in vitro* asymbiotic culture from seeds (Lesa et al., 2012; Balilashaki et al., 2015). However, propagation through *in vitro* seed culture has not yet been achieved in *P. labukensis* orchid. The success of seed germination through *in vitro* culture is influenced by several factors, including the types of culture media (Vudala and Ribas, 2017), plant growth regulators (Vogel and Macedo, 2010; Paudel et al., 2012) and the most influential factor is seed maturity (Suzuki et al., 2012; Udomdee et al., 2013).

Asymbiotic seed germination is one of the efficient and effective techniques for orchid micropropagation, as well as to conserve many rare and endangered orchid species (Harrison and Arditti, 1978; Vij et al., 1994; Shimora and Koda, 2004; Zeng et al., 2012). Capsule age plays an important role in determining the success of seed germination in orchid. Learning proper harvesting time and optimum age capsules are very useful to avoid seed dormancy to develop an efficient protocol on seed germination. In the present study, we evaluated the effects of different capsule age and basal medium on seed germination of *P. labukensis*. The reproducible protocol for the production of seedlings from seeds by *in vitro* culture of this species has been established.

Materials and Methods

Plant samples

Three healthy plants of *P. labukensis* (1-2 m) at their full bloom stage were used in the present study. The plants were grown in the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

Pollination, capsule development, and harvesting period

The hand-pollination procedure of *P. labukensis* flowers was carried out manually by transferring pollen onto stigma on the same flower to achieve self-pollination. The capsule length, diameter, and colour were evaluated weekly until full maturity reached. The period for each capsule to open was also recorded. The freshly collected undehised capsules at three different stages (60, 90, and 120 days) after pollination were used for seed germination. Length and diameter of the capsule at each stage were also recorded by using a ruler and Vernier calliper.

Sterilization of capsules

The capsules were washed thoroughly under running tap water and brushed to remove any solid particles that adhere to the surface. Next, the capsules were surface sterilized by dipping it into 30% (v/v) for 30 s, followed by agitation for 20 min in 5.25% (w/v) of sodium hypochlorite solution plus two drops of Tween 20 in a completely aseptic condition. The capsules were then rinsed three times with sterile distilled water in laminar flow. The sterilized capsules were cut longitudinally into two and the seeds were taken out and sowed immediately onto culture media.

Seed viability test and in vitro seed germination

To determine the viability of seeds, approximately 600 seeds were used for 2,3,5-triphenyltetrazolium chloride (TTC) staining tests, and 200 seeds were estimated for each replicate. The seeds were incubated in 1% (w/v) of TTC solution for 48 h in the incubator at 28°C. The percentage of viable TTC-stained embryo seeds was calculated as the viability percentage of seeds. The seeds were then placed in a Petri dish and counted by using a microscope. For *in vitro* germination, seeds were sowed in Knudson C (KC) basal media and pH was adjusted between 5.6 to 5.8 before added with 3 gL^{-1} of agar Duchefa prior to autoclaving at 121°C for 20 min. Media were poured and seeds were inoculated evenly on the culture media to examine seed germination.

Statistical analysis

There are six replicates for each treatment and the experiments were carried out in a completely randomized design (CRD). The data on germination and viability percentage of the seeds were subjected to an analysis of variance (ANOVA) and T-test. Subsequently, the Duncan's Multiple Range Test (DMRT) was used to determine the significance different at $p<0.05$.

Results and Discussion

Pollination of flowers and capsules development

The present experiment showed some interesting results on capsule formation and developmental stages. Figure 1 showed the development of *P. labukensis* capsule after pollination which took approximately 125 days (18 weeks). Hand pollinated flower of *P. labukensis* started to develop capsules 30 days after pollination (DAP). During the development, the diameter of capsules increased during the first 21-67 days and it remained constant until 125 days. The initial capsule length of *P. labukensis* was only 2 cm and its diameter was 2.1 mm. Determining proper harvesting time and optimum capsule age is very useful to avoid seed dormancy in order to develop an efficient protocol on germination (Birhalawati et al., 2014). Blooming of *P. labukensis* was observed during early and end of the year. Then, the length of the capsule was constantly increased from day 25 until day 80. Both diameter and pod length remained constant when it reached its maturation period. The maximum length recorded at day 100 was 10.5 cm and its diameter was 14.0 mm.

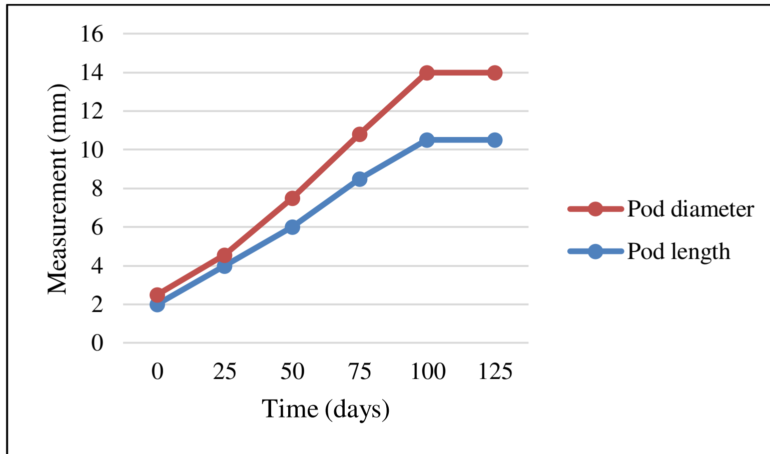

Figure 1: The diameter measured in capsules of *P. labukensis* during development.

Starting from hand-pollination until capsule maturity stages it took approximately 125 days. It takes a long time (4 to 10 months) for the orchid pod to be matured after pollination (Norberto, 2010). Meanwhile, time for the orchid seeds and pods to be matured are depending on genus and species of orchids (Lo et al., 2004). Optimum harvesting time for achieving full maturity takes it times according to genus and species of orchids such as *Phragmipedium humboldtii* in between (70 DAP), *P. longifolium* (112 DAP), and *P. pearcei* (224 DAP) (Munoz and Jimenez, 2008).

Capsules of *P. labukensis* were dark green at 60 DAP (Figure 2A), indicating immaturity and seed is very compact, difficult to be spread onto media culture. At mature stage, capsules were red in colour before opening and the seed is in dust-like structure (Figure 2C). Norberto (2010) reported that the capsule maturity also can be determined when it's green colour turn yellowing or there is a presence of crack.

Figure 2: Characteristic of *P. labukensis* capsule and seed based on different pod age (60 days, 90 days, and 120 days). [Bar: Figure A = 0.5 mm, Figure B and C = 1.0 mm]

Seed viability test

In the present study, Table 1 shows the result of a viability test on three different capsule age. Sixty days after pollination capsule showed the lowest viability percentage or less red-stained on the seed embryo. The viability percentage of fresh and immature seeds was only 2.5%. The embryo of fresh seeds is a group of living things which will be stained by TTC. Hence, the viability test was not suitable for immature seed. Embryogenesis of orchid seeds is completed immediately three months after pollination. Consequently, the immature seed of this species is considered to exhibit a low germination percentage and when embryogenesis is completed, high germination percentage will be achieved (Nagashima, 1982). In most orchids with mature embryos, the germination percentage is high (Lee et al., 2005).

Table 1: Effect of time harvest during seed formation on stainability of embryos of *P. labukensis* by using 1% TTC.

	Days after pollination (days)		
	60	90	120
Stainability (%)	2.5[a]	34.6[b]	78.8[b]

The same letters indicate not significantly different at $p<0.05$ *by* DMRT.

For 90 DAP capsule, the viability percentage slightly increased to 34.6% and followed by 120 DAP capsule with 78.8% (Table 1). Hence, seeds harvested after 120 DAP were recommended for seed germination as it has the most red-stained on the seed embryo (Figure 3C). The seeds were inoculated on KC basal media and after 35 days of culture, seeds started to swell in which germination occurs. Germination was considered to occur when the embryo has swollen. Hence, 120 days capsule was used as the explant for *in vitro* seed germination of *P. labukensis*.

With increasing maturity, the seed germination percentage gradually increased (Wu et al., 2018). Generally, orchid seeds are lacking with endosperm and have an undifferentiated embryo.

Figure 3: Red-staining of *P. labukensis* seeds after undergoes TTC test. (A) No stained observed for immature 60 days capsule, (B) 90 days capsule less-stained, and (C) Most red-stained seeds in 120 days capsule. [Bar = 0.5 mm].

Effect of capsule age on germination of P. labukensis

Most embryos become more viable after capsule ripening (Lo et al., 2004; Thakur and Dongawar, 2012). Seeds were swollen and germinated within three weeks after culture. The seeds completely developed into protocorm at stage 3 after 70 days of culture. The effect of different capsule age was tested. Three different capsule ages were used as the source of explant in this study (60, 90, and 120 days old capsules). As a result, four months old (120 DAP capsule) showed the highest percentage of seed germination after culture. This may be due to capsule maturity which is ready to be germinated. The germination percentage was the highest in 120 DAP capsule followed by 90 DAP capsule and immature capsule (60 DAP) showed the least or the lowest germination rate. Both capsule has slightly increased and changes towards the germination of *P. labukensis.* Hence, 120 DAP capsule was chosen as explant for seed germination of *P. labukensis*.

Effect of basal media on germination of P. labukensis

Seed germination and seedlings development of *P. labukensis* were highly influenced by the mineral composition of the basal medium. Three basal medium were tested in this study, which are Murashige and Skoog (MS), Knudson C (KC), and Vacin & Went (VW). As a result, KC basal medium showed the best germination rate, followed by MS and VW media recorded the lowest. It was demonstrated that KC medium could inhibit the germination of *P. labukensis*. Hence, the medium composition in those basal medium plays a crucial role in the development and seed germination of *P. labukensis*. All tested media contain variations of mineral salts, not only in terms of its concentrations but also the

mineral forms such as variation of nitrogen (Sonia et al., 2012). Seed germination in KC and MS basal media has been influenced by the suitability and presence of significant elements as compared to VW media. KC media contain only inorganic nitrogen, which is ammonium and nitrate, while MS basal media composed of inorganic and organic elements. Nitrogen is important element in developing the cell wall. Lack of nitrogen ions could inhibit the growth of seeds.

Table 2: Effect of basal media on seed germination of *P. labukensis*.

Basal media	Germination (days)			
	70	90	110	130
KC	74.13±8.58[b]	82.91±5.20[b]	88.03±2.62[b]	93.61±3.45[a]
MS	48.90±11.76[b]	63.75±7.39[a]	71.73±4.30[b]	88.05±4.45[b]
VW	44.65±16.46[a]	48.58±14.93[b]	66.56±7.71[a]	73.45±6.62[a]

Different letter in the same row shows significantly different at p<0.05 by DMRT. *KC: Knudson C; MS: Murashige and Skoog; VW: Vacin & Went. Mean±standard deviation.*

Seed germination and seedling development of *P. labukensis* took approximately 130 days to complete in which the seedlings produced has at least two leaves and one root, as shown in Figure 4F. Seed started to swell and break the seed coat in KC media (Figure 4B) after 14 days and formed protocorm after 30 days of culture (Figure 4C). The highest formation of green protocorms can be seen on the KC and MS media. The green colour indicated that the protocorm grows healthy. This is due to the presence of magnesium and potassium that stimulate the development of chlorophyll (Birhalawati et al., 2014). Hence, low concentration of nutrient preferred to support the germination of *P. labukensis* seeds.

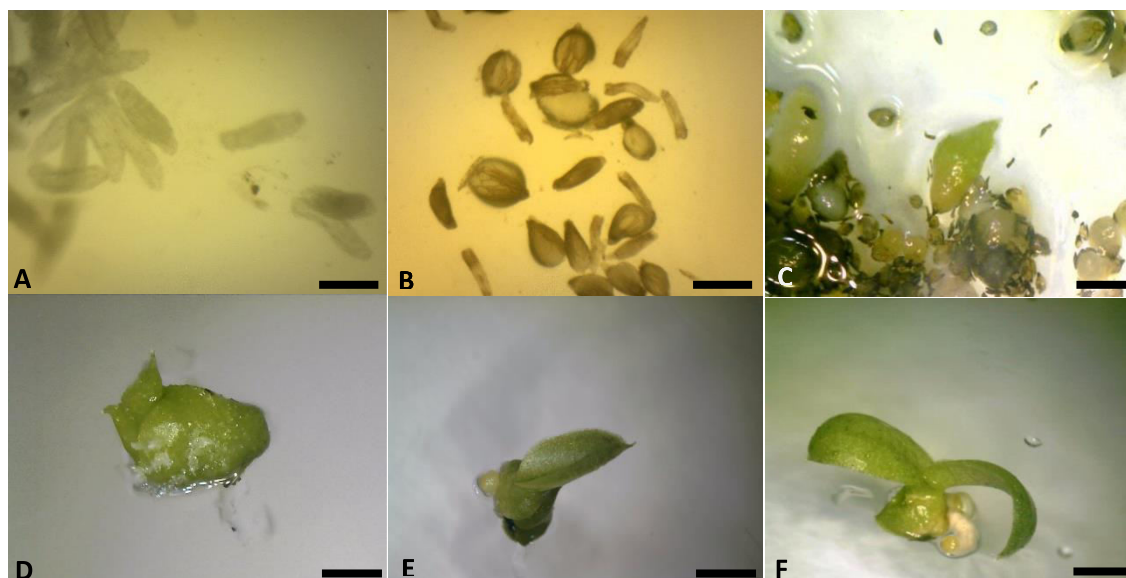

Figure 4: Seed germination and seedlings development of *P. labukensis* after 130 days of culture.

The process of seed germination to the seedling formation of *P. labukensis* within 142 days after culture has been summarized in Table 3. Based on full observation, it can be classified into five stages of embryos development stages.

Table 3: The seed germination, protocorm formation, and seedling development of *P. labukensis*.

No.	Description	Age (days)
1	Non germinated seeds	0
2	Protocorm with appointed shoot and rhizoid	37-40
3	Protocorm with the emergence of the primary leaf	55-78
4	Seedling with two leaves and one root	82-118
5	Seedling with three leaves and two roots	125-142

Conclusions

The best capsule age is important to achieve the best germination rate in *P. labukensis* species. This research indicates that by using 120 DAP of capsule, it can boost the germination efficiency when cultured on KC basal media for germination.

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Effect of Different Basal Media on Seed Germination of an Epiphytic Orchid, *Aerides odorata* Lour. var. Alba

Norisa, J.[1,2] and Nor Azizun, R.[1,*]
[1]Unit for Orchid Studies, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia.
[2]Faculty of Natural Science and Sustainability, University College Sabah Foundation, Jalan Sanzac, 88100 Kota Kinabalu, Sabah, Malaysia.
**E-mail: azizun@ums.edu.my*

Introduction

Aerides odorata is a wild epiphytic and tropical orchid with monopodial growth habit. It is widely distributed from Southeast Asia ranging from India to Papua New Guinea (Chris, 1996; Devi et al., 2013). In recent years, orchids have been producing a high economic interest in many countries, especially in horticulture, floristry, pharmaceutical, and fragrant industries. The epiphyte *A. odorata* is among important orchid in Malaysia. This orchid species produces a refreshing aroma, and its scent has made it valuable sources for the extraction of fragrances. Additionally, the flowers of this species are attractive in colors and beautifully arranged along the stalk (Hongthongkam and Bunnag, 2014). It has the potential to be commercialized as fragrance-cut flowers.

Ecologically, epiphyte orchids grow on trees for support, and it also has low water and nutrient availability in their growth habitat. In the wild, the major threat for most wild orchid species includes habitat disturbance, over-harvesting for trade and illegal human activities (Sathiyadash et al., 2014). In conserving natural resources, appropriate strategies and conservation plans need to be made, which will keep the orchid biodiversity in this region. Thus, plant tissue culture and micropropagation are the options to reduce pressure from the illegal collection, to meet commercial needs and to re-establish threatened species back into the wild. Hence, the *in vitro* propagation technique through seed culture is considered as an effective technique or strategy to multiply this orchid species and to maintain variability of plant genetic, either for conservation purposes or for horticultural activities (Utami and Hariyanto et al., 2019). Apart from that, *in vitro* seed germination which involved symbiotic or asymbiotic represents the most efficient method of wild orchid propagation for conservation purposes over the world (Sainiya et al., 2014).

In nature, orchid seeds germinate symbiotically which involved a compatible mycorrhizal fungus that can provide embryos with water, carbohydrates, minerals, and vitamins (Kauth et al., 2008). However, according to Bijaya (2013), orchids are inherently slow growers and the rate of seed germination in nature is very low, which is only 2-5%. Orchid produces a huge number of seeds, but it is an incredibly tiny, dust-like and nonendospermic that lacks of enzyme which cannot grow directly. The introduction of an asymbiotic method by Knudson (1922) made it possible for orchid seed to be germinated. Asymbiotic seed germination is a more straightforward process. It may require specific growth media with appropriate culture conditions (Arditti, 1967; Zeng et al., 2013) as well as seed maturity and origins (Chen et al., 2015). Therefore, this study was carried out to develop an efficient and effective *in vitro* asymbiotic seed germination as well as the determination of the best basal media. Towards the end, this data can be used as the sources and information reports towards orchid growth and development. The result generated by this analysis should help to determine the potential basal medium to propagate more orchid species by *in vitro* propagation method.

Materials and Methods

Plant material

Aerides odorata was obtained from the greenhouse located at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. To increase capsule productivity, hand pollination was performed at the full bloom period of *A. odorata* flowers (Figure 1A). The immature capsules of 4-months after hand pollination was harvested (Figure 1B) and the capsules were brought to the laboratory for the surface sterilization process.

Surface sterilization

Surface sterilization was conducted to remove any small particle or other contaminants on the capsules (Figure 1B). The procedure of surface sterilization was summarized in Figure 1. The capsules of *A. odorata* were immersed in a 0.5% fungicide solution for 10 minutes. Then, the capsules were gently scrubbed with a toothbrush in a soap solution for another 10 minutes before immediately washed under running tap water till all the detergent was washed off clearly (Figure 1C). After that, the capsules were surface-sterilized sequentially with 70% of sodium hypochlorite solution containing one or two drops of Tween-20 for 10 minutes and rinsed three times with distilled water (Figure 1D).

Seed culture

After surface sterilization, the capsules were dipped into 95% ethanol before passing it briefly through the flame (Figure 2A) (David et al., 2015). The capsule was cut longitudinally into halves (Figure 2B) and then inoculated into three different basal media: MS (Murashige and Skoog, 1962), KC (Knudson, 1922) and VW (Vacin and Went, 1949), respectively (Figure 2C). All experiments consist of three independent replicates with five culture plates per replicate and placed under a 24-hour photoperiod at constant temperature (25ºC). Each petri dish contains approximately 800 seeds, which are determined by calculating the individual number of seeds that examined under a light microscope. The average number of seeds for each plate was calculated. Seed germination and protocorm development were examined weekly.

Observation and data collection

The process of seed germination was divided into five categories according to the developmental stages of the embryo, as characterized by Steward and Kane (2007) in Table 1. Seed germination percentages for each developmental level were calculated by dividing the number of seeds in a particular stage by the total number of seeds sown and multiplied by 100. Seed germination and protocorm development were examined by weekly intervals.

Table 1: Development stages of asymbiotically cultured of *Aerides odorata* seeds and protocorm.

Stage	Description
0	No germination, testa intact
1	Embryo swollen (the indication of germination)
2	Continued embryo enlargement testa ruptured, rhizoids present
3	Appearance of protomeristem
4	The emergence of the first leaf
5	Elongation of first leaf and further development

Statistical analysis

All experiments were carried out in a Completely Randomized Design (CRD) and were repeated in five replicates. Then, the cultures were observed under Carl Zeiss Microscope and Dinolite Capture TM (UK) starting from seed germination, growth, and development. Analysis of variance (ANOVA)

was analyzed statistical package, and mean values were compared by using the Duncan Multiple Range Test (DMRT) at p<0.05 (Khamchatra et al., 2016).

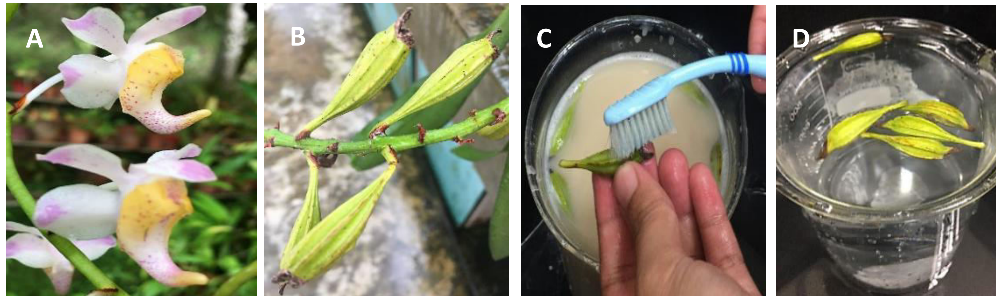

Figure 1: Surface sterilization of *A. odorata* capsules. (A) The flower of *A. odorata*. (B) *A. odorata* immature capsules. (C) The capsules immersed under fungicide solution and scrubbed with a toothbrush. (D) The capsules immersed in distilled water.

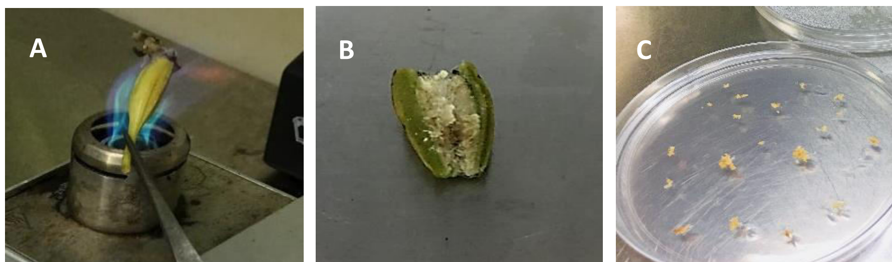

Figure 2: (A) Capsule dipped in 90% ethanol and flamed on a burner. (B) The capsule cut longitudinally into two halves. (C) The seed was inoculated and cultured on different basal media.

Results and Discussion

The effect of different types of basal media towards seed germination was monitored and evaluated after sowing for 120 days (Table 2). The immature capsule was selected because it shows better germination response for certain orchid species (Pant and Swar, 2011; Parmar and Pant, 2016). The germination was achieved by testa rapture which indicates spherule formation (stage 1: spherical stage), followed by a protocorm formation (stage 2: development of a promeristem).

Table 2: Effect of different basal media on *in vitro* seed germination of *A. odorata* after 120 days of culture.

Media	The average percentage of seed germination (%)					
	20 days	40 days	60 days	80 days	100 days	120 days
MS	20.19±2.31^{a}	50.98±4.83^{a}	76.32±2.62^{a}	100.0±0.00^{a}	100±0.^{00a}	100±0.00^{a}
KC	0.0±0.00^{b}	21.61±3.67^{b}	34.14±2.94^{b}	41.22±2.30^{b}	54.92±6.54^{b}	62.75±8.04^{b}
VW	0.0±0.00^{b}	0.0±0.00^{c}	0.0±0.00^{c}	0.0±0.00^{c}	0.0±0.00^{c}	0.0±0.00^{c}

Values are mean (n = 5) ± standard deviation. Different letter in the same row shows significant different at p<0.05. MS; Murashige and Skoog. KC; Knudson C. VW; Vacin and Went.

Not all seeds germinated in the basal media tested, but a varied response in terms of seed germination and differentiation was observed. In this study, seed germination was significantly influenced by medium composition and capsules condition. The maximum percentage of seed germination was recorded in MS basal medium (100%), followed by KC (62.57%) and VW (0%). According to Arditti and Ernst (1993), seed germination could be influenced by several factors including a specific nutritional and medium condition that was needed for *in vitro* seed germination. Xu et al. (2001) also described the importance of nitrogen source and organized elements for seed germination and culture development in *D. chrysotoxum.*

The percentage of seed germination of *A. odorata* was recorded starting for an interval of 20 days. Seeds on MS medium started to germinate after 20 days of culture and some seeds were developed into stage 2 which is enlargement of globular wall structures due to swelling embryos. The physical changes of seed indicate the first visible sign of the germination of cultured seeds (Khamchatra et al., 2016). After 80 days, the seeds on MS media were fully germinated. This is indicated by swelling of the embryo and seed coats rupture (Figure 3B) followed by embryo enlargement (Figure 3C) and finally, the transformation of embryos to an early stage of protocorm (Figure 3D). The color of cultured seeds has been changed from light yellowish into light green and finally to green in color during the development of protocorms (Figure 3). Most of the seeds on MS media were still maintained in stage 2, where the embryo was continued enlarging.

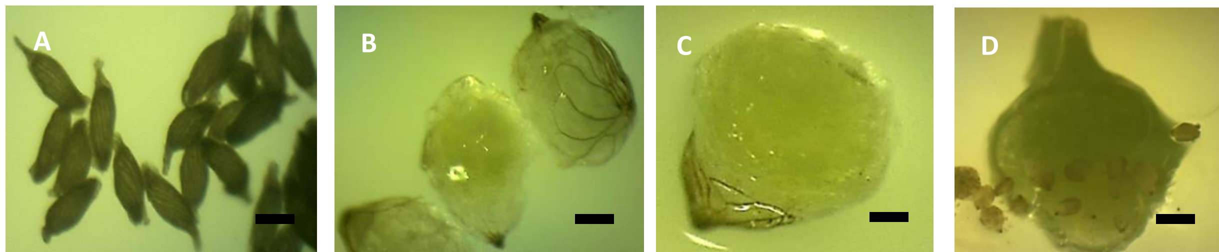

Figure 3: *In vitro* seed germination and protocorm development stages of *A. odorata* in MS medium. (A) The seed on day 1. (B) The seed started to germinate and grow from seed coat after 20 days. (C) The embryo continues to enlarge after 50 days. (D) The embryo was developed into a protocorm stage after 80 days. Scale bar = 0.25 mm (A) and 0.50 mm (B–D).

In contrast, the seeds of *A. odorata* in the KC medium was started to germinate at day 40 where seeds have begun to swell and break out from the seed coat (Figure 4). Only 21.61% of seed germination was achieved in the KC media after 40 days of culture. Within 120 days of observation, although 62.75% of the seeds germinated in KC medium, none of these entered stage 3 (appearance of protomeristem). The color of seeds on the KC medium was maintained in light yellowish since the first day of culture. This study showed that the KC medium gave satisfactory results of seed development, but the germination took a longer time compared to the MS medium.

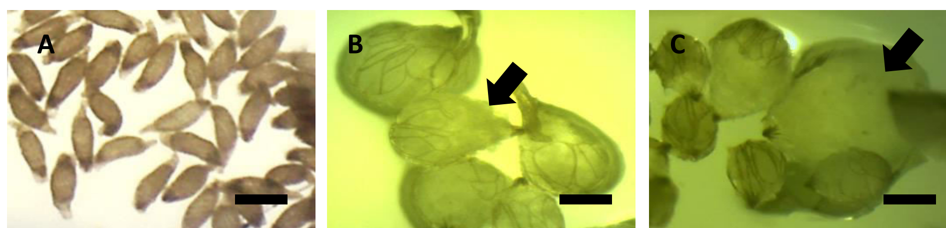

Figure 4: *In vitro* seed germination and protocorm development stages of *A. odorata* in the KC medium. (A) Stage 0 (arrow) no germination. (B) Stage 1 (arrow) embryo swollen after 30 days of culture. (C) Stage 2 (arrow) embryo enlargement; testa ruptured after 49 days of culture onto the KC medium. Scale bar = 0.25 mm (A) and 0.50 mm (B – C).

Observations on seed germination of *A. odorata* on the VW medium failed to show any sign of germination within 120 days of culture (Figure 5). The differences in types of chemical composition and concentration of each element play a vital factor in seed germination. MS medium is highly enriched with macro and microelements with different vitamins, whereas KC and VW media contained a comparatively amount of macro and microelements without vitamins (Shreeti et al., 2013). Seeds germinated on the KC medium had shown not much effect on protocorm development, although a high percentage of seed germination was observed. Hence, KC media is not suitable for protocorm development because the nutrients in the KC medium were not enough to support the growth of protocorm. Production of a huge number of protocorms indicates that culture seeds require enough

nutrients. There are several reports on asymbiotic germination of immature seeds, which vary from species to species (Arditti and Ernst, 1984; Zeng et al., 2013). Besides, mature orchid seeds may have a more significant potential for propagation and storage because of fully developed testa and lower water content (Fu et al., 2016).

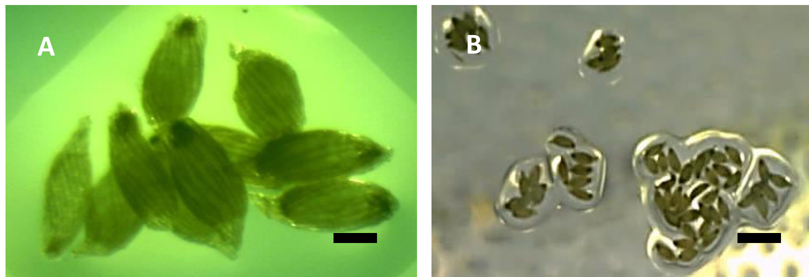

Figure 5: Asymbiotic seed germination of *A. odorata* in the VW medium. (A) The seed on day 1, (B) Stage 0 (no germination after 120 days of culture). Scale bar = 0.25 mm (A–B).

Conclusions

A simple protocol for *in vitro* asymbiotic seed germination of *A. odorata* has been developed. The rate of germination was recorded within 120 days with MS medium was found to be the most effective for seed germination compared to other media.

Acknowledgments

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Effect of Irrigation Frequency on Growth and Biomass of Cocoa (*Theobroma cacao*) Seedlings

Rozita, O.[*], Saiful Mujahid, A.R. and Sharih, S.
Malaysian Cocoa Board, Locked Bag 211, Wisma SEDCO, 88999 Kota Kinabalu, Sabah, Malaysia.
**E-mail : orozita@koko.gov.my*

Introduction

Water scarcity is one of the main threats in agriculture. Due to global warming and uncertainty of weather conditions, knowledge on the needs for water regimes in plant are important. In many localities, water is still the limiting factor for most agricultural crops and this will affect yields. Plant water status usually controls the physiological processes and growth performance of the plant. Due to the current situation, farmers need to improve their irrigation strategies in order to save water consumption and at the same time to give adequate water supply to the plant. Several studies showed that shorter interval of irrigation application will positively influence plant growth and increase yield production (Adejomubi et al., 2015). Cocoa is a drought sensitive crop and it has been grown in many parts of the world that have a distinct alternation in water supply between wet and dry seasons. With the threat of extreme climate conditions, inadequate rainfall in the tropics, higher temperature and drier air conditions these cocoa production and subsequently yield production are difficult to forecast. Inadequate water supply in cocoa plants can results in premature leaves fall, the yellowing of basal leaves, wilting, small leaves and slow trunk growth (Carr and Lockwoods, 2011).

Research on mature cocoa trees in the field related to water requirements are scarce as they are usually planted under different shade conditions and using diverse varieties and other intercropping practices such as cocoa interplanted with coconut, banana, fruits tree etc. All these factors may affect the requirements of water in plant. Therefore, to understand these effects, an experiment in greenhouse study was carried out to examine the effect of irrigation frequency on cocoa seedlings growth.

Materials and Methods

The study was conducted under rain shelter house of Malaysian Cocoa Board (MCB) Research and Development Centre (CRDC), Jengka, Pahang. This study was started with the sowing of cocoa seedlings. After three months, the seedlings were grafted with KKM 22 clones. All cocoa seedlings were placed under 70% shade. Irrigation was given at uniform level for all grafted seedlings up to three months. After three months, the grafted seedlings were treated with different frequency of irrigation. There were four groups of irrigated seedlings: a) watered daily, b) watered at alternate day, c) watered at every two days and d) watered at every three days. The amount of water given to each treatment during their irrigation schedule is equal distributed except the frequency as mentioned.

All treatments were replicates four times and arranged in Randomized Complete Block Design (RCBD). Parameters that have been recorded including plant height, plant girth, fresh and dry weight of the plant and root volume. A destructive samplings was taken every month for five consecutive months.

Statistical analysis

All data collected from this study was analysed using ANOVA and the difference between the means using SAS software version 9.2 in Duncan's Multiple New Range Test (DMNRT).

Results and Discussion

At the beginning of the experiment, there was no significant difference of all parameters between treatments. However, after a month of irrigation treatments, the plant height of the seedlings which received water only every three days shows stunted growth and this result showed continuation growth until months two (Figure 1). At the fourth months, mortality for all seedlings watered at three days interval was high. This indicates that seedlings survival (with stunted symptom) and growth can be sustained of up to three months for plants received water at three days interval. Thereafter, after fifth months, the seedlings that were water daily and at alternate days showed high survival while those watered at every two days showed mortality.

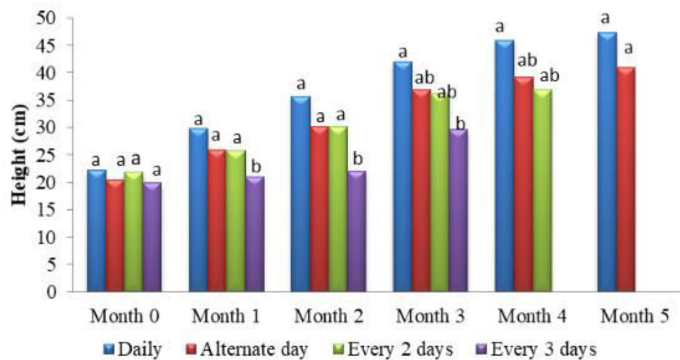


Figure 1: Effects of different frequency of irrigation on plant height.

Height and girth growth

Plant girth was significantly bigger when watered daily compared to other frequency of irrigation (Figure 2). However, continuously water deficiency may cause the cocoa seedlings to die in week four. These two parameters (height and girth) are significantly affected by the frequency of water regimes. Result from this study also shows that there was no significant difference in plant height and girth growth when he plants were irrigated in either daily or alternate days.

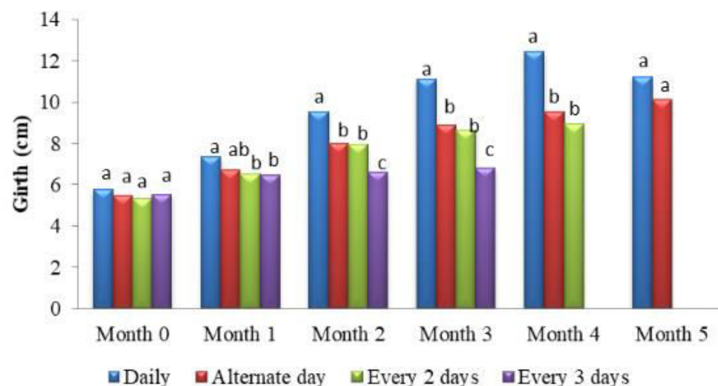
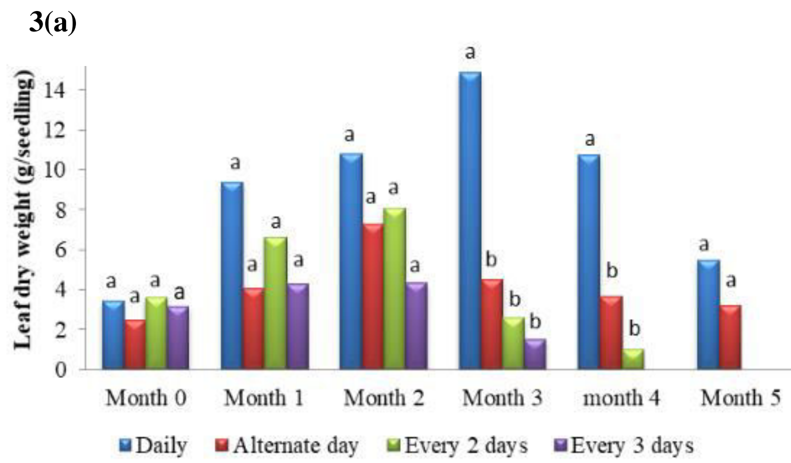


Figure 2: Effect of different frequency of irrigation on plant girth.

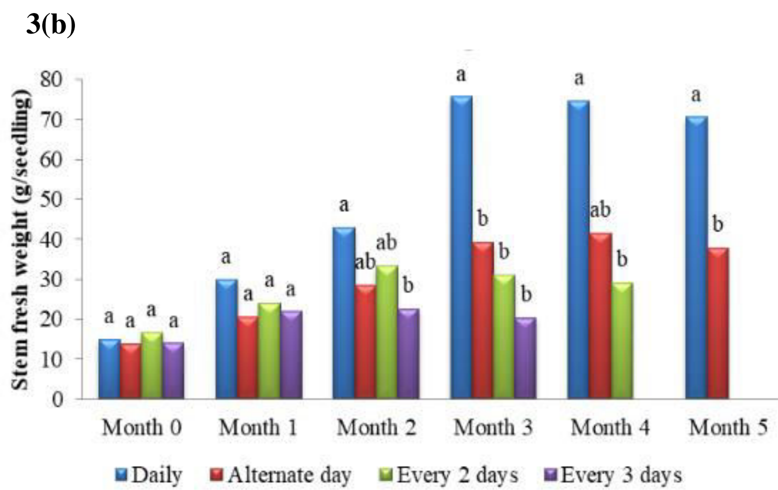
Leaf, stem and root growth

Leaf fresh and dry weight, stem fresh and dry weight as well as root fresh and dry weight show similar trend where the seedlings which water daily have a significantly heavier biomass than other frequency of irrigation as shown in Figure 3a, 3b, 3c, 3d, 3e and 3f.

3(a)



3(b)



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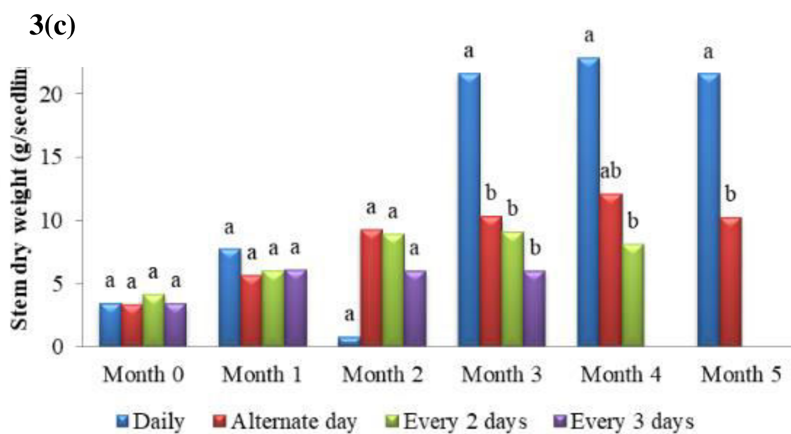
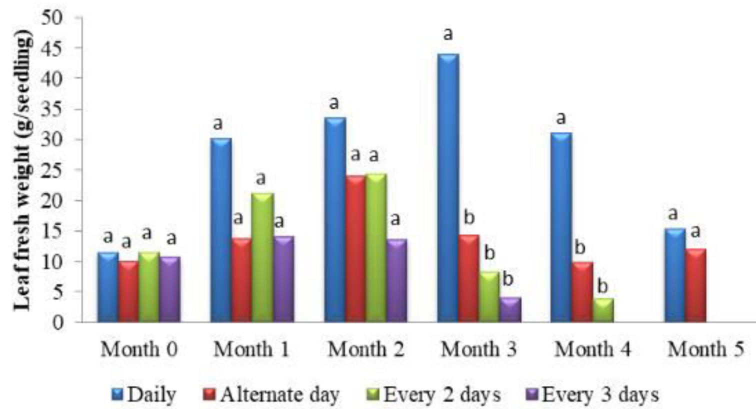
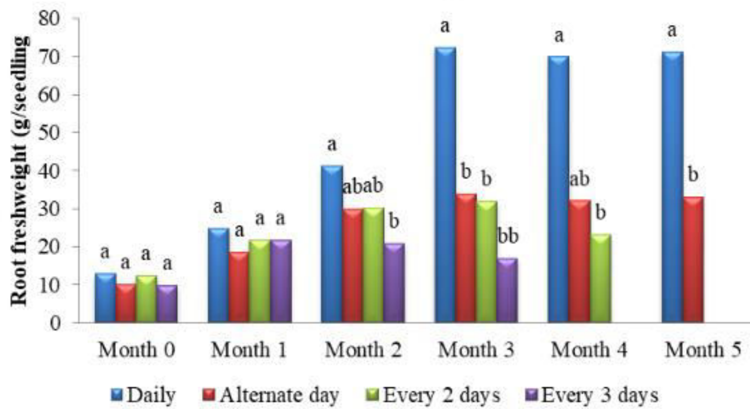


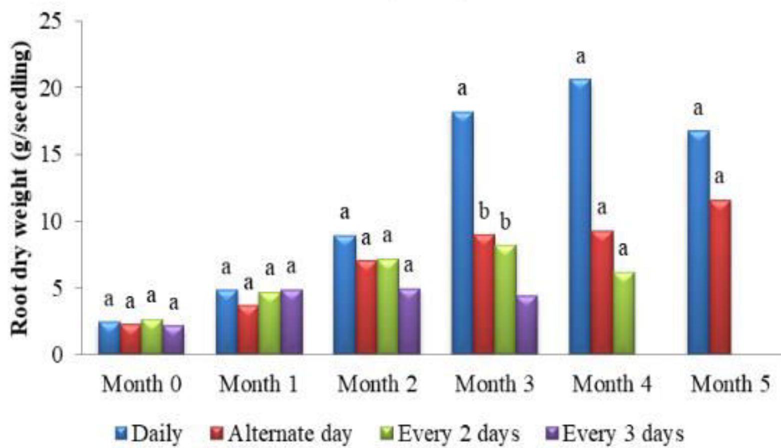
3(d)



3(e)



3(f)



Figures 3: Effects of different frequency of irrigation on plants growth.

Root volume

Root volume was also measured. For the first three month of the treatments (including the zero month), all seedlings shows no significant different among different frequency of irrigation (Figure 4). However, at the third month of the treatment application, the seedlings with deficit irrigation demonstrated the stunted growth. For plants growing at water deficits, root growth is slowed down and there are considerable apoplastic barriers blocking the hydraulic route.

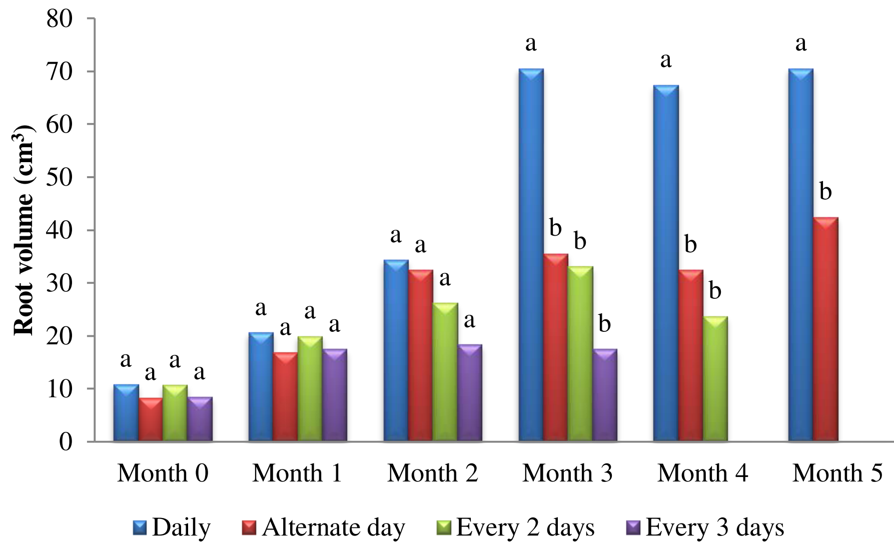


Figure 4: Effect of different frequency of irrigation on plant growth.

Conclusions

In this study, the results showed that the seedlings should be watered daily in order to enhance growth in cocoa plants. However, the cocoa seedlings started to show symptoms in the third months after receiving inadequate water supply as these include every alternate days, every two days and every three days. Any prolong water deficiency for more than three months may result in the mortality of all the seedlings.

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Study on Interactions Between Growth Parameters of Harumanis Mango Seedlings at Nursery Stage

Zul Helmey, M.S.[1,*], Muhammad Najib, O.G.[2], Muhamad Hafiz, M.H.[1] and Mohd Shahril Firdaus, A.R.[3]
[1]Horticulture Research Centre, MARDI Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia.
[2]Industrial Crop Research Centre, MARDI Headquarters, Persiaran MARDI-UPM 43400 Serdang, Selangor, Malaysia.
[3]Biotechnology and Nanotechnology Research Centre, MARDI Headquarters, Persiaran MARDI-UPM 43400 Serdang, Selangor, Malaysia.
**E-mail: zulhelmey@mardi.gov.my*

Introduction

Mango (*Mangifera indica* L.) belongs to the family Anacardiaceae is an important tropical fruit grown in more than 100 countries (Alam et al., 2006; Ram et al., 2012). In 2017, there was about 6048.29 ha under mango cultivation in Malaysia with production of 16,912.59 metric tons (DOA, 2017). Among the well-known cultivars are Golek, Masmuda, Maha 65, Chok Anan, Nam Dok Mai, Sala and Harumanis (DOA, 2017). Harumanis is the most popular clone, registered as MA128 on May 28, 1971 by the Department of Agriculture, Malaysia (DOA, 1995) with oblong fruit shape, has a prominent beak, the skin colour is green with a little bit of glossyness and turn yellowish green when ripe. Its fruit size varies ranging from 300 to 650 g and has 16-17°Brix and the flesh is yellowish to orange colour with sweet aroma (Mohd Mokhtar, 2014). This variety of mango is one of the famous fruits that has high economic demand and potential to be exported especially from Perlis Malaysia (Farook et al., 2013).

Usually, mango can be propagated using seeds or by grafting technique (Ram et al., 2012). However, propagation by seeds is not true to type. Harumanis mango is classified as polyembryonic, which enables each seed to produce several seedlings (Mohd Asrul et al., 2018). Identification of zygotic seedlings (referred to as off type) from nucellar seedlings (true to type) is important for maintaining genetic homogeneity for uniform field performance of rootstocks (Rao et al., 2008; Simon et al., 2010). In polyembryonic cultivars, nucellar seedlings can be identified by the uniformity in the colour of the emerging leaves that are not present in zygotic seedlings (Zakaria et al., 2002). However, only one embryo is zygotic in origin which then degenerates or becomes weak and stunted seedling (Litz, 1997). Seedling detection, whether zygotic or nucellar, and separation at the seedbed stage before transplanting into nursery polybags is important to minimize the number of abnormal (crooked) seedling rootstocks or zygotic seedlings (Zakaria et al., 2002; Simon et al., 2010). With these facts in view, the present investigation was undertaken to appraise the interaction among growth parameters of Harumanis mango seedlings after germination.

Materials and Methods

Plant materials and growth conditions

Seeds for the experiment were collected from Harumanis mango fruits obtained from Malaysian Agricultural Research and Development Institute (MARDI) station, Sintok, Kedah. Fifty five mature fruits of Harumanis mango were removed of flesh and seed coat and washed with clean water and soaked in 0.2% Benomyl before the seeds were sown in the sandy seedbed. The seedbed was shaded with black netting and watered daily. Seedlings germinated from the seeds were labelled with different colours of tag according to sequence of germination.

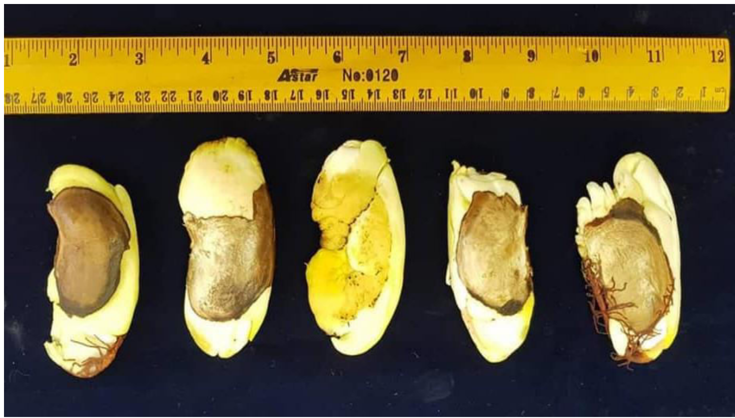

Figure 1: Seeds of Harumanis mango after removal of the seed coat.

Measurements of growth

Seedlings at the age of 30 days after germination were randomly sampled for measurements of plant height, stem diameter, leaf number and leaf area (Figure 2). Measurement of plant height was taken from the soil surface to the highest shoot tip using a measuring tape. Stem diameter was measured at the lowest part of stem using Electronic Digital Caliper (Model SCM DIGV-6) while the leaf number was manually counted based on fully expanded leaves. Leaf areas were measured and recorded as total leaf area per plant using an automatic leaf area meter (MODEL LI-300, LI-COR).

Statistical analysis

The data were analyzed using SAS Statistical Software (SAS 9.4) and correlation analysis was carried out to determine the relationship among the growth parameters.

Results and Discussion

Correlation analysis of growth parameters of Harumanis seedlings

In this study, the germination sequence had significant at ($p \leq 0.05$) and negative correlation with leaf number ($r=-0.38$), plant height ($r = -0.44$), stem diameter ($r=-0.45$) and total leaf area ($r=-0.29$). This result showed that germination sequence thus did not affect the leaf number, plant height, stem diameter and total leaf area of Harumanis seedlings. According to Zakaria et al. (2002), the first seedling that emerged was not always the most viable or vigorous in growth. However, leaf number had significant at ($p \leq 0.05$) and showed positive correlation with plant height ($r=0.60$), stem diameter ($r=0.53$) and total leaf area ($r=0.44$). These results showed that leaf number increased with plant height, stem diameter and total leaf area. The present results agree with Shaban (2010) that the number of leaves was correlated positively with total leaf area and plant height and total leaf area for Zebda mango seedlings from Egypt. In term of plant height, there were significant at ($p \leq 0.05$) and high positive correlation with stem diameter ($r=0.77$) and total leaf area ($r = 0.63$). The stem diameter also had significant at ($p \leq 0.05$) and strong positive correlation with total leaf area ($r=0.67$). Rocha et al. (2004) found that the seedlings from each seed differed in terms of vigour, plant size or height depending on whether they were nucellar or zygotic in origin. Zakaria et al. (2002) and Muralidhara et al. (2015) suggested that the removal of seed coat may be gave superior response in all initiations of plant height, stem diameter, number of leaves per plant and leaf area. At the same time, the different growth responses of seedlings produced after germination and emergence might be caused by competition between seedlings for nutrient uptake, light and space.

Table 1: Correlation analysis of growth paramaters for Harumanis mango seedlings.

	Leaf number	Plant height	Stem diameter	Total leaf area
Germination sequence	-0.38 *	-0.44 *	-0.45 *	-0.29 *
Leaf number		0.60 *	0.53 *	0.44 *
Plant height			0.77 *	0.63 *
Stem diameter				0.67 *

** Correlation is significant at 0.05 level of probability.*

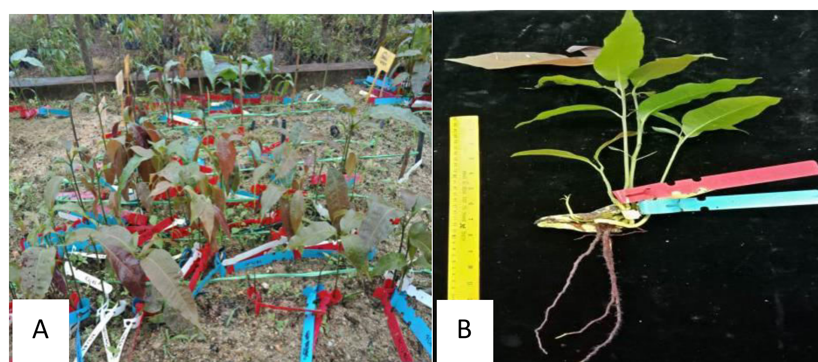

Figure 2: (A) Seedlings of Harumanis mango germinated in sandy seedbed and (B) Seedlings of Harumanis mango at 30 days after germination.

Conclusions

Based on the results, leaf number showed significant positive correlation with regards to plant height, stem diameter and leaf area and between plant height and stem diameter. Hence, the preliminary results of this study provide useful information to identify the genetic origin, zygotic or nucellar, of seedlings of Harumanis mango for the production of good quality planting materials.

Acknowledgements

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Effect of Growth Media on Seed Germination and Seedling Growth of Rambai (*Baccaurea motleyana*)

Faizah Salvana, A.R.[1,*], Khadijah, A.[1], Mohd Shukri, M.A@I.[2], Muhammad Najib, O.G.[1] and Ariff M.[3]
[1]Industrial Crops Research Centre, [2]Horticulture Research Centre, [3]Seed, Planting Materials and Livestock Production Programme, Technology Commercialization and Business Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400, Serdang, Selangor, Malaysia.
**E-mail: fsalvana@mardi.gov.my*

Introduction

Baccaurea motleyana or commonly known as rambai is a tropical fruit species which grows in warm humid areas. *B. motleyana* belongs to family Phyllanthaceae and a native of Sumatra, Borneo and Java. It found in the tropical forests of south and south-east Asia and is an important tree for local people and forest ecology. It is widely cultivated throughout Peninsular Malaysia, Sumatra, Borneo, Java and Bali, and has found its way to neighbouring countries like Thailand and the Philippines (Li, 1999). It is reported that *Baccaurea* species has potential in providing nutritional and pharmaceutical value. The fruits are reported to have high antioxidant activities while its skin has antimicrobial properties (Nurhazni et al., 2013; Hock et al., 2016).

Rootstock plays a very important role in influencing height and vigour of plants, yield, size, maturity, colour and storability of fruits and also affects disease resistance, salt tolerance and adverse weather conditions (Samaddar and Chakrabarti, 1988). There are several types of media that are available for use in propagation (Richard et al., 1964). These media vary widely but generally include fine sand mix in varying proportions of materials such as soil, peat moss, saw dust, rice hull, etc. Perlite and vermiculite are also used, but all these low nutrient mixtures require added fertilizer supplements and continuous feeding of plants until the plants become established in the nursery. They also play an important role in improving germination and seedling growth (Binyamin, 2017).

Use of suitable media or substrate is essential for production of high quality horticultural crops. It directly affects the development and later maintenance of the extensive functional rooting system (Bhardwaj, 2014). A good growing medium would provide sufficient anchorage or support to the plant, serves as a reservoir for nutrients and water, allow oxygen diffusion to the roots and atmosphere outside the root substrate (Abad et al., 2002). Nursery potting media influence the quality of seedlings produced (Agbo and Omaliko, 2006) whereas growing media plays an important role for seed germination. Growing medium not only acts as a growing place but also as a source of nutrient for plant growth. Media composition used will influence the quality of seedling (Wilson et al., 2001).

Production of high quality rootstock in large quantities is important for nurserymen to produce grafted rambai seedlings. The determination of the best growth media is a crucial part of seedling production and can save huge amounts of money and time for the nurserymen. Hence, the objective of this study is to determine the germination percentage and growth seedlings of *B. motleyana* on different media compositions.

Materials and Methods

Plant materials

Fresh fruits of *B. motleyana* were collected from Kuala Nerang, Kedah. The seeds were extracted and cleaned with water to remove their mucilaginous layer. The processed seeds were stored under shade house before sowing and were sown within 5 to 6 days after extraction. The selected seeds were sown in germination tray for observation on germination percentage. For evaluation of seedling growth,

seeds were sown at a depth of 1.0 cm in vertical position in the polybags. Light and irrigation were provided just after sowing.

Planting media for germination evaluation

There were seven treatments conducted for germination study. The treatments include T1; Soil: Cocopeat: Sand (3:2:1), T2; Soil: Cocopeat: Sand (2:1:1), T3; Soil: Peat moss: Sand (3:2:1), T4; Soil: Peat moss: Sand (2:1:1), T5; Soil: Jutani: Sand (3:2:1), T6; Soil: Jutani: Sand (2:1:1) and T7; Sand. These media mixtures were placed in germination tray according to treatments.

Planting media for growth evaluation

Six treatments were used for seedling growth study. The treatments were T1; Soil: Cocopeat: Sand (3:2:1), T2; Soil: Cocopeat: Sand (2:1:1), T3; Soil: Peat moss: Sand (3:2:1), T4; Soil: Peat moss: Sand (2:1:1), T5; Soil: Jutani: Sand (3:2:1) and T6; Soil: Jutani: Sand (2:1:1). Media mixtures were filled in polyethylene bags of 8 x 12 cm size.

Growth parameters evaluation

Germination percentage, germination index and mean germination time were recorded everyday commencing from the date of sowing till germination had completely ceased. Percentage of germination (GP), mean germination time (MGT) and germination index (Gi) were calculated as below:

GP = Total number of germinated seeds x 100
MGT = Σ Fx / Σ F, where F is number of seed germinated on day x
Gi = $N_1 / D_1 + \ldots + N_L / D_L$, where N_1: Number of seeds germinated on 1st count, D_1: Days to 1st count, N_L: Number of seeds germinated on last count, D_L: Days to last count

Seedling height was measured from the ground level to the growing tip using a measuring tape. Girth of seedling was measured at 5 cm above the soil by using veneer callipers. The number of leaves present on each observational seedling was counted. The length of roots after germination were measured and recorded. All seedling growth parameters were collected monthly for 6 months which is after the seedling started to produce true leaves.

The experiments were arranged in Completely Randomized Design (CRD) with four replications. Both germination test and seedling growth were carried out in 4 replicates. The data obtained was subjected to the Analysis of Variance (ANOVA) and differences among treatments means were compared using Least Significant Design (LSD).

Results and Discussion

Effects of different media on seed germination

Figure 1 shows the effect of different media mixture on germination of *B. motleyana*. Germination percentage was highest (93.7%) in T4 and significantly higher than other treatments. No germination was recorded in both T5 and T6. The highest germination index (Gi) was recorded in T4 (1.43) and significantly higher compared to other treatments. From Figure 1, seeds subjected to T4 treatment took only 15 days to germinate, which is faster than other treatments. Peat moss is known to help improve soil aeration, add substance to sandy soils, help soil hold nutrients more effectively, and help retain soil moisture without being waterlogged (Crouse, 2018).

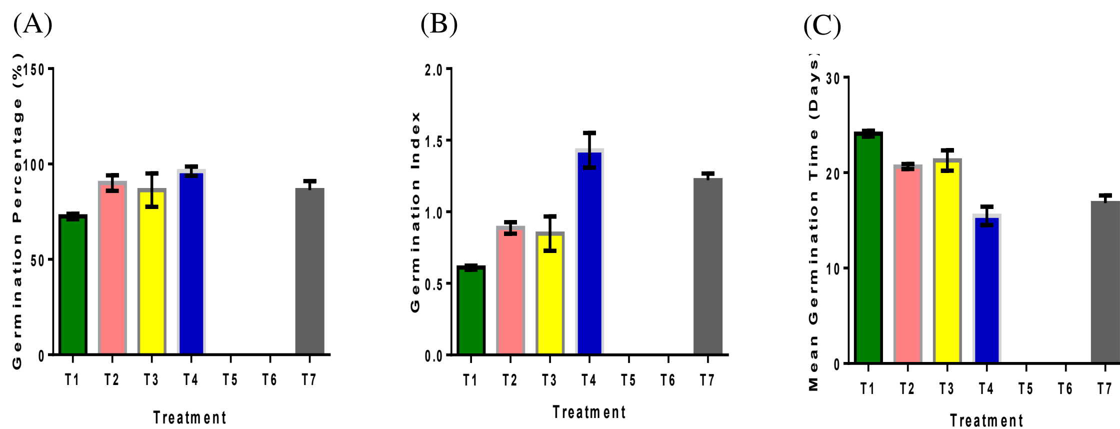

Figure 1: (A) Germination Percentage (%), (B) Germination Index and (C) Mean germination time (days) in *B. motleyana* seed under different media treatments (T1; soil: cocopeat: sand (3:2:1), T2; soil: cocopeat: sand (2:1:1), T3; soil: peat moss: sand (3:2:1), T4; soil: peat moss: sand (2:1:1), T5; soil: jutani: sand (3:2:1), T6; soil: jutani: sand (2:1:1) and T7; sand).

Effects of different growing media on growth parameters

Shoot parameters of B. motleyana

Compared to other treatments, the highest number of leaves per seedling (14.1), seedling height (42.4 cm), stem girth mean (8.46 mm) and shoot fresh weight (64.6 g) were observed in T2. Meanwhile, no growth data recorded for T5 and T6 because the seedling could not survived in both treatments (Table 1). Plant size was affected by different types of media. The shoot parameters of *B. motleyana* seedlings were better using cocopeat media. This could be due to the fact that soil and cocopeat are high organic matters which could increase water and nutrient holding capacity of the medium for supply to the plant. Organic matter may improve nutrient availability (Karama et al., 1990).

Table 1: Effects of different media on shoot parameters of *B. motleyana.*

Treatment	Number of leaves	Seedling height (cm)	Stem girth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)
T1	13.9^{a}	42.0^{a}	8.11^{a}	60.1^{a}	15.5^{a}
T2	14.1^{a}	42.4^{a}	8.46^{a}	64.6^{a}	15.8^{a}
T3	13.8^{a}	41.4^{ab}	7.99^{a}	54.2^{ab}	17.7^{a}
T4	12.4^{a}	36.5^{b}	7.07^{b}	42.4^{b}	11.3^{a}
T5	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{b}
T6	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{b}

Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's LSD.

Root parameters of B. motleyana

Table 2 shows the effect of different planting media on root parameters of *B. motleyana.* The length of roots was maximum (40.0 cm) in T2 which was statistically different from other treatments. Although root fresh weight recorded at T3 was significantly higher than others, the dry root dry weight was the highest (9.52 g) at T2 treatment.

Treatment T2 which consists of a mixture of soil: cocopeat: sand (2:1:1) provides support for roots and enhance seedling growth. Beneficial effect of cocopeat on the root system was observed on nutmeg seedlings by Novak (2004). The porosity of cocopeat helps in easy penetration of the root, resulted in higher root length and higher root dry weight of the seedlings.

Table 2: Effects of different media on root parameters of *B. motleyana.*

Treatment	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
T1	32.7^{b}	16.7^{b}	7.34^{ab}
T2	40.0^{a}	20.9^{b}	9.52^{a}
T3	35.7^{ab}	21.5^{a}	9.47^{a}
T4	36.9^{ab}	15.1^{c}	6.53^{b}
T5	0.00^{c}	0.00^{d}	0.00^{c}
T6	0.00^{c}	0.00^{d}	0.00^{c}

Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's LSD.

Conclusions

Media mixtures are very important to provide growth support for plants. The right media used determines survivability and speedy growth of seedlings. From this study, it can be concluded that media mixture of soil:peat moss:sand (2:1:1) was suitable for *B. motleyana* seed germination whereas media mixture of soil:cocopeat:sand (2:1:1) was preferable for seedling growth.

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Cryopreservation Attempts on the Potential Rare Fruit Species (*Lepisanthes fruticosa*)

Suryanti, B.[1,*], Mohd Shukri, M.A@I.[2], Nur Atisha, S.[1], Abdul Muhaimin, A.K.[3] and Noor Camellia, N.A.[3]
[1]Agrobiodiversity and Environment Research Centre, MyGeneBank™ Complex, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Horticulture Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[3]Industrial Crop Research Centre, MyGeneBank™ Complex, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: suryanti@mardi.gov.my*

Introduction

Lepisanthes fruticosa or ceri terengganu is fruit tree from the Sapindaceae family that is mainly distributed in the Southeast Asia regions such as Malaysia, Thailand, Indonesia, Myanmar, Indochina and Borneo (Abd. Latif et al., 2016). Due to its esthetical values that lies on the attractive tree shapes and fruit colour that turns into shiny bright red when fully ripens, this species is often used as an ornamental tree (Rukayah, 2006). The species is considered as underutilized as only few know that the fruit can be consumed fresh when ripe and the seed is consumed when roasted. Traditionally, it is used as medicine by the rural people. For an instance, the root is used in a compound to cure itching and it is also used to reduce the body temperature during fever. This species is considered potential as the fruits was proven rich with antioxidant and the value was higher than apples and the other well-known commercialized fruits like guavas and oranges (Mirfat and Salma, 2015). This species has the potential for preventing postprandial hyperglycaemia in diabetic patient (Zhang et al., 2016). With regards to its potential, this species has the good prospect in future to be exploited for commercial production. Since this species is an underutilized fruit, conservation methods of the plant genetic resources should be developed to avoid extinction. However, this tropical fruit species has the recalcitrant seed storage behaviour, i.e. sensitive to drying and storage at low temperatures which are critical criteria in prolonging the storage life.

Recalcitrant seeds cannot be conserved through the normal seed storage technique for the orthodox seeds where they can be maintained satisfactorily for long when dried and stored at low temperature (Hong and Ellis, 1996). Methods for conserving genetic resources of these plants are usually carried out by storing living plants in the field genebanks and also storing plant tissues or seedlings *in vitro*. Living plant conservation in field gene banks is difficult and requires high maintenance costs as well as being exposed to loss due to deforestation and natural disasters such as pests and diseases, flash floods, droughts, heat waves and extreme weather. The conservation through *in vitro* is also exposed to loss due to microbial contamination, somaclonal variations and human error during subculture. Cryopreservation is the most promising option for conserving the species with the recalcitrant seed storage behaviour (Englemann, 1992). In theory, storage at a very low freezing temperature of -196°C during cryopreservation enables the sample to be stored for an unlimited time. During cryopreservation, cellular divisions and metabolic process in the conserved tissues stop thus reducing cell and tissue deterioration (Chaudhury, 2003). In order for achieving success in cryopreservation, it is crucial to remove all the freezable water in the tissue to avoid the intracellular ice formation during the freezing stage which can cause damage to the cell membrane and lead to death. Removal of freezable water can be done by dehydration of sample before freezing by exposing cells or tissues to highly concentrated cryoprotective solutions and/or to physical drying condition. This technique is known as the vitrification based procedure (Engelmann, 2009). Vitrification is the physical process where transition of a highly concentrated aqueous solution directly from the liquid phase into an amorphous or glassy state during cooling occurs, avoiding ice crystal formation (Fahy et al., 1984). This current work highlights the long-term germplasm conservation of *L. fruticosa* zygotic embryo

(ZEs) through cryopreservation. The objectives of this study were to identify the suitability of the vitrification based technique namely vitrification and encapsulation-vitrification on the cryopreservation of *L. fruticosa* ZEs. Prior to that, preliminary study on the development of the tissue culture techniques for ZEs excised from seeds was done in order to prepare the suitable sample for the cryopreservation study.

Materials and Methods

Preparation and sterilization of plant materials

The fruits of *L. fruticosa* were obtained from the 4 years old plant in MARDI, Serdang Field Germplasm. Only ripe fruits at maturity indexes 7–8 (>5 weeks after flower bloom) (Figure 1a) were used in the study. Seeds (Figure 1b) were extracted from the fruits, washed under running tap water to remove traces of flesh prior to the disinfection using Dettol solution for 30 seconds. The seeds were rinsed with distilled water and subsequently blotted dry using tissue paper and were left dried in the air-conditioned laboratory (25±2°C, 60% relative humidity) for 30 minutes. The seeds were taken into the laminar air flow cabinet. Subsequently, seeds were soaked with 30% Clorox mixed with 3 drops of tween 20 for about 30 minutes. Then, the seeds were washed with sterilized distilled water thrice. The ZEs (Figure 1c) were then carefully excised from the seeds without any injury.

The effects of ZEs cutting sizes on regeneration

The ZEs were cut into 4 different sizes namely 7–8 mm, 4–5 mm, 3 mm and 2 mm. The ZEs were placed in a 50 mL conical flask. The ZEs were then washed with 70% (v/v) ethanol for 30 second followed by 10% (v/v) Clorox mixed with 3 drops of tween 20 for 5 minutes. Five ZEs were then singly cultured onto basal Murashige and Skoog (MS) media prepared in culture tubes and replicated for three times. Cultured ZEs were placed in a culture rack in a room with 25±2°C temperature with 16/8 hours of light and dark photoperiod provided by LED light. The regeneration percentage of ZEs was recorded after 4 weeks of culture.

The effect of different sucrose pre-culture concentration treatment on cryopreservation of ZEs through vitrification technique

The ZEs measuring 3 mm excised from seeds were used as sample. The ZEs were placed in a 50 mL conical flask. The ZEs were then washed with 70% ethanol for 30 second followed by 10% Clorox mixed with 3 drops of tween 20 for 5 minutes. The ZEs were pre-cultured overnight in the dark in MS medium supplemented with 0, 0.2, 0.4 and 0.6 M sucrose. The pre-cultured ZEs were then placed into the cryotubes and then loaded with Loading solution (2 M glycerol + 0.4 M sucrose) for 20 minutes and subsequently dehydrated with PVS2 solution (2 mL: 30% glycerol + 15% ethylene glycol + 15% DMSO-Dimethyl sulfoxide + 0.4 M sucrose, pH 5.8) for 5 minutes and then PVS2 solution was replaced with 2 mL fresh PVS2 and held for 20 minutes at 0°C (ice bucket). The ZEs in the cryotube were suspended in 0.5 mL PVS2 solutions, tied with cryocane and were directly plunged into liquid nitrogen (LN)/Cryopreservation for 1 hour storage. LN-non-exposed ones were kept as control and processed in the same way as LN-exposed samples except plunging into LN/Cryopreservation. After LN exposure, rewarming was performed by rapidly immersing the cryotubes containing ZEs in a water bath at 40°C for 90 seconds. PVS2 were replaced with washing solution (MS + 1.2 M sucrose) twice at 10 minutes intervals, blotted on filter paper layer on MS basal medium for one day and then transferred to recovery (MS media + 0.5 mg/L BAP + 0.1 mg/L GA_3) in dark. After 7-10 days incubation in the dark, the ZEs were transferred to standard conditions of illumination. The ZEs were transferred after 4-6 weeks to medium supplemented with 1.0 mg/L BAP and 0.5 mg/L NAA. Each sucrose concentration treatment consisted of 10 samples and was replicated for 5 times. Weekly observations were done on the survival of ZEs for 20 weeks.

The effect of different duration of PVS2 exposure treatment on cryopreservation of ZEs through encapsulation vitrification technique

The ZEs measuring 3 mm excised from seeds were used as sample. The ZEs were encapsulated with Sodium Alginate and Calcium Chloride solution forming Calcium-Alginate beads. The encapsulated ZEs were pre-cultured overnight in the dark in MS medium supplemented with 0.4 M sucrose. Similar procedure was applied in vitrification technique step except on the duration of PVS2 exposure where the PVS2 exposures were done for a different duration namely 0, 10, 20, 30, 40 and 50 minutes. Each PVS2 duration treatment consisted of 10 samples and was replicated for 5 times. Weekly observations were done on the survival of ZEs for 20 weeks.

Data analysis

All data were analysed by analysis of variance (ANOVA) using Statistical Analyses System Software (SAS) release 9.4. This study adopted the Completely Randomized Design (CRD). Means was differentiated at P≤0.05 level of significance using Tukey's Studentized Range (HSD) Test.

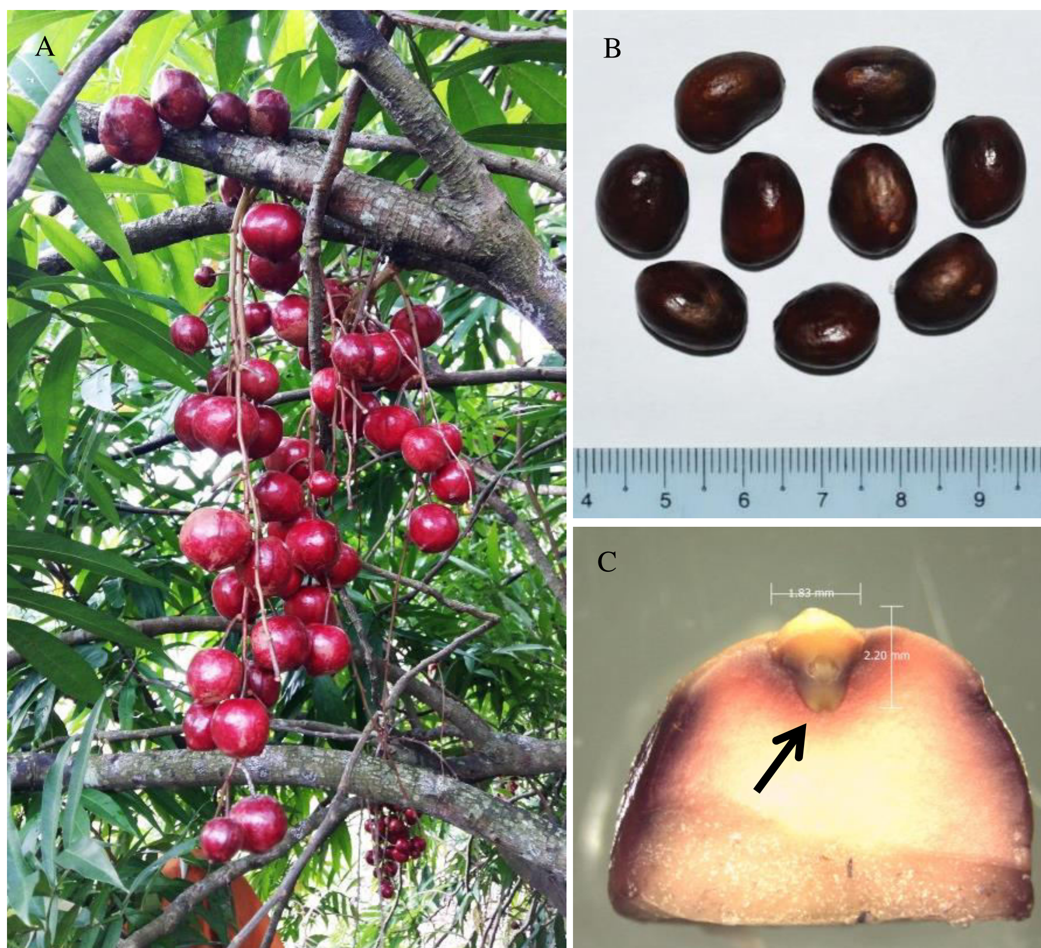

Figure 1: Fruits (a), seeds (b) and ZEs (arrow) (c) of *L. fruticose.*

Results and Discussion

Figure 2 showed that ZEs sized from 4–8 mm resulted in 100% regeneration producing complete plantlet with healthy shoot and root due to the normal developmental stages of ZEs (Figure 3). However, these sizes are too large and are not suitable to be used as a sample for cryopreservation. In order to obtain success in cryopreservation, sample should be dried prior to storage in the LN (cryopreservation). The smaller the size the more uniform the desiccation would be, thus granting

better chance of survival after exposure to LN. In this current study, no survival was recorded from the 2 mm ZEs. 2 mm was too small and in order to get a very small size, the ZEs might be injured during excision causing the failure for ZEs to form complete plantlets. Although 3 mm ZEs resulted in lower survival percentage (87%) as compared to 4–8 mm (100%), however, large size sample are not recommended to be used as cryopreservation sample. Thus 3 mm ZEs was advisable to be used for cryopreservation due to the small size.

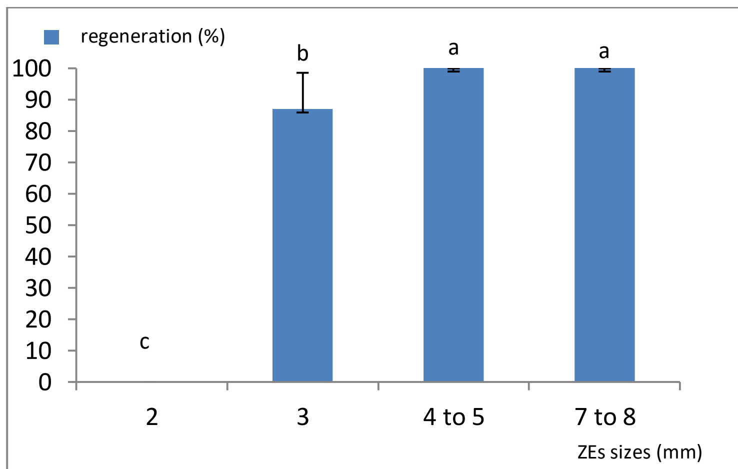

Figure 2: The effects of ZEs cutting sizes on regeneration percentage; means with different letters within the survival percentage are significantly different at p≤0.05.

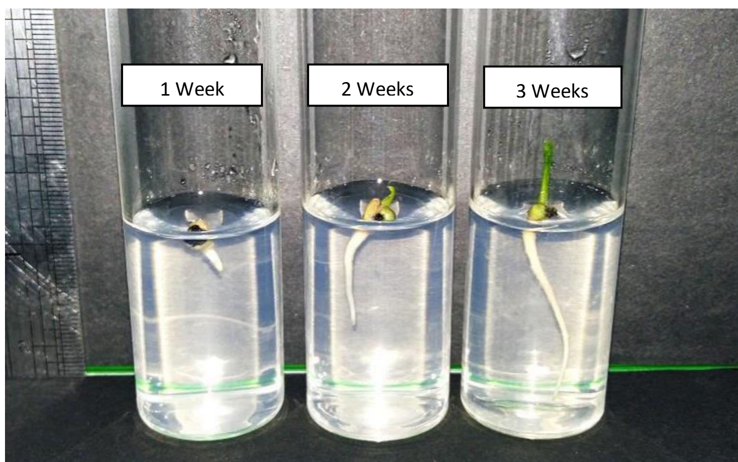

Figure 3: The normal developmental stages of ZEs of *L. fruticosa* in tissue culture to form complete plantlet (regeneration) after 3 weeks.

The key for successful cryopreservation by vitrification is to optimize the steps involved in the vitrification technique. This current study was done in order to determine the best concentration of sucrose pre-culture media prior to cryopreservation (+LN) which resulted in highest survival percentage. Based on Table 1, ZEs with all concentrations recorded total viability percentage (100%) without cryopreservation (without LN exposure / -LN). However, for cryopreservation, 0.4 M sucrose pre-culture recorded highest viability percentage (66.57%) after cryopreservation. Based on Figure 4, the growth of ZEs after vitrification treatment before and after cryopreservation (-LN/+LN) were unable to produce plantlet but producing multiple globular somatic embryos. This was a common phenomenon faced in cryopreservation studies where abnormal seedlings were produced in many species following cryopreservation and the conversion of these seedlings into normal ones remains a major challenge for many cryopreservation protocols (Peran et al., 2006; Steinmacher et al., 2007). In coconut, apart from the 20% soil-established coconut seedlings, they produced a further 23% recovered embryos which were viable. However, the embryos were lacking roots or had stunted shoot

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and were unable to produce normal plantlets (Sisunandar et al., 2010). The abnormal growth produced in this current study might be due to the toxic effect of the vitrification solution at full strength (100%) and therefore care must be taken to standardize the time and temperature of treatment as precisely as possible in order to get the normal growth after cryopreservation (Mandal, 2003). Trial on the cryopreservation of ZEs through the encapsulation-vitrification was unsuccessful as all of the ZEs tested on different durations of vitrification solution (PVS2) exposure lost their viability after cryopreservation, while all treated samples could grow when they were not subjected to cryopreservation.

Table 1: The effect of different sucrose treatments prior to cryopreservation on the survival of ZEs after 12 weeks through vitrification technique.

Concentration of sucrose pre-culture	Viability (%)	
	Without cryopreservation (-LN)	After cryopreservation (+LN)
0 M	$100{\pm}0^{a}$	$20{\pm}0^{c}$
0.2 M	$100{\pm}0^{a}$	$13.33{\pm}5.77^{c}$
0.4 M	$100{\pm}0^{a}$	$66.67{\pm}5.77^{a}$
0.6 M	$100{\pm}0^{a}$	$40{\pm}0^{b}$

Means with different letters within the same column are significantly different at $p \leq 0.05$.

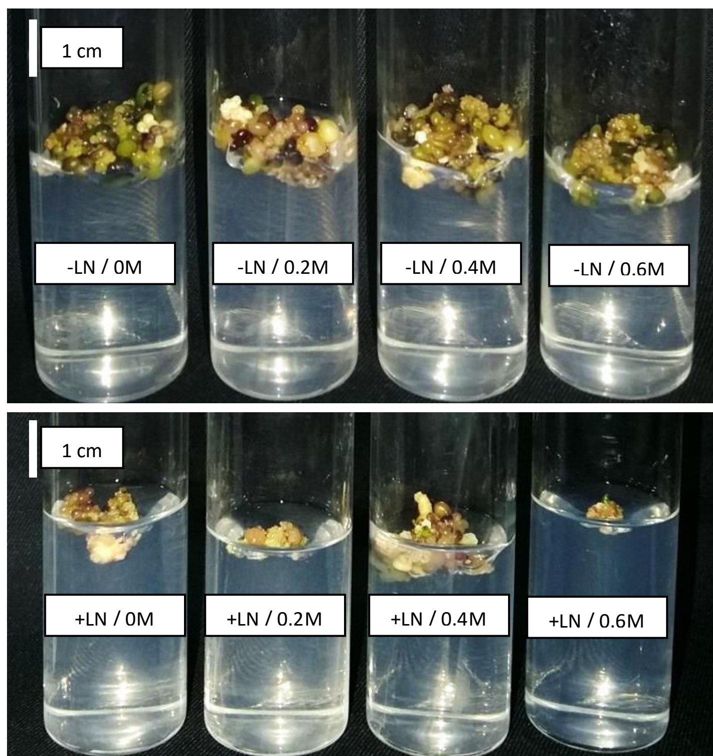

Figure 4: The growth of ZEs after vitrification treatment without (-LN)/with (+LN) after 20 weeks.

Conclusions

This current study showed that 13–66.7% of viability was obtained when the ZEs were cryopreserved through the vitrification technique. The best results were obtained (66.7%) when the ZEs were pre-cultured with 0.4 M sucrose prior to exposure to vitrification solution (PVS2) and cryopreservation. Trial on the cryopreservation of ZEs through the encapsulation-vitrification was unsuccessful as all of the ZEs tested on different durations of vitrification solution (PVS2) exposure lost their viability after cryopreservation. This study provides the information on long-term conservation technique for *L. fruticosa* which might be useful for researchers, planters and plant conservators of this species.

Acknowledgements

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Effects of Desiccation on Seed Quality of Two *Baccaurea* Species

Muhammad Najib, O.G.[*], Faizah Salvana, A.R., Noor Camellia, N.A., Khadijah, A. and Mohd Shukri, M.A@I.
Industrial Crop Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: najibo@mardi.gov.my*

Introduction

In conserving plant genetic material, particularly seed, it is essential to know whether the species shows orthodox, intermediate or recalcitrant seed storage behavior. Orthodox seeds face dehydration at the point of maturation and can be preserved at low moisture content and low temperature for extended lengths of time. These seeds are often found in the temperate quarters of our planet (Roberts, 1973). Recalcitrant seeds show no occurrence of maturation-related drying and the seeds are shed off at moderately high moisture content (Chin, 1988). They are incapable to withstand extensive desiccation. Since they exhibit vulnerability to chilling, storing them using conventional seed storage methods is almost impossible. The term intermediate seed on the other hand was coined by Ellis et al. (1990) for seeds that did not account satisfactory for all observations on seed storage behavior; orthodox or recalcitrant. Recalcitrant and intermediate seeds usually originated in the tropics such as oil palm, coffee, cacao, rubber and some tropical fruits. To maintain the viability of this type of seeds, the factors that determine the level of dehydration sensitivity have to be considered and the seeds preserved in a moist and moderately warm environment; nonetheless, even under such favourable conditions, the lifespan of these seeds is restricted to days and infrequently months.

Baccaurea polyneura and *Baccaurea motleyana* or their respective common name jentik-jentik and rambai which belong to the Phyllantaceae family are few of the indigenous crops in Malaysia. These species can be easily found in Kedah, Terengganu, Kelantan and Negeri Sembilan. Throughout the world, there are 42 species of *Baccaurea* with 31 species can be found in Malaysia (Khadijah et al., 2014). The edible fruit from *Baccaurea* species serve as source of additional nutrients and was reported to be high in antioxidant activities (Nurhazni et al., 2013; Khadijah et al., 2018). Sustainable utilization of *Baccaurea* species in the supply chain of supplement and medicine is highly dependent on the successful propagation of this tree species. Currently, no other means of propagation is feasible for this species except via seed. Since *Baccaurea* species produce fruit seasonally, there is a need to store the seeds to ensure production can be carried out all-year round. However, information on storage methods of their seed is still lacking. Previous study by Normah et al. (1997) had reported seed viability of *B. polyneura* and *B. motleyana* is subjected to desiccation. However, no information on response of seedling vigour was affirmed. Data on seedling vigour would serve a better prospect to classify seed storage behavior of *B. polyneura* and *B. motleyana.* In view of that, a study was conducted to evaluate seed quality; viability and vigour of *B. polyneura* and *B. motleyana* as affected by desiccation, in order to obtain preliminary information on their storage behaviour.

Materials and Methods

Seeds preparation

Fresh ripe fruits of *B. polyneura* and *B. motleyana* were collected around Kuala Nerang, Kedah in August 2018. The pericarp was removed from the fruits manually by washing under running water. The mucilaginous seed coat was removed by soaking the seeds overnight in water before being scrubbed with metal mesh sponge.

Measurement of moisture content

Moisture contents of both species before and after desiccation were determined using the low constant temperature oven method and percentage of moisture content was calculated on a fresh weight basis. Four replicates of 30 seeds were used for *B. polyneura* in determining the moisture content. The aluminium containers were weighed (W_1), the pericarp was removed and the seeds were then cut into small pieces and placed into the container. The fresh weight of the seeds and the container were recorded (W_2), and placed in the oven for 17±1 hours at temperature of 103±1°C. After drying completion, the weight of dried seeds and the container were recorded (W_3). The same procedure was done for *B. motleyana* four replicates of 20 seeds. The sample size for *B. motleyana* was smaller due to the size of its seeds that is bigger than *B. polyneura*. The moisture content, expressed in fresh weight basis is calculated as below:

% Moisture content (MC) = $(W_2 - W_3) / (W_2 - W_1) \times 100$
W_1 = Weight of aluminium container (g)
W_2 = Weight of aluminium container + fresh seeds (g)
W_3 = weight of aluminium container + dried seeds (g)

Desiccation treatment

Seed of *B. polyneura* and *B. motleyana* with removed pericarp were placed on a modified culture rack in the laminar air flow and the sample weight were monitored periodically until the seeds moisture content reduced to 40, 30, 20 and 10%. When the sample weight had reached the targeted weight, the seed lots were sealed in hermetic storage and the actual moisture content of the seeds were determined using low constant temperature oven method. Desiccation period (hour) for the seeds to reach targeted moisture content was recorded and the desiccation rate was calculated and compared between the species. The weight of sample seeds for targeted moisture content was calculated as below:

Target weight, W_2 (g) = $W_1 - [W_1 \times (MC_1 - MC_2) / (100 - MC_2)]$
W_1 = Initial weight (g)
MC_1 = Initial MC (%)
MC_2 = Target MC (%)

Seed germination and vigour test

The sterilized sand was moistened using distilled water to 50% of the sand water holding capacity and was placed in plastic boxes. The seeds were then treated with powdery Benlate accordingly. Four reps of 20 seeds for *B. motleyana* and 4 reps of 30 seeds for *B. polyneura* were spaced out in the media and covered with 10 to 20 mm of loose sand. The seeds were kept in germination chamber (25±2°C, 75±2% RH) under 12 hours daylight photoperiod with light photon flux density of 26-30 µmol $m^{-2} s^{-1}$ and were watered daily using distilled water. The numbers of germinated seeds for both species were recorded at 30 days of germination. The seeds were considered as germinated when the seedlings emerge from sand surface. Percentage of germination (G%), mean germination time (MGT) and germination index (Gi) were calculated as below:

G% = Total number of germinated seeds x 100
MGT = $\Sigma Fx / \Sigma F$, where F is number of seed germinated on day x

Gi = $N_1 / D_1 + \ldots + N_L / D_L$, N_1: Number of seeds germinated on 1st count, D_1: Days to 1st count, N_L: Number of seeds germinated on last count, D_L: Days to last count.

Experimental design and data analysis

Treatments comprising two species and five desiccation levels were arranged in a Complete Randomised Design (CRD) with four replications comprise of 2200 seeds per replication. The data obtained was analysed using ANOVA in the Statistical Analysis Software (SAS) (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Tukey's Honest Significant Difference (HSD) at P≤0.05. Pearson correlation coefficient (r) was determined between the variables in each species at P≤0.05.

Results and Discussion

Desiccation rate for B. polyneura and B. motleyana

When desiccated over time, both species followed exponential decay pattern where increased in desiccation hour resulted in decreased of moisture content exponentially. The overall rate of desiccation however was different between the species. Desiccation rate in *B. polyneura* was 3.11 times faster as compared to desiccation rate in *B. motleyana*. Seeds of *B. motleyana* took 52 hours to be desiccated from initial MC, i.e. 54% to targeted MC of 20%, whereas in *B. polyneura*, the seeds only took less than 15 hours (Figure 1). Our results are in agreement with results reported by Normah et al. (1997) where *B. polyneura* desiccated faster compared to *B. motleyana.*

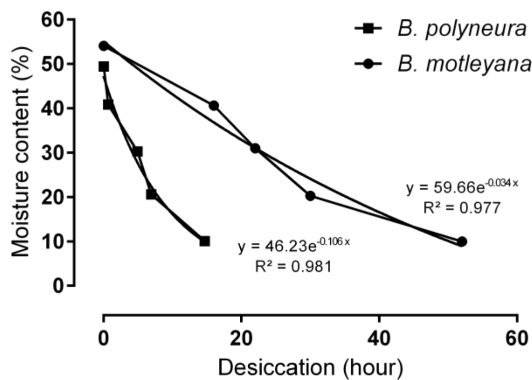

Figure 1: Desiccation period for *B. polyneura* and *B. motleyana* seeds to achieve targeted moisture content (%).

When investigating pattern of desiccation rate in each species, the desiccation rate for both species was slightly higher during the first quarter of desiccation period which declined at a rate of 12.76% and 1.61% moisture content per hour for *B. polyneura* dan *B. motleyana* respectively. However, towards the fourth quartile of desiccation period, we found that desiccation rate had decreased tremendously to only 1.35% (*B. polyneura*) and 0.46% (*B. motleyana*) moisture content per hour. This pattern of loss in moisture content can be related to the water chemistry where the rapid lost during the first quarter is due to the lost in free water, meanwhile the slower rate during the fourth quartile is attribute to lose in either loosely bound or tightly bound water. From the pattern of declining in moisture content, it will be very hard to further desiccate *B. motleyana* seed as the moisture content will hardly decline whereas in *B. polyneura* seeds, further desiccation would still be possible.

Effects of desiccation on seed quality

Both species and moisture content significantly (P<0.05) affected germination percentage, MGT and Gi. Significant interactions (P<0.05) between the species and moisture content were recorded in all parameters taken except Gi. Regardless of the species, desiccated the seeds from initial moisture content (fresh seeds) to 40% moisture content significantly reduced the Gi by 27.6%. Desiccation to

20% moisture content resulted to another significant reduction of Gi by 61.8% from initial moisture content. When further desiccated to 10% MC, the Gi in both species reduced significantly by 85.1% of Gi at initial moisture content (Table 1).

Table 1: Main and interaction effects of *Baccaurea* species and percentage of moisture content (MC) on germination (G), mean germination time per day (MGT) and germination index (Gi).

Factor		G (%)	MGT (day)	Gi
Species	*B. polyneura*	76.80^{a}	16.41^{b}	1.37^{a}
	B. motleyana	68.50^{b}	21.60^{a}	0.75^{b}
Moisture content (%)	Fresh	98.50^{a}	13.51^{c}	1.81^{a}
	40	96.50^{a}	18.36^{b}	1.31^{b}
	30	91.38^{a}	19.11^{b}	1.21^{b}
	20	49.25^{b}	20.26^{b}	0.69^{c}
	10	25.13^{c}	23.79^{a}	0.27^{d}
Species		**	**	**
Moisture content (%)		**	**	**
Species*Moisture content		**	**	ns

*Note : **Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at P≤0.05% level according to Tukey's HSD (Mean; n=4).*

Significant interaction recorded in germination percentage revealed that effects of desiccation on germination percentage were highly depended on the species. Desiccating the seeds from 30% to 20% MC did not significantly reduce the germination percentage in *B. polyneura.* In *B. motleyana* however, desiccation from 30% to 20% moisture content significantly reduced germination percentage by 72.15%, compared to germination percentage at 30% moisture content (Figure 2A). This suggested that desiccation seems to affect seed viability of both *Baccaurea* species, but to a greater extent in *B. motleyana.* These results is in agreement with the study of Normah et al. (1997), where the decreased in moisture content reduced the germination percentage significantly in *B. motleyana* but not in *B. polyneura.* The study however did not report any seedling vigour data of the desiccated seeds. Both species had reduced vigour as desiccation progressed, but the effects were more explicit in *B. motleyana* compared to *B. polyneura.*

MGT is one of the parameters to determine seed vigour viz; seeds having low MGT can be corresponded to low deterioration seeds (Amirmoradi and Feizi, 2017). In this study, the same pattern of mean difference classification for MGT can be observed in both species as desiccation progressed. However, degree of MGT that showed drastic increased was at difference desiccation interval in both species. Drastic increased (30.7%) in MGT for *B. polyneura* seeds can be observed at the further end of desiccation interval; when the seeds were desiccated from 20% moisture content to 10% moisture content. On the other hand, in *B. motleyana*, drastic increased (48.75%) in MGT was recorded at early desiccation interval; when the seeds were desiccated from initial moisture content to 40% moisture content (Figure 2B). This showed that in view of MGT, *B. motleyana* seeds were more sensitive to desiccation. This can be supported by its higher value of correlation coefficient between moisture content and MGT in *B. motleyana* ($r = -0.87$, $p \leq 0.01$) as compared to *B. polyneura* ($r = -0.82$, $p \leq 0.01$). Reduction in all parameters recorded as desiccation progressed can be explained by the properties of water in seed tissue during desiccation. Vertucci (1990) has identified five types of water hydration levels in seed tissues. At the different hydration levels, different metabolic processes can take place. At the high water contents, full normal metabolism occurs and seeds are able to germinate. As the water content becomes lower, protein and nucleic acid synthesis, together with respiration, are possible, but there is inadequate water for cell growth and germination. At lower water contents protein and nucleic acid synthesis are not possible, but some respiration can be detected. At lower water contents, low level of catabolic events will occur (Vertucci, 1990).

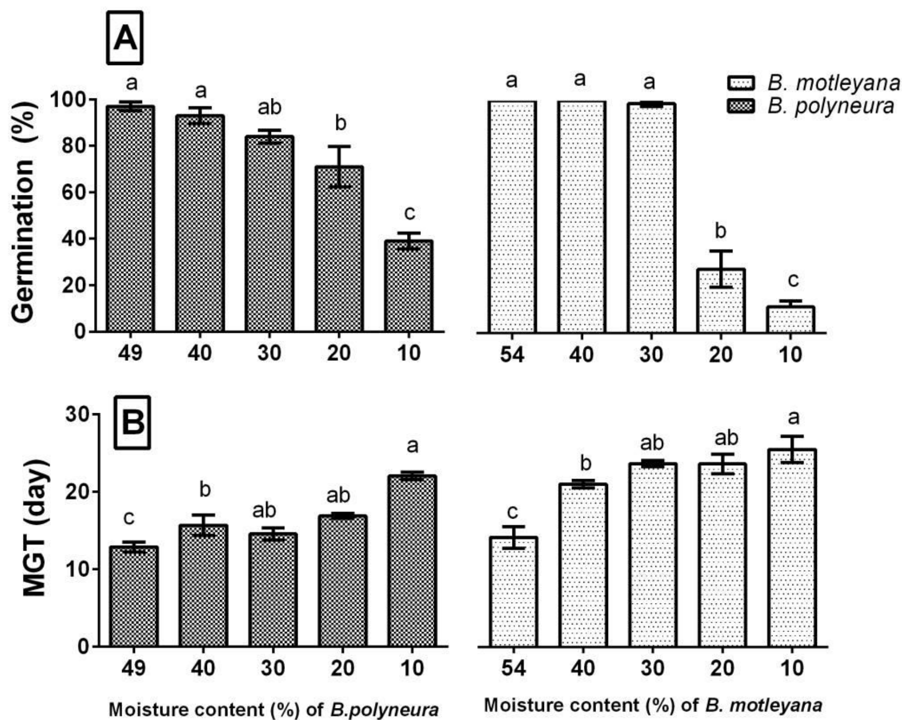

Figure 2: Effects of desiccation on mean germination percentage (A) and mean germination time (B) of *B. polyneura* and *B. motleyana*. Means in each graph with the different letters indicate significant differences at P≤0.05 l according to Tukey's HSD. (Mean; n=4). Vertical bars represent standard errors of the means.

The results of *B. motleyana* being sensitive to desiccation can be associated with the desiccation rate of *B. motleyana* which is slower compared to *B. polyneura.* Pammenter et al. (1998) stated that the drying rate of whole seeds could influence the response to desiccation, with rapidly dried seeds surviving lower moisture contents. Slow drying contributed to homogeneous dehydration, leading to considerable membrane degradation. In fact, membranes are particularly susceptible to damage during slow drying, because the degradation processes appears to be aqueous-based and oxidative in nature (Pammenter et al., 1998). However, if material is dried rapidly, only a short time is spent at this intermediate water contents, and thereby little damage will occur. Thus the faster the drying, the less damage will occur and the lower the water content can be tolerated.

Conclusions

Baccaurea polyneura and *B. motleyana* seeds were differ in response to removal of MCT, desiccation rate and desiccation tolerance. Seeds of *B. motleyana* took longer time to be desiccated and were more sensitive to desiccation as compared to *B. polyneura*, as indicated by the deterioration of its viability and vigour. Based on seed storage behavior, the desiccation tolerance *of B. motleyana* can be classified as recalcitrant while *B. polyneura* as intermediate.

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Chapter 7

Biotechnology

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In vitro Micropropagation Techniques of Two Local Taro Cultivars

Noor Camellia, N.A.[1,*], Abdul Muhaimin, A.K.[1], Nur Atisha, S.[2], Mohd Norfaizal, G., Suryanti, B.[2] and Umikalsom, M.B.[2]
[1]Industrial Crop Research Center, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Agrobiodiversity and Environment Research Center, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: camellia@mardi.gov.my*

Introduction

Taro or also known as keladi [*Colocasia esculenta* (L.)] is an edible plant primarily grown for its corm. It is immensely cultivated in the Southeast Asia, East Asia and the Pacific Islands (Macharia et al., 2014). Taro contributes significantly the people's diet in terms of the carbohydrate nourishment in many regions in developing countries. Taro can serve as food security should the production of staple food industry fails. It is ranked 14th as the most cultivated staple food around the world (Oscarsson and Savage, 2007; Hutami and Purnamaningsih, 2013). Either the corms or all the other parts of plant such as stalk, leaves and flower are edible depending on the cultivars (Gonçalves et al., 2013). The corms contain plenty of starch. Although it is less significant than other tropical root crops such as yam, cassava and sweet potato, it stills a major staple in some parts of the tropical and sub-tropical like the African country and The Pacific Islands (Manju et al., 2017). Taro also being a significant export commodity in numerous countries and it is highly priced in the market. Taro has been widely used in rural agro-industrialisation for raw material product like syrup, gum, edible film for modified atmosphere packaging (MAP) and renewable energy source. In 2010, taro world production is approximately 9,006,116 metric tonnes (Mg). Only 133,676 Mg of taro from the world production was exported worldwide (Joe, 2012).

There are demands from countries such as United States of America, Japan, China and these are some of the world's top global importers. In 2010, production of taro in the Asia region was approximately 2.1 million Mg about 23% of global production (Joe, 2012). In Malaysia, production of taro hugely decreased from 4,856.81 Mg in 2015 to 4,129.15 Mg in 2016 and 4,634.8 Mg in 2017 respectively (DOA, 2015; 2016; 2017). The constraints for taro production are mainly pest and diseases and low supply of planting materials that are disease-free (Mbong et al., 2013). The plant that belongs to Araceae family is commonly propagated vegetatively from suckers. It has been reported that cultivars that produced large size of corms will produce very few suckers (Sivan, 1984; Sivan and Liyanage, 1992). Through this conventional method, the amount of taro planting materials yielded is low and probably disease-carrying. The crop is susceptible to fungal, viral and nematode infections (Gadre and Joshi, 2003). Taro leaf blight disease, which caused by *Phytophthora colocasiae* is one of the major important economic diseases of taro. It reduces corm yield of up to 50 percent (Singh et al., 2006). Other than that, pest and disease such as Dasheen Mosaic Virus Disease (DMV), the Alomae/Bobone Virus Disease Complex and the taro beetles (*Papuana woodlarkiana*, *Papuana biroi*, *Papuana huebneri*, and *Papuana trinodosa*) also reduced the yield. Plant tissue culture techniques have become a powerful tool for propagation of taro to overcome many problems from the conventional methods of propagation.

The tissue culture technique provides a convenient yet phytosanitary method for international transfer of germplasm (Inno, 1999). The technique provides an economical, mass propagation, space and labour saving method for the preservation of germplasm. They can be stored as tissue culture in nutrient medium rather than repeatedly growing germplasm collections in the field. It is only need to be sub-cultured once in several months (Inno, 1999). The method described in this study for *in vitro* production of two local taro cultivars is the first report to increase productivity of mass, disease-free planting material of taro in Malaysia. Therefore the main aim of this study is to develop an efficient *in vitro* micropropagation protocol for two local taro cultivars using shoot tip from the corm as explants.

Materials and Methods

Plant materials and surface sterilization of taro corm

Corms of Keladi Wangi and Keladi Putih (*Colocasia esculenta*) were obtained from MARDI's (Malaysian Agricultural Research and Development Institute) aroids germplasm collection (Mohd Norfaizal et al., 2016). Experiments were performed in the laboratories of the MyGeneBank Complex in MARDI, Serdang. To obtain initial cultures of taro, corms were cut and trimmed to about 4-5cm sizes with 5-6 leaf sheaths. The explants were then washed with Dettol and rinsed with tap water. Cut and trimmed the explants into smaller pieces leaving only 3-4 leaf sheaths. The explants were then washed under running tap water for 1 hour and rinsed with double distilled water (ddH_2O). Then, under the laminar airflow chamber, explants were soaked in 0.3 g/100 mL streptomycin for 30 minutes and rinsed for three times with ddH_2O. The explants were then transferred and soaked into 50% commercial bleach (Clorox) with three drops of Tween 20 for 15 minutes under laminar airflow chamber followed by rinsing three times with ddH_2O. Then, the explants were excised to 0.40-0.60 cm of size. The explants were further disinfected with 70% ethanol (30 sec) and 10% Clorox (5 min) followed by rinsing with ddH_2O for three times and blotted dry before cultured to MS media (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose and solidified with 3 g/L gelrite.

Shoot multiplication

In vitro shoot tips were cultured in MS medium supplemented with various concentrations of plant growth regulators such as 6-Benzylaminopurine (BAP) and Thiadizuron (TDZ) for shoot induction and multiplication. Each experimental condition consisted of 10 samples and was repeated thrice. Cultures were kept under 18/6 h photoperiod at 25±2°C. Mean of shoot numbers, shoot length, root numbers and root length were recorded after 4 or 6 weeks of cultures.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) using Statistical Analysis System Software (SAS) release 9.3. The experiment was set up in a Randomized Complete Block Design (RCBD). The significance of differences among means was done by using Least Significant Difference (LSD).

Results and Discussion

Effect of BAP and TDZ concentrations on shoot multiplication and growth of keladi wangi

From nine treatments of hormones concentrations, only control does not induce shoot proliferation (Figure 1). Of all concentrations tested, the highest multiplication of shoots was found on MS media containing 2 mg/L BAP or 0.5 mg/L TDZ with a mean number of shoots 2.10±0.33 and 2.18±0.36 respectively. The results also indicated that supplementation of BAP and TDZ hormones shorten the shoot and root length and reduced the number of roots produced in keladi wangi *in vitro* plantlets. Table 1 showed a highly significant difference at $P<0.01$ between treatments for all parameters observed. Without any additional hormones, keladi wangi *in vitro* plantlets produced sufficient number of roots and length. The plantlets produced average of 21.25 numbers of root and 11.25 cm root length. This indicated that keladi wangi did not require any rooting or additional hormones for normal growth in the MS medium. High concentration of TDZ hormone suppressed the root formation in taro similar to the study done by Jackson et al. (1977). Chand et al. (1999) similarly reported that 1 mg/L BAP or TDZ supplemented as single hormone, resulted in 2 to 2.5 shoots of cv. Niue. TDZ application in micropropagation might also produce somaclonal variation depends on the concentrations used (Ko et al., 2008).

Figure 1: Effects of different concentrations of single hormones (BAP and TDZ) on keladi wangi after 4 weeks of culture in MS medium.

Table 1: Effects of different concentrations of single hormones (BAP and TDZ) on number of shoot and root, shoot height and root length of keladi wangi after 4 weeks of culture in MS medium.

Concentrations of single hormones (BAP/ TDZ)	Number of shoots	Shoot height (cm)	Number of roots	Root length (cm)
Control (without hormone)	0.32 ± 0.12^{d}	13.11 ± 0.36^{a}	21.25 ± 1.10^{a}	11.25 ± 0.69^{a}
0.5 mg/L BAP	0.96 ± 0.23^{cd}	4.18 ± 0.61^{f}	12.14 ± 0.93^{c}	2.34 ± 0.22^{bc}
1.0 mg/L BAP	1.32 ± 0.24^{bc}	6.21 ± 0.48^{e}	14.32 ± 1.00^{b}	2.86 ± 0.21^{b}
2.0 mg/L BAP	2.10 ± 0.33^{a}	6.54 ± 0.53^{de}	3.46 ± 0.58^{f}	0.54 ± 0.09^{e}
3.0 mg/L BAP	2.04 ± 0.27^{ab}	7.61 ± 0.47^{cd}	5.96 ± 0.89^{e}	0.99 ± 0.14^{ed}
0.5 mg/L TDZ	2.18 ± 0.36^{a}	8.68 ± 0.61^{c}	9.11 ± 0.99^{d}	2.45 ± 0.17^{b}
1.0 mg/L TDZ	2.0 ± 0.20^{ab}	10.71 ± 0.40^{b}	2.04 ± 0.29^{gf}	2.11 ± 0.21^{bc}
2.0 mg/L TDZ	1.50 ± 0.36^{abc}	5.64 ± 0.39^{e}	1.04 ± 0.24^{g}	1.14 ± 0.2^{de}
3.0 mg/L TDZ	1.46 ± 0.30^{abc}	5.22 ± 0.48^{ef}	1.64 ± 0.33^{gf}	1.64 ± 0.22^{cd}

Data followed by different letters show significant difference at P≤0.01.

Effect of BAP concentrations on shoot multiplication and growth of keladi putih

Table 2 showed a highly significant difference at P<0.01 between treatments for all parameters observed. From six treatments of hormones concentrations, only control does not induced shoot proliferation (Figure 2). Same as keladi wangi, the highest multiplication of shoots was found on MS media containing 2 mg/L BAP. The results showed that keladi putih produced more shoots compared to keladi wangi. Keladi putih produced 3 to 4 (3.57±0.26) shoots compared to 2 shoots obtained in keladi wangi. The intensification in BAP concentrations beyond 2 mg/L has not improved the

multiplication of shoots and did not affect the shoot length, root numbers and root length. In contrast to the study of Adelegn (2018), taro (cv. Bolosso I) supplemented with BAP ranging from 4 mg/L to 8 mg/L concentration resulted in 3.83 up to 6.13 number of shoots.

Table 2: Effects of different concentrations of BAP on number of shoot and root, shoot height and root length of keladi putih after 6 weeks of culture in MS medium.

Concentrations of BAP	Number of shoots	Shoot height (cm)	Number of roots	Root length (cm)
Control (without hormone)	$0.03{\pm}0.03^{d}$	$10.23{\pm}0.31^{a}$	$12.27{\pm}0.40^{ab}$	$12.85{\pm}0.50^{a}$
0.5 mg/L BAP	$2.27{\pm}0.28^{c}$	$7.40{\pm}0.41^{b}$	$12.57{\pm}0.62^{a}$	$7.90{\pm}0.91^{b}$
2.0 mg/L BAP	$3.57{\pm}0.26^{a}$	$5.97{\pm}0.34^{c}$	$10.67{\pm}0.74^{b}$	$1.41{\pm}0.19^{c}$
4.0 mg/L BAP	$3.10{\pm}0.28^{ab}$	$4.63{\pm}0.32^{d}$	$4.43{\pm}0.67^{c}$	$0.53{\pm}0.09^{cd}$
6.0 mg/L BAP	$3.00{\pm}0.19^{ab}$	$4.43{\pm}0.31^{d}$	$3.17{\pm}0.56^{c}$	$0.26{\pm}0.05^{d}$
8.0 mg/L BAP	$2.53{\pm}0.20^{bc}$	$3.90{\pm}0.20^{d}$	$2.83{\pm}0.60^{c}$	$0.21{\pm}0.04^{d}$

Data followed by different letters show significant difference at $P \leq 0.01$.

Figure 2: Effects of different concentrations of BAP on keladi putih after 6 weeks of culture in MS medium.

Conclusion

An effective protocol needs to be established to provide a rapid technique for mass multiplication of these potential crop cultivars. Initiation of keladi wangi and putih through micropropagation begin after 1 week in culture. At multiplication stage, both cultivars multiplied when culture in MS medium supplemented with selected cytokinin (BAP/TDZ) during this study. MS medium supplemented with 2 mg/L BAP showed the highest number of shoots produced for both cultivars. At rooting stage, the highest number of roots was achieved on MS medium without hormone. After acclimatization, 100% of seedlings were survived in green house.

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Effect of Different Plant Growth Regulators on Shoot Multiplication of *Zingiber officinale* (Ginger)

Muhd Fuad, Y.*, Nor Hasnida, H., Mohd. Saifuldullah, A.W., Nazirah, A., Rozidah, K., Rohani, A. and Sabariah, R.
Tissue Culture Laboratory, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.
**E-mail: muhdfuad@frim.gov.my*

Introduction

Zingiber officinale or widely known as ginger is an herbaceous perennial that is widely used across the globe as spice for culinary and medicinal purposes. This plant is indigenous to Southeast Asia and being cultivated at India, China, Indonesia and many other countries including Malaysia. Ginger can be harvest as soon as six months for young ginger. But to produce old ginger and planting material, it is need to be cultivated for nine months. Swarnathilaka et al. (2016) reported that ginger is propagated exclusively through underground rhizome with low proliferation rate (10-15 buds from one unit per year), a significant fraction of the total production is utilized as seed material for the subsequent season.

Using tissue culture technique to produce ginger planting material is much faster compared to the conventional method. It can be multiplied throughout the year and the quantity will not be an issue. The most important factor is the planting material produced using *in vitro* technique is free from any disease and contaminants. Besides that, conventional vegetative propagation technique of ginger has higher risk of spreading pest and systemic infections (Swarnathilaka et al., 2016). Pathogens such as *Fusarium oxysporum* f. sp. zingiberi causing rhizome rot and ginger yellows, *Pseudomonas solanacearum* causing bacterial wilt, and *Pythium* spp. and nematodes (Meloidpgyne spp.) causing soft rot in storage and under field conditions can affect ginger farm which using rhizomes produced from field grown plants (Sharma and Singh, 1995).

To be able to increase the multiplication rate during *in vitro* culture, the best micropropagation media need to be identified. By using the best media and plant growth regulator, good quality plantlets can be produced. The objective of the study was to determine the effects of different plant growth regulators and its concentration on ginger shoot multiplication rate *in vitro*.

Materials and Methods

In vitro young shoots of *Z. officinale* (Halia Bentong) were used as an explants for this study. Each young shoots were inoculated into Murashige and Skoog (MS) (1962) basal medium with different plant growth regulators (PGR) and its concentration with total of 8 different treatments. Benzyl amino purine (BAP) singly (0, 0.1, 0.5, 1.0, 2.0 and 3.0 mg/L) or in combination with Naphthalene acetic acid (NAA) (0.1 mg/L NAA + 0.5 mg/L BAP, 0.1 mg/L NAA + 1.0 mg/L BAP and 0.1 mg/L NAA + 2.0 mg/L BAP) were used throughout the experiment. 30 replicates were prepared for each treatment.

Final observations were recorded after 8 weeks of culture. Number of new shoots, height of new shoots and root existence were recorded. All the data were analyzed using R statistical package. ANOVA was applied to compare values obtained for the different measured parameters ($P<0.01$). Mean values were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussion

In this study, we could observed the response of *Z. officinale* towards different types of plant growth regulators in MS basal medium. In term of shoots multiplication, MS medium suplemented with 3.0 mg/L BAP produced the highest mean number of new shoots (5.5) followed by MS supplemented with

2.0 mg/L BAP (5.28) and MS added with 1.0 mg/L BAP (3.41) (Figure 1). Based on the observation data, there was no significant difference for the multiplication rate when NAA was added to the culture medium. Based on the study by Kambaska and Santilata (2009), MS medium without any plant growth regulator failed to produce any new shoot. Our results showed different observation as the explant in MS basal still produced new shoots and roots with no significant difference with other medium except to the two medium with highest number of shoot produced.

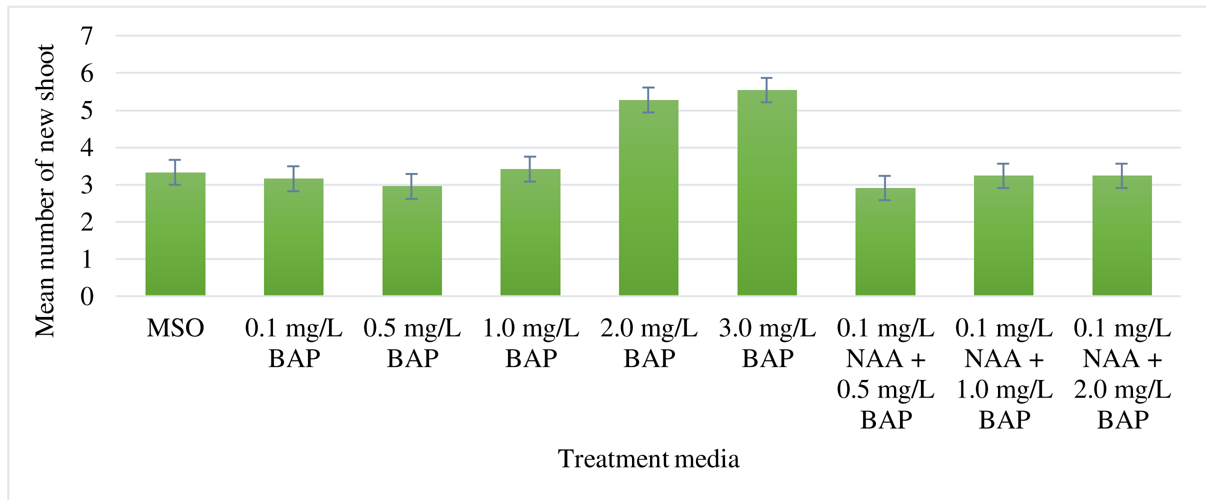

Figure 1: Effect of different PGR concentrations on the ginger shoot production.

In term of height and elongation, MS medium supplemented with 0.1 mg/L BAP produced the highest mean height (9.16 cm) compared to other treatments (Figure 2). Followed by MS with 0.1 mg/L NAA + 1.0 mg/L BAP and MS0. In spite of having the most mean shoot, MS medium added 3 mg/L BAP produce the lowest mean height compared to other treatment. These showing that the mean height of *Z. officinale* is inversely proportional to the mean new shoot produced. In spite of that, there is no significance different for the mean shoot length for all the treatments. Comparing the results of mean shoot length between medium with NAA and without NAA, it showed that 0.1 mg/L NAA help in producing higher plantlet.

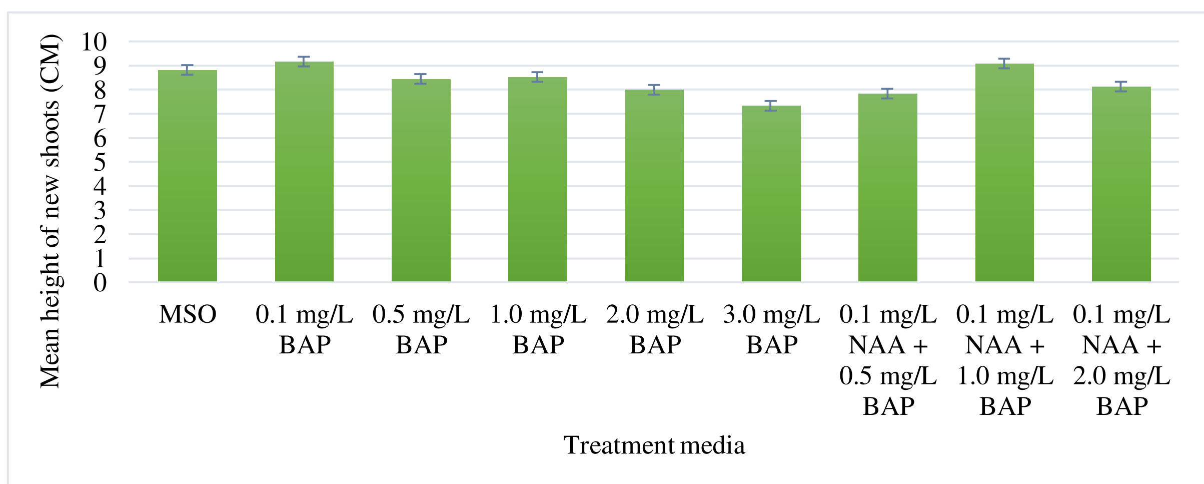

Figure 2: Effect of different PGR concentration on shoot height.

Table 1: Effect of different hormone concentrations on the production of root.

Treatment (Media)	Callus	Roots
MSO	None	Yes
MS + 0.1 mg/L BAP	None	Yes
MS + 0.5 mg/L BAP	None	Yes
MS + 1.0 mg/L BAP	None	Yes
MS + 2.0 mg/L BAP	None	Yes
MS + 3.0 mg/L BAP	None	Yes
MS + 0.1 mg/L NAA + 0.5 mg/L BAP	None	Yes
MS + 0.1 mg/L NAA + 1.0 mg/L BAP	None	Yes
MS + 0.1 mg/L NAA + 2.0 mg/L BAP	None	Yes

Having roots is critical to a plant as for it to be able to absorb nutrient from the soil. Based on the observation, *Z. officinale* in all treatments media able to produce roots (Table 1). Even the media with only BAP have roots. In spite of that, none of it produce callus. With these results, we can shorten the production time by skipping the *in vitro* rooting process and proceed with acclimatization after the multiplication process.

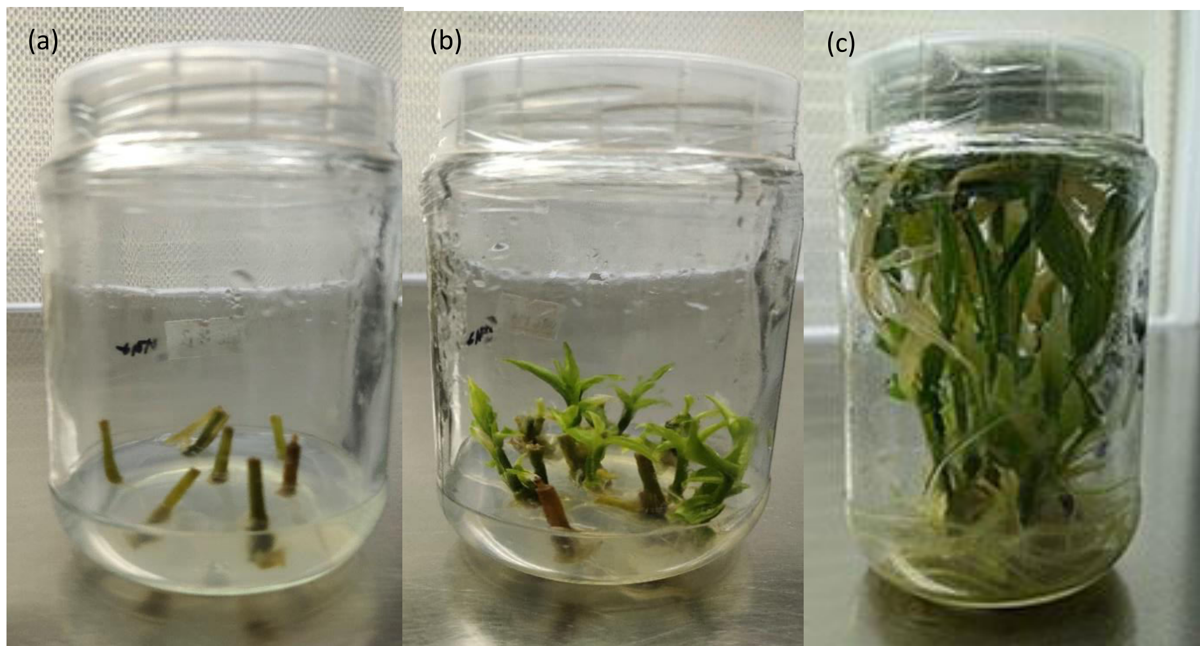

Figure 3: *Zingiber officinale* shoot multiplication. (a) Initial (left), (b) 1 month of culture and (c) 3 months of culture.

Conclusion

From this study, it is concluded that MS medium added with 3 mg/L BAP is the best for *Zingiber officinale* planting material production.

Acknowledgement

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A Cross-reactivity Study of Dithiocarbamate Inhibition by using Dehydrogenase Immobilized on Modified Screen-printed Carbon Electrode

Nurul Hidayah, A.P.[1,*], Salmah, A.A.[1], Khairatul Azmah, M.[2], Azima, A.[1], Siti Noraini, B.[1], Erna Mutiara, M.[1], Sahira Akmar, Z.[1] and Faridah, S.[1]
[1]Biotechnology and Nanotechnology Research Centre, [2]Soil Science, Water and Fertilizer Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: nurulhidayah@mardi.gov.my*

Introduction

Dithiocarbamates (DTCs) are extensively used in the agricultural industry as an insecticide, herbicide and fungicide. In Malaysia, farmers used DTCs for control of various fungal diseases on seeds, fruits and vegetables. The general formula of DTC is characterised by the presence of carbon disulfide (CS^2) group. Under certain circumstances, DTCs are decomposed into several compounds, such as sulfur, ethylenethiourea (ETU) and ethylenediamine (EDA), which have been classified by the United States Environmental Protection Agency (EPA) as a probable human carcinogen (Gupta, 2011). Besides, the residue of DTCs in food and fodder is harmful that they could pass through the soil and leach into the groundwater. Although these compound is low in toxicity, their degradation products are suspected to be carcinogenic, goitrogenic and mutagenic (Knio et al., 2000).

The maximum requirement performance limits (MRPLs) are specified worldwide as mg CS^2/kg food, for example, the level of ethylenethiourea (ETU) of certain crops (after cooking) such as spinach and carrots are below 0.1 mg/kg product (Goranka and Wolfgang, 2009). It was reported that human exposure to ETU was in the range of 0.24 µg/kg to 3.65 µg/kg per day in the USA. Besides, 1.0 µg/kg body weight per day was estimated for the Canadian population (WHO, 1988).

Commonly, DTCs are determined by standard analytical methods such as UV-Vis spectrophotometer, inductive coupled plasma emission spectrometry, atomic absorption spectrometry, gas chromatography, liquid chromatography (LC), high-performance liquid chromatography coupled to selective detectors and LC-MS/MS (Santino and Diana, 2013). However, these methods are time-consuming, expensive, use hazardous reagent and require skilled technicians for application (Sharma et al., 2005). Therefore, a rapid and simple, inexpensive, sensitive and reliable method that can be used for onsite monitoring is needed. Hence, enzyme-based biosensor technology offers a practical and rapid approach for DTC residues detection in vegetables (Ozlem and Nuri, 2013). The general principle of an enzyme-based electrochemical biosensor involves an analytical device that combines an enzyme with a transducer to produce a signal proportional to target analyte concentration (Mulchandani, 1998).

This study aims to investigate the specificity of the developed enzyme biosensor for the detection of DTCs. It was performed through cross-reactivity study against other pesticides such as dimethoate, imidacloprid, buprofezin and azoxystrobin in free solution. Four types of DTC fungicides have been studied, which include zineb, maneb, mancozeb and thiram.

Materials and Methods

Electrode surface modification

The screen-printed carbon electrode (SPCE) was purchased from Scrint Technology (M) Sdn. Bhd., a manufacturer membrane switch company, located in Kedah, Malaysia. The transducer comprised a working electrode (WE), reference electrode (RE) and counter electrode (CE). Both electrodes (WE and CE) were made from carbon paste, while silver chloride for RE. The working electrode (WE)

surface is essential in the electrochemical study, in which it provides a medium for enzyme-substrate and inhibitor reactions. In this study, the electrode's surface was modified with the nano-composite (polypyrrole, graphene and nano-gold) by electro-deposition method at 0.9 V for 900 s using the chrono amperometric technique. Moreover, 0.1 M potassium chloride (KCl) was used as a working buffer. The electrochemical measurements and characterisations were performed by using Autolab PGSTST 128N (Eco-Chemie, The Netherlands).

Enzyme immobilisation

Dehydrogenase enzyme, which was locally isolated, was immobilised and incorporated into the thin layer films of polypyrrole, graphene and nano-gold mixtures via the entrapment method. Then, 20 uL of 0.1 M KCl electrolyte was added prior to the chrono amperometry measurement at 0.9 V for 900 s. The SPCE was allowed to dry, followed by rinsing the modified surface with double distilled water for further use.

Electrochemical measurement for cross-reactivity

The cross-reactivity of zineb, maneb, mancozeb and thiram (dithiocarbamates) against other pesticides include dimethoate, imidacloprid, buprofezin and azoxystrobin was carried out using serial dilutions of pesticide standard solution. A stock of standard solution (100 mg/L) was prepared in 80% methanol and further diluted into a series of concentrations ranging from 0 to 10 mg/L. The enzymatic inhibition was measured by the chrono amperometric technique at -0.2 V, for 200 s using the modified and enzyme-immobilised SPCE. The percentage of enzyme inhibition (I%) at various concentrations of pesticide standard solution was plotted, where the calculated rate of enzyme inhibition (I%) was referred to the equation below:

$$\text{Percentage of enzyme inhibition, I\%} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

A_0 = Reading without dithiocarbamate/inhibitor
A_1 = Reading with dithiocarbamate/inhibitor

The measurement was carried out in triplicates for each concentration of pesticide standard solution.

Results and Discussion

Modification of surface electrode

The surface of the screen-printed carbon electrode was modified with polypyrrole, graphene and colloidal nano-gold solutions to increase its conductivity, selectivity and active area for enzyme-substrate reactions and further inhibition study (Nurul Hidayah, 2014). The addition of 0.002% graphene solution onto the surface of SPCE is effective for biosensor probe development because it provides a large active surface area with high electrocatalytic activities, which also provides an ideal site for enzyme immobilisation (Săndulescu et al., 2015).

The nano-gold particles were electro-deposited together with the polypyrrole and graphene because of its unique characteristics to amplify the current signal; provides a friendly and efficient loading platform for enzyme immobilisation with a high surface-to-volume ratio. Besides, it could afford the attached enzyme more freedom of orientation and increase the electron transfer rate (Putzbach and Ronkainen, 2013). All three components are biocompatible, environmentally friendly and comprise non-toxic materials. Compared to the unmodified electrode, the working electrode's surface became darker after modification with nano-composite and immobilised with an enzyme. Meanwhile, the active surface area was approximately 41.60% and the current density was about 0.077 $mAcm^{-2}$. These calculations were carried out based on the Randles–Sevcik equation (Wang, 2000).

Cross-reactivity of dithiocarbamates against other pesticides

The cross-reactivity study was carried out against other pesticides such as dimethoate, imidacloprid, buprofezin and azoxystrobin at various concentrations. All of the experiments were carried out using a modified electrode that has been immobilised with dehydrogenase enzyme in triplicates. This experiment was performed by the chrono amperometric technique at a potential of -0.2 V for 200 s, in which the current (I) had been measured in microampere (µA). Consequently, the percentage of enzyme inhibition (I%) for each pesticide was plotted as shows in Figure 1.

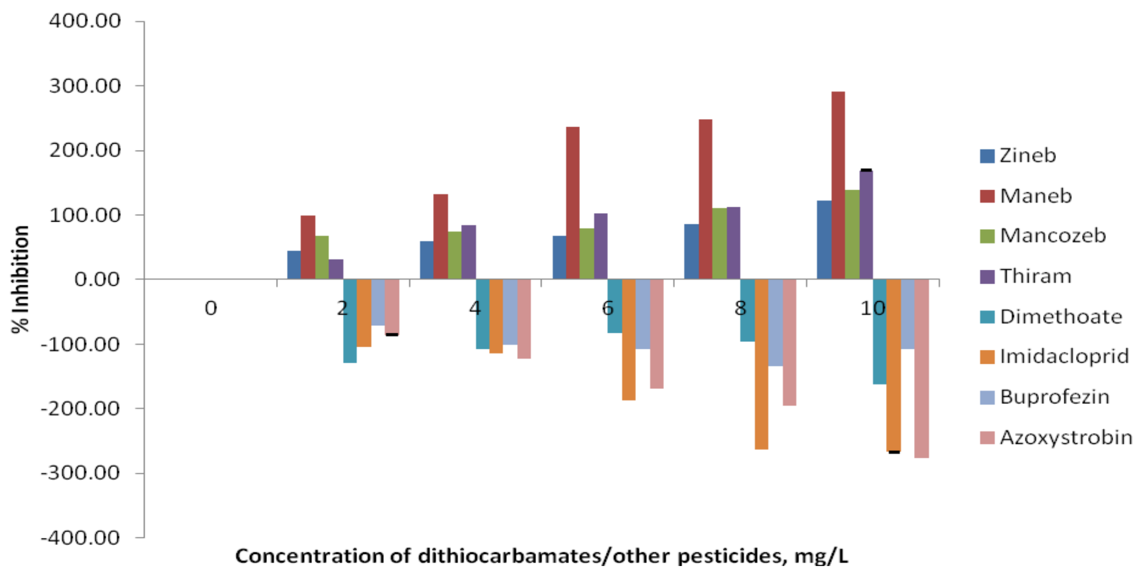

Figure 1: The percentage of enzyme inhibition (I%) for each DTC and pesticide.

The enzymatic inhibition was excellently performed by zineb, maneb, mancozeb and thiram (Figure 1). However, there was no inhibition observed for other pesticides such as dimethoate, imidacloprid, buprofezin and azoxystrobin. The percentage of enzyme inhibition (I%) at 10 mg/L was 122.46%, 290.46%, 139.52% and 169.43% for zineb, maneb, mancozeb and thiram, respectively, which indicated the ability of enzyme-based biosensor to detect zineb, maneb, mancozeb and thiram in vegetables with high sensitivity and precision.

Conclusion

In conclusion, under optimum conditions, the dehydrogenase enzyme-modified screen-printed carbon electrode (SPCE) could precisely detect DTCs such as zineb, maneb, mancozeb and thiram in free solution in the range of 0 to 10 mg/L. Interestingly, there was no enzymatic inhibition exhibited by the other groups of pesticides, thus providing a promising platform for developing a rapid biosensor device for the detection of dithiocarbamate fungicides in vegetables.

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PCR Detection of *Fusarium solani* from Pepper Farms in Sarawak

Lai, P.S.[*], Lau, E.T., Angela, T.H.G., Sulok, K.M.T. and Khew, C.Y.
Malaysia Pepper Board, Lot 1115, Jalan Utama, 93450 Kuching, Sarawak, Malaysia.
**E-mail: laips@mpb.gov.my*

Introduction

Pepper farms in Sarawak are frequently infected by a pathogenic fungus, *Fusarium solani,* which cause slow wilt disease. The initial symptoms are general yellowing and drooping of leaves in the whole canopy. Moreover, brownish and black lesions can be observed within xylem vessels of the vascular bundles for severe cases (Paulus et al., 2011). The progress of slow wilt disease in pepper tree could take months, or even years, after the infection until the death of the tree. Subsequently, this condition affected the quality and quantities of pepper berries produced and resulted in economic losses to the farmers, especially those in the rural community. Meanwhile, the conventional method of detection based on morphological characteristics is time-consuming and requires mycological expertise. Casualties such as culture failure may lead to the pending production of an accurate result (Haghani et al., 2015). Hence, the development of early detection and fast laboratory diagnostic is an essential step that should be conducted. The accurate result allows effective and targeted treatment to prevent yield deterioration over time. Molecular method has been commonly used for the identification and detection of plant pathogens due to its specificity, especially using the polymerase chain reaction (PCR) method (Silvar et al., 2005). This technique allows amplification from a minimal amount of DNA, which determines the sensitivity of this method (Kuzdraliński et al., 2017). Internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) were used for identification of many fungi at species level as the sequences of these regions are highly variable (Shahnazi et al., 2012). A simple, rapid and accurate method that incorporates these features can be used for proper management of plants infested by the invasive fungus (Iwen et al., 2002). This project aims to develop a primer set for the detection of *Fusarium solani* in pepper plants or soils using the PCR method.

Materials and Methods

Sample collection and fungal isolates

Soil from 5 to 10 cm depth and roots of pepper plants with disease symptoms were collected. Soil samples were processed using the soil dilution plate method. Ten grams of soil sample was diluted with 90 mL distilled water and serially diluted up to 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. A total of 100 µL of each dilution was plated on potato dextrose agar (PDA) to grow the fungus. Root segments were washed, sterilised, cut into small pieces and placed on PDA. Then, the PDA plates were incubated at 25°C in the darkness for approximately four to seven days. Sub-cultures were conducted until pure cultures were obtained from single-spored isolates on PDA.

DNA extraction

Fungal DNA extraction was performed using AllPrep Fungal DNA/RNA/Protein Kit (Qiagen, Netherlands), following the manufacturer's instruction. A total of 1.8 mL of fungal culture was used for DNA extraction per sample, and the extracted DNA was stored in -20°C. The quality and concentration of DNA was determined by UV visible spectrophotometer (IMPLEN Nanophotometer P330, Germany).

PCR amplification and sequencing

The internal transcribed spacer (ITS) regions of the extracted DNA was amplified by PCR using primer pair ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-

TCCTCCGCTTATTGATATGC-3'). For PCR amplification, 1 µM of both forward and reverse primers, 10 µL of 2X GoTaq® Colorless Master Mix (Promega, USA) and 1 µL of the sample were mixed in a total volume of 20 µL supplemented with the appropriate amount of nuclease-free water. PCR amplification was conducted using a thermocycler (Applied Biosystems, USA) with the thermal cycling profile as following: initial denaturation at 94°C for 2 min, followed by 38 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 1 min, with the final extension step at 72°C for 5 min. Amplified PCR products were visualised through gel electrophoresis.

Fungal identification

Positive amplified products were sequenced using BigDye Terminator v3.1 and ABI 3730xl DNA Analyzer (Applied Biosystems, USA). The sequences of the ITS region were submitted for a BLAST search using NCBI GenBank database to obtain species-level information.

Fungal specific primer design

ITS region sequences of the isolated fungus were aligned together with other fungal nucleotide sequences retrieved from NCBI. Specific nucleotide regions were selected to design the primers for the specific detection of *F. solani* using the Primer3 program. The specificity of the primer was confirmed by screening the primer sequences with Primer-BLAST from NCBI (Ye et al., 2012).

Specificity, reproducibility and sensitivity of primer

In specificity and reproducibility assessment, the designed primer sets were used to amplify DNA extracted from *F. solani* and non *F. solani*, and visualised using 2% agarose gel. A negative control is added as an indicator if any contamination occurs. In the sensitivity test, different concentrations of DNA were applied for the PCR with the same profile. The concentration used are 20, 10, 1, 0.1, 0.01 and $1x10^{-3}$ ng/µL. All PCR reactions were conducted in triplicates.

Results and Discussion

Sample collection

Soil and root samples from the pepper farms suspected to be infected by *F. solani* were collected. In most of the cases, the root of the trees was rotten, and brownish or black lesions within xylem vessels of the vascular bundles can be observed obviously in the unrotten part (Figure 1).

Figure 1: Root of pepper plants with disease symptoms.

Specificity, accuracy and sensitivity of specific primers

A total of five (5) sets of primers were designed (Table 1). The primers were designed based on the conserved regions of the gene found in *F. solani*. The conserved regions were identified by DNA sequence alignment between *F. solani* and other fungi found in GenBank database. The specificity of

the primer was further confirmed by screening the primer sequences with Primer-BLAST from NCBI to overcome the non-species-specific problem.

Table 1: List of primers developed in this study.

Primer name	Primer sequence (5' – 3')	Bases	Tm
B4	Forward: TGCCTGTTCGAGCGTCATTA Reverse: CTCTCCAGTTGCGAGGTGTT	20 20	56.6 57.2
B5	Forward: CCAGAGGACCCCCTATCTCT Reverse: ACTGTATTTGGGGGACGGC	20 19	57.3 57.6
B6	Forward: CAGACGGCCCTGAAACGAT Reverse: CGAGGTGTTAGCTACTACGCA	19 21	57.7 56.3
B12	Forward: GCCTTTGGTCTTGTTCTCTCA Reverse: CGTCAATTATTGCCTCTTTCAA	21 22	55.0 51.5
B14	Forward: CTGCTTATCTCGGGTCGTGG Reverse: GGCGTCTGTTGATTGTTAGCTG	20 22	57.6 56.4

All primers were successful for PCR amplification. Interestingly, one set of primer, namely B5 primer, showed a unique amplification just for *Phytophthora capsici* and *F. solani* with different fragment size (Figure 2). The amplicon size was 1200 bp and 300 bp for *P. capsici* and *F. solani*, respectively. This primer set provides considerable potential for species identification between both *P. capsici* and *F. solani* as the fragment sizes were in great difference. Following DNA sequencing, the exact amplicon size of *F. solani* was determined to be 297 bp. BLAST search indicated that the fragment DNA sequence was highly identical to the reference sequence of *F. solani* with 99.33% identity (Figure 3).

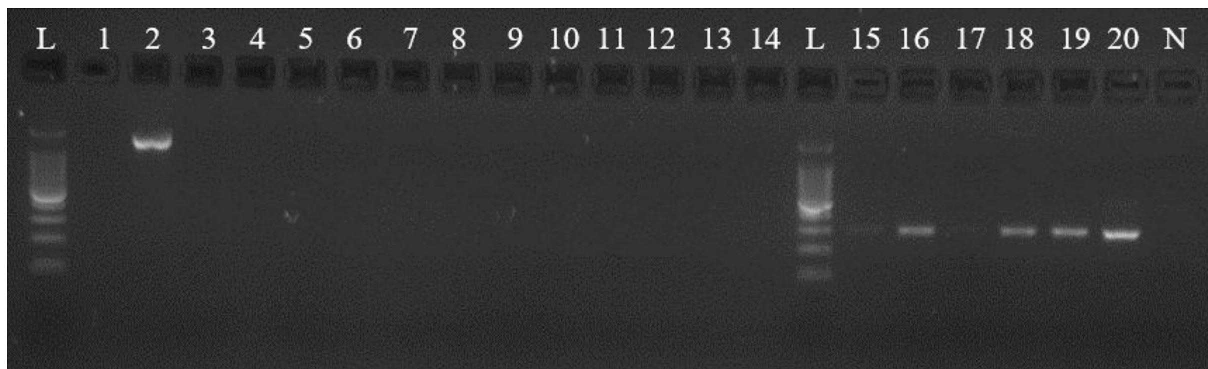

Figure 2: PCR amplification of several species of fungus using primer B5. L: 100 bp ladder; 1: *Colletotrichum gloeosporioides*; 2: *Phytophthora capsici*; 3: *Phytophthora nocatianae*; 4: *Rigidoporus microporus*; 5: *Talaromyces fumiculosus*; 6: *Penicillium citrinum*; 7: *Purpureocillium lilacinum*; 8: *Rhizoctonia solani*; 9: *Trichoderma lixii*; 10: *Trichoderma spirale*; 11: *Trichoderma harzianum*; 12: *Trichoderma reesei*; 13: *Pleosporales sp*.; 14: *Talaromyces pinophilus*; 15-20: *Fusarium solani;* and N: negative control.

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Fusarium solani isolate BARI 5PU internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer	538	538	100%	5e-149	99.33%	MF281192.
Fusarium solani strain YMZ1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and intern	538	538	100%	5e-149	99.33%	KY245947.
Fusarium solani isolate AS086 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, com	538	538	100%	5e-149	99.33%	KU507202.
Fungal sp. isolate E11623A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trans	538	538	100%	5e-149	99.33%	KT995774.1
Fungal sp. isolate E11621D internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal trans	538	538	100%	5e-149	99.33%	KT995772.1
Fusarium solani isolate AS410 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte	538	538	100%	5e-149	99.33%	KU382638.
Fusarium solani isolate AS272 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inte	538	538	100%	5e-149	99.33%	KU382637.
Fusarium solani isolate AS274 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, c	538	538	100%	5e-149	99.33%	KU382606.
Fusarium solani isolate AS275 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, c	538	538	100%	5e-149	99.33%	KU382600.
Fusarium solani isolate AS276 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, c	538	538	100%	5e-149	99.33%	KU382595.

Figure 3: Blast result for DNA fragment amplified by primer set B5 using *F. solani* sample.

As this project was focusing on *F. solani*, PCR assessment using five more samples of the *F. solani* were conducted for reproducibility assessment. As predicted, all samples showed positive results consistently (Figure 2). Thus, subsequent sensitivity assessments were conducted by using gradually reduced DNA concentration. A total of three different *F. solani* samples were used to determine the detection limit of the primer set. Based on the result, the detection limit was determined to be 1 ng/µL (Figure 4).

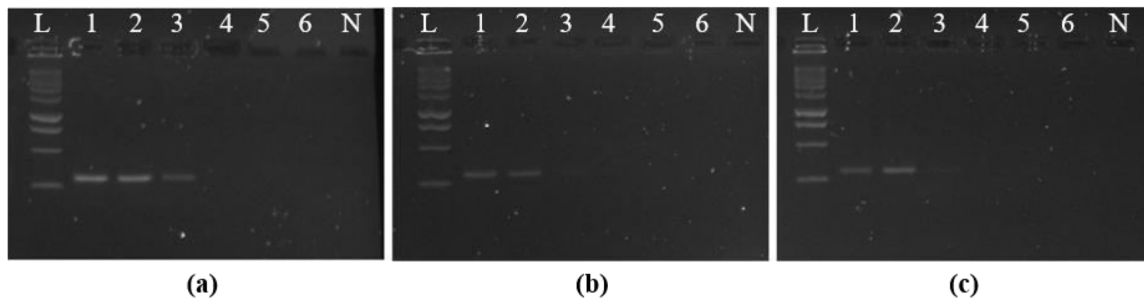

Figure 4: Sensitivity test by using different concentration of template DNA from three different *Fusarium solani* samples (a), (b) and (c). L: 1 kb ladder; 1: 20 ng/µL; 2: 10 ng/µL; 3: 1 ng/µL; 4: $1x10^{-1}$ ng/µL; 5: $1x10^{-2}$ ng/µL; 6: $1x10^{-3}$ ng/µL; and N: negative control.

Conclusions

In this study, a primer set, B5, has been discovered to uniquely differentiate *F. solani* and *P. capsici.* After several assessments, the primer set was found to be specific, reproducible and sensitive at 1 ng/µL for *F. solani* detection.

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Investigating the Roles of miRNA in Secondary Metabolite Production in *Mitragyna speciosa* Through Next Generation Sequencing (NGS)

Atiqah, S.[1], Ismail, I.[1,2] and Che-Othman, M.H.[1,*]
[1]Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
[2]Institute of System Biology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
**E-mail: hafiz87@ukm.edu.my*

Introduction

Ketum is getting much attention due to its psychoactive activity and its use in traditional medicine. The effect of ketum consumption on human depends on the dosage, which at small doses can cause stimulatory effects of drugs such as cocaine while at high dosage may cause sedative-narcotic effects of drugs like opiates (Cinosi et al., 2015). Ketum has been known to be consumed traditionally by locals in Malaysia and Thailand to improve work-productivity to combat fatigue either by chewing the raw leaves or drinking the leaf extracts due to its opium and coca-like effects. Due to its opioid-like characteristics, this plant has also been misused in so many ways and becoming a global health concern (Saingam et al., 2016). The leaves of ketum contain various phytochemicals in which more than 40 alkaloid related compounds have been identified and nearly 25 alkaloids have been isolated (Singh et al., 2017). Most of these alkaloids are used in traditional medicine to cure muscle pain, intestinal infections, to reduce coughing and diarrhoea (Hassan et al., 2013).

Mitragynine and 7-hydroxymitragynine are the main alkaloids that contribute to the psychoactive activities of ketum (Warner et al., 2016). Mitragynine and 7-hydroxymitragynine exhibit an ability to interact with the monoaminergic and opioid receptors conferring its analgesic effects (Wungsintaweekul et al., 2012; Stolt et al., 2014; Warner et al., 2016). Studies showed that the level of 7-hydroxymitragynine in ketum leaves was much lower compared to mitragynine, but the potency of 7-hydroxymitragynine is 13- and 46- fold higher compared to morphine and mitragynine, respectively, due to the presence of a hydroxyl group at C-7 in the structure (Matsumoto et al., 2006; Cinosi et al., 2015). Even though psychoactive properties have been well characterised for ketum alkaloids, the biosynthetic pathways and their regulations are still not fully understood.

Perturbation of normal state in plant cells through elicitation can either upregulate or downregulate the expression of certain genes and can be useful to study the function of the genes involved in the secondary metabolite biosynthesis (Wungsintaweekul et al., 2012; Mohamad Zuldin et al., 2013; Othman et al., 2017). Previous studies also showed that the exogenous application of tryptophan, methyl jasmonate and yeast extract had induced the production of mitragynine in the suspension culture of ketum (Mohamad Zuldin et al., 2013). Tryptophan was known as the precursor of monoterpene indole alkaloid biosynthetic pathway while yeast extract and methyl jasmonate acted as the elicitors to increase the production of mitragynine. Such elicitation approaches have been used in many studies to explore genes that are involved in secondary metabolite biosynthesis and the regulatory network (Aizat et al., 2018; Qi et al., 2018; Figueroa-Pérez et al., 2019).

Recent studies demonstrated the involvement of microRNA (miRNA) as one of the key regulators in the expression of genes involved in the secondary metabolite biosynthesis by targeting specific messenger RNAs (mRNAs) and transcription factors (TFs) (Samad et al., 2017; Samad et al., 2019). miRNAs which are small non-coding RNAs with the lengths of 21 to 24 nucleotide and have the ability to target single or multiple target transcripts mostly transcription factors that have important function in regulating plant growth and development. miRNA may act as a downregulator of mRNA in plants either by suppressing the translational process of the mRNA or cleaving the mRNA sequence in the post-transcriptional level at a specific target sequence. A wide array of techniques involving forward genetic, molecular cloning, bioinformatic analysis, and the latest technology, deep

sequencing, have greatly advanced miRNA discovery. Novel and conserved miRNA in plants can be discovered through the applications of Next Generation Sequencing (NGS). Hence, this study aimed to identify miRNA involved in regulating the production of alkaloids in ketum. The finding of this study can be used to understand the regulation of biosynthetic pathways of the key bioactive phytochemicals in ketum.

Materials and Methods

Elicitor treatments

Ketum leaves were treated *in situ* at Pulau Pinang, Malaysia (5° 32′ 50.9″ N, 100° 24′ 17.1″ E). Matured, undetached leaves of ketum were sprayed either with 5 mL distilled water (control), 5 mL of 3 µM tryptophan (Trp-treated) or 5 mL of 100 mg/L of yeast extract (YE-treated). The elicitor concentrations used were based on the most effective elicitor concentrations as described in Wungsintaweekul et al. (2012) and Mohamad Zuldin et al. (2013). Each treatment consisted of three biological replicates. All leaves were collected 24 hours after spraying and were stored at -80°C until further use for metabolite and total RNA extractions.

Liquid chromatography-mass spectrometry (LC-MS)

Treated and control leaves were ground in liquid nitrogen. Freshly prepared ice-cold methanol (5 mL) was added to 100 mg of powdered samples, immediately vortexed and centrifuged at 6000 rpm for 10 min at 4°C. The resulted supernatant was collected and filtered with a 0.2 µm polytetrafluoroethylene (PTFE) syringe filter. Then, 1 mL of the filtrates were transferred into vials and stored at −80°C until being used for LC-MS. Prior to LC-MS analysis, the samples were spiked with 100 mg/L of umbelliferone as an internal standard. Chromatographic separation of samples was performed using Thermo Scientific C18 columns (Acclaim™Polar Advantage II, 3 × 150 mm, 3 µm particle size) with an Ultimate UHPLC system (Dionex). High resolution mass spectrometry was carried out using a MicroTOF-Q III Bruker Daltonic using an ESI positive ionization. The mass range was 50 to 1000 *m/z*. Nine technical runs were performed for each of the three biological replicates for every treatment. The accurate mass data of molecular ions provided by the TOF analyzer were processed by Compass Data Analysis software (Bruker Daltonik GmbH). The MS raw dataset obtained was prepared using ProfileAnalysis 2.0 (Bruker Daltonic, Germany) for data bucketing. The *m/z* value of a molecular ion of interest was searched against online databases namely METLIN and METFRAG (Guijas et al., 2018). XCMS Online was used to statistically analyse the changes in the metabolites abundances (Huan et al., 2017).

Next generation sequencing (NGS)

Prior to NGS analysis, total RNA from 100 mg of ground leaf for each sample was extracted using plant RNA reagent PureLink® Plant RNA (Ambion, USA) according to the protocol provided. RNA samples that have an RNA Integrity Number (RIN) ≥ 7 were sent for NGS analysis at Beijing Genomics Institutes (BGI). Small RNA sequencing was carried out using the BGISEQ-500 Small RNA platform. The sequence reads from all samples were mapped to *Coffea arabica* data for annotation. Known miRNAs were identified using miRBase database (Kozomara and Griffiths-Jones, 2014). RIPmiR was used to predict novel miRNA by exploring the characteristic hairpin structure of the miRNA precursor (Breakfield et al., 2012). In order to find more accurate miRNA targets, psRobot and TargetFinder software were used to predict plant targets (Fahlgren and Carrington, 2010; Wu et al., 2012). Pathway Enrichment Analysis of the target transcripts were performed using KEGG (Kanehisa et al., 2008). KEGG terms with the corrected P-value less then or equal to 0.05 were defined as significantly enriched KEGG terms.

Results and Discussion

The m/z values and the retention time from the LC-MS analyses were used to identify the unique compounds in the samples. There were three groups of pairwise comparisons consisting of control-tryptophan (Ctr-Trp), control-yeast extract (Ctr-YE) and tryptophan-yeast extract (Trp-YE). Based on the abundance comparison analyses, 125 unique compounds were significantly changed in abundance in pairwise comparison across the three treatments. The number of compounds with significant differences in abundance for each pairwise comparison has been summarised in Table 1.

Table 1: Number of compounds with significant differences in abundance for each pairwise comparison.

Group	Higher in	No. of Compound	Higher in	No. of Compound	Total Compound
Ctr-Trp	Trp	37	Ctr	13	50
Ctr-YE	YE	72	Ctr	7	79
Trp-YE	YE	38	Trp	10	48

Figure 1 showed the number of compounds with significant changes in abundance for three-group comparison. In control, there were three compounds that showed significantly high in abundance when compared to both Trp and YE treatments, while one compound showed significantly high abundance in both control and YE treatment compared to Trp treatment. Upon treatment of Trp and YE on ketum leaves, each treatment showed that there were five and 18 compounds that showed significantly high changes in abundance when compared to two other treatment groups. There were 14 compounds that showed significant increases in abundance in both Trp and YE treatments when compared to control.

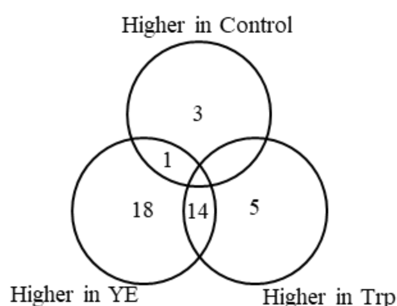

Figure 1: The Venn diagram of compounds showing significantly high abundance in each treatment group.

The *m/z* value of the compounds that showed significant differences in abundance have been used to identify the putative compounds using METLIN. The *m/z* values together with the retention times that corresponded to several previously reported alkaloids including mitragynine, were identified. Putative metabolites such as mitragynine and 7-hydroxymitragynine have been identified using their *m/z* values (mitragynine: 399.23 *m/z*; 7-hydroxymitragynine: 415.21 *m/z*). Figure 2a showed one prominent peak at retention time (RT) of 5.5 minutes which represented mitragynine and a large number of smaller peaks. The large peak was found in all samples and the RTs for this peak for all samples were in the range of 5.0 and 6.0 minutes. On average, mitragynine and 7-hydroxymitragynine constituted about 69.70% and 3.93% of the measured metabolites in the ketum leaves, respectively, which were consistent with previous studies (Takayama, 2004; Kikura-Hanajiri et al., 2009).

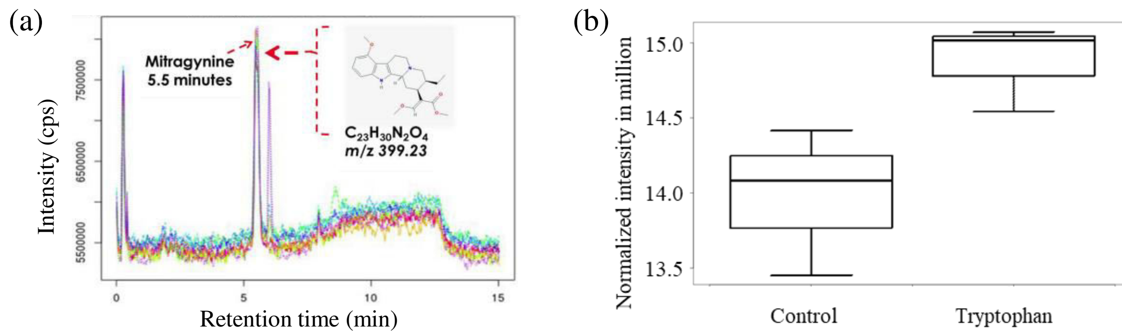

Figure 2: (a) The chromatogram showed the mitragynine peak as the highest peak of all the metabolites and (b) mitragynine showed a significant increase in abundance upon the tryptophan treatment.

Based on the pairwise abundance comparison analyses, mitragynine abundance was significantly higher in the leaves treated with tryptophan compared to control with the increment of about 11% on average (Figure 2b). There was no significant difference in the abundance of 7-hydroxymitragynine observed across all treatments. In a previous study using suspension cell culture, Mohamad Zuldin et al. (2013) found that the abundance of mitragynine showed a substantial increment upon the tryptophan treatment. This observation might probably be due to the characteristic of the suspension cells that were not differentiated to a specific function which led to low mitragynine production under normal condition. The cells in matured leaves were already differentiated to specific functions and were producing a high amount of mitragynine even before the leaves were treated with tryptophan as observed in this study. This may explain why the mature leaves ketum are widely used for medicinal purposes. In another study (Wungsintaweekul et al., 2012), the production of mitragynine was induced using methyl jasmonate and yeast extract in the shoot cultures of ketum. From the study, it was found that the highest production of mitragynine was at 24 hours after the treatment. These findings indicated that the perception of elicitation and production of mitragynine in ketum cells was highly dependent on the types of cells and the developmental stages of the leaves as observed in other plants (Giri and Zaheer, 2016; Gobbo-Neto et al., 2017). Thus, to induce higher accumulation of mitragynine in mature ketum leaves, higher elicitor concentration or longer exposure time may needed.

NGS analyses of miRNA were conducted using the same samples used in the LC-MS analyses. The annotation and miRNA prediction of the small RNA sequences obtained from control, tryptophan- and yeast extract-treated leaves samples resulted in the identification of 3237 known miRNAs and 34 putative novel miRNAs respectively. The target transcripts for all known and putative miRNA were predicted using psRobot and TargetFinder software. There are 3220 out of the 3271 known and putative miRNA identified were predicted to target 8269 transcripts in *Coffea arabica* transcriptome. The number of the filtered miRNA-targets pairs identified using both miRNA target prediction softwares is summarised in Figure 3.

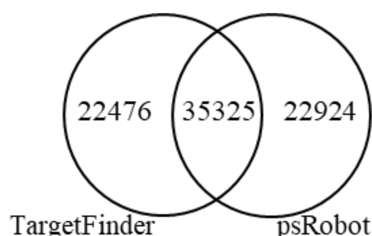

Figure 3: Venn statistics of target predictors.

Pairwise comparison analysis of miRNA abundance was performed between tryptophan-treated samples against control samples through DESeq2 analysis in order to find differentially expressed miRNA between samples. There are a total of 334 miRNAs were significantly changed in abundance

in the tryptophan-treated samples compared to control samples. About 128 miRNAs were down-regulated, whereas 206 miRNAs were up-regulated.

Pathway enrichment analysis of the target genes was performed based on KEGG database to see the roles of the differentially expressed miRNA in the biological functions. There were 1072 targeted genes with pathway annotation identified. The number of target transcripts involved in the metabolism pathway that may be affected by the differentially expressed miRNA was summarised in Figure 4. There were 31 target genes involved in the biosynthesis of other secondary metabolites while five of them were involved in the biosynthesis of alkaloid compounds in which four genes encoded polyphenol oxidase while another gene encoded tyrosine aminotransferase.

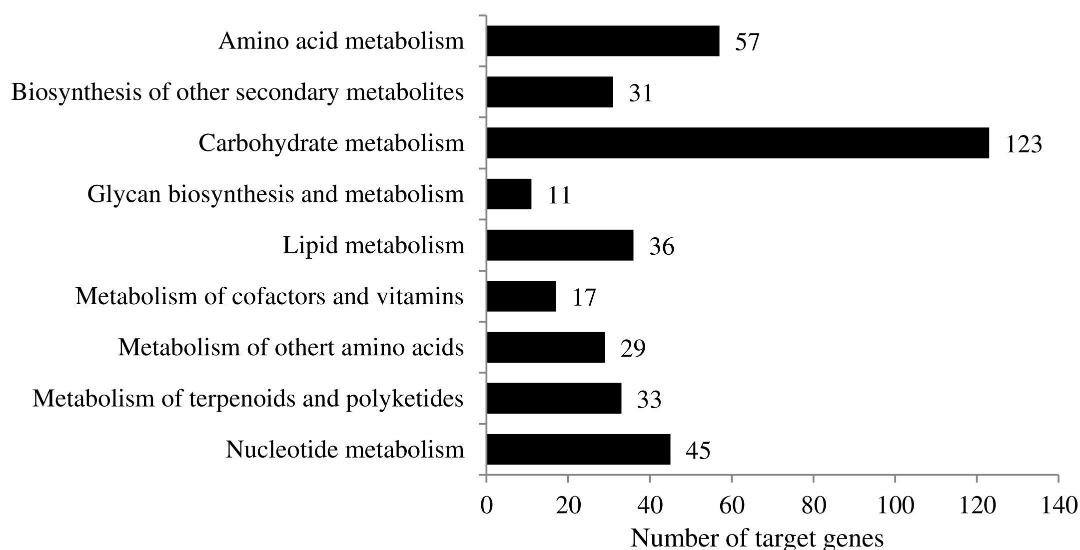

Figure 4: Classification of differentially expressed genes with pathway annotation in ketum.

Conclusions

Putative mitragynine and some previously reported alkaloids were identified and the differences in abundance between treatments were observed. Mitragynine showed a significant increase in abundance upon the tryptophan treatment, while 7-hydroxymitragynine, a more potent mitraginine derivative, showed no significant change in abundance in both tryptophan and yeast extract treatments. NGS analysis with reference to *Coffea arabica* data showed that 334 miRNAs were differentially expressed and may affect the expression of 1072 pathway annotated genes. Upregulation of miR393 and miR396 miRNAs were identified which targeted genes encoding polyphenol oxidase and tyrosine aminotransferase, respectively. Both enzymes are involved in the upstream part of the isoquinoline alkaloid biosynthesis pathway.

Acknowledgement

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Encapsulation Efficiency of SAR-inducing Recombinant Protein in Chitosan Nanoparticles at Different Chitosan:TPP:Protein Ratio

Wahid, N.S.[1,*], Mohd Nor, N.S.[1], Bashirah, R.A.[2], Abu Bakar, N.[1], Masdor, N.A.[1] and Masaruddin, M.S.[2]
[1]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI) Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
**E-mail: nsabrina@mardi.gov.my*

Introduction

A formulation of several recombinant proteins secreted by *Erwinia mallotivora*, the causal pathogen of papaya dieback infection, has shown potential in eliciting systemic acquired resistance (SAR) in papaya plant as a defence strategy against papaya seed-borne pathogens. Despite their applicability and efficiency, protein formulations as a novel mode-of-action plant protection approach faces challenges regarding their on-field stability and shelf life. One way to maintain and improve the stability of biological compounds is the through the application of nanotechnology. Encapsulation has been found to be a promising method to minimize degradation or loss during processing and storage. Studies have also shown that encapsulation is beneficial and can be used as a technique of slow releasing compounds to their environment in a timely and stable manner (Pal et al., 2011).

With regard of the types of nano formulations for biomolecules encapsulations, biodegradable polymers of natural origins are of increasing interest in line with the motivation to develop nanotechnological formulations that are less harmful to the environment. Thus for this study, chitosan, a natural non-toxic biopolymer derived by the deacetylation of chitin has been chosen. Chitosan based nanoparticles has gained considerable interest due to its biocompatibility, biodegradability, high permeability cost effectiveness and non-toxic properties (Shu and Zhu, 2000). It has immense structural possibilities for chemical and mechanical modification to generate novel properties and functions to be use in the field of agriculture (Katiyar et al., 2015; Kasyap et al., 2015). Higher physiological and biochemical responses of chitosan-based nanoparticles as compared to bulk chitosan is due to its high surface to volume ratio and surface charge (Gan and Wang 2007).

The objective of this study is to incorporate a selected protein, hrpS, which have shown potential in prompting SAR in papaya against dieback and produced through recombinant technology, into chitosan nanoparticles (CNP-P) via ionic gelation and to optimize the factors influencing the size distribution, stability and encapsulation efficiency of the CNP-P formulation.

Materials and Methods

Materials

Low molecular weight chitosan and sodium tripolyphosphate (TPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant protein, hrpS, was isolated and purified using previously developed method by Abu Bakar et al. (2017).

Preparation and characterization of CNP and CNP-P samples

The chitosan nanoparticle (CNP) and protein incorporated chitosan nanoparticles (CNP-P) were prepared using ionic gelation method as reported by Masaruddin et al. (2015). A master solution of 1.0% chitosan was prepared by dissolving 0.025 g chitosan powder in 1 mL of 1% glacial acetic acid,

which was then top up with 9 mL distilled water and stir on a hot plate (30°C) overnight. 15 mL of distilled water was then added and the solution was diluted further to 0.5%. The pH of the 0.5% chitosan solution and 0.7% TPP solution was then adjusted to pH 5 with 1 M NaOH and pH 2 with 1 M HCl, respectively. The solutions were then centrifuged at 4000 rpm for 45 min at room temperature. To prepare CNP, 600 uL of 0.5% chitosan solution was mixed with 250 uL of 0.7% TPP solution, while CNP-P was prepared by adding 250 uL of 0.5 mg/mL and 1 mg/mL protein hrpS to 600 uL of 0.5% chitosan solution before adding 250 uL of 0.7% TPP solution to the mix.

The particle size and polydispersity index (PDI) of the samples were measured using Nanobrook 90Plus PALS Particle Size Analyzer (*Brookhaven* Instruments Corp., Holtsville, NY, USA). Prior to analysis, all samples were centrifuged at 13,000 rpm for 20 min at room temperature and the supernatant was then collected and sonicated for 20 min. Three different synthesis batches were analyzed to obtain the average particle size and PDI.

The encapsulation efficiency was calculated by comparing the differences of theoretical protein amount and free protein, which are protein there were not encapsulated and remain in the supernatant after centrifugation. The chitosan nanoparticles loaded with recombinant protein hrpS were separated from the solution by ultracentrifugation at 30,000 rpm at 10°C for 30 min. The protein content in the supernatant was analysed with UV spectrophotometer at 690 nm using the protein A assay. The hrpS protein encapsulation efficiency was calculated using Equation 1.

Equation 1:

EE (%) = ((Theoretical protein amount – free amount of protein in supernatant) / Theoretical protein amount) x 100% (1)

Morphological characteristics of the samples were examines with a high resolution Transmission Electron Microscope (TEM) machine. One-drop of sample was pipette placed on a carbon coated copper grid and was allowed to air dried before viewing.

Results and Discussion

Optimizations of the factors determining the size distribution and stability of the chitosan nanoparticles loaded with selected recombinant protein, hrpS (CNP-P) prepared through ionic gelation has been carried out. The CNP-P formed instantly when polyanionic sodium tripolyphosphate (TPP) is added to readily mixed chitosan (CS)-hrpS protein (P) solutions; and showed an increase in particle size compare to unloaded chitosan nanoparticles. It was determined that the optimum ratio for chitosan:TPP:protein is 2.4:1:1 with 0.5% chitosan (pH5), 0.7% TPP (pH2) and protein concentration 1 mg/mL. This ratio allows for the size of the chitosan nanoparticles with encapsulated protein to be below 150 nm with a low dispersity index and an encapsulation efficiency of 80% (Table 1, Figure 1). Evaluation of protein encapsulation has also been conducted.

The chitosan nanoparticles and chitosan nanoparticles loaded with hrpS protein were further characterized with FTIR and TEM. The FTIR spectra of chitosan and chitosan nanoparticle are shown in Figure 3. The peak at 3356 cm^{-1} is attributed to $–NH_2$ and –OH groups stretching vibration. The peak at 1657 cm^{-1} and 1590 cm^{-1} are attributed to the $CONH_2$ and NH_2, respectively. These peak shift hyposochromically to 1627 cm^{-1} and 1529 cm^{-1} which caused by the interaction between NH^{3+} groups of chitosan and phosphate group of TPP. The other significant band for chitosan nanoparticles was observed at 1433 cm^{-1} owing to $–CH_2$ wagging. Furthermore, the peak at 945 cm^{-1} which appears in the FTIR spectra of chitosan nanoparticle shows characteristic of P=O stretching vibration from phosphate groups. Similar results of formation of chitosan nanoparticles treated TPP were reported in previous studies (Bhumkar and Pokharkar, 2006; Sarkar et al., 2013; Luistriane et al., 2018).

Table 1: Effective diameter, polydispersity index and encapsulation efficiency of protein at different protein concentration and CS:TPP:P ratio.

Chitosan (%) and TPP (%)	[Protein hrpS], mg/mL	Ratio (CS : TPP : P)	Effective diameter (nm)	Polydispersity index	Encapsulation efficiency (%)
0.5 CS and 0.7 TPP	0	2.4:1	74.92	0.14	-
0.5 CS and 0.7 TPP	0.5	2.4:1:1	365.05	0.47	-
0.5 CS and 0.7 TPP	1.0	2.4:1:1	110.61	0.34	70 – 88
0.5 CS and 0.7 TPP	0	5:1	133.58	0.32	-
0.5 CS and 0.7 TPP	0.5	5:1:1	444.30	0.42	-
0.5 CS and 0.7 TPP	1.0	5:1:1	153.71	0.40	35 – 60

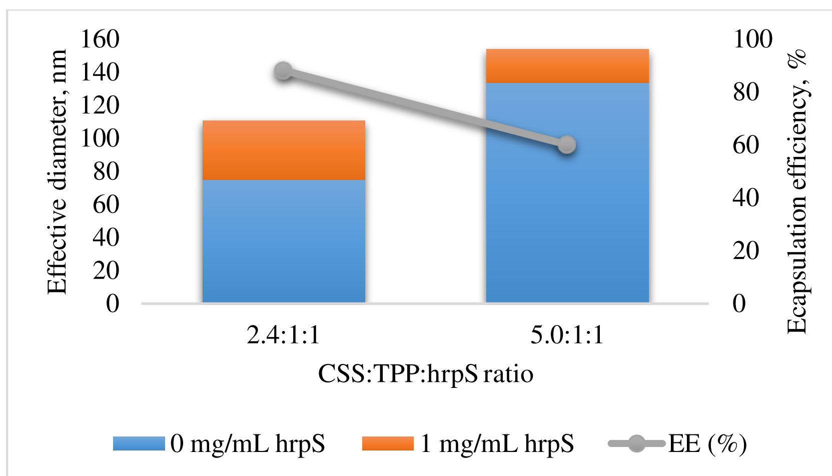


Figure 1: Increase in chitosan nanoparticle effective diameter and encapsulation efficiency of encapsulated 1 mg/mL protein hrpS at different chitosan:TPP:protein ratio.

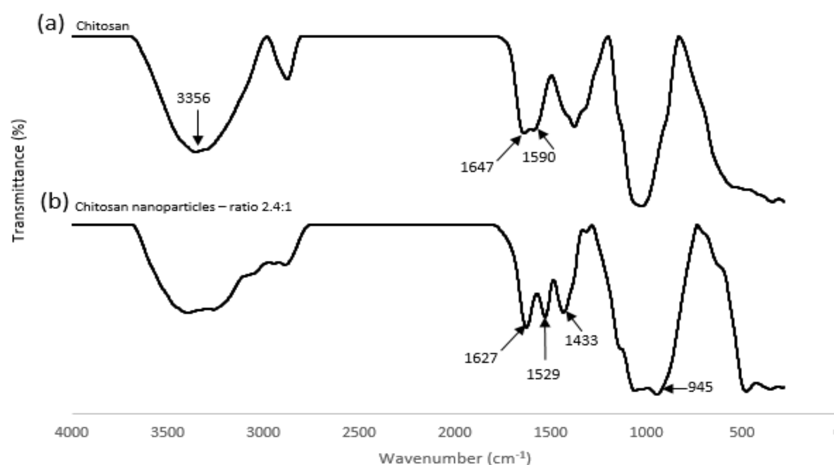
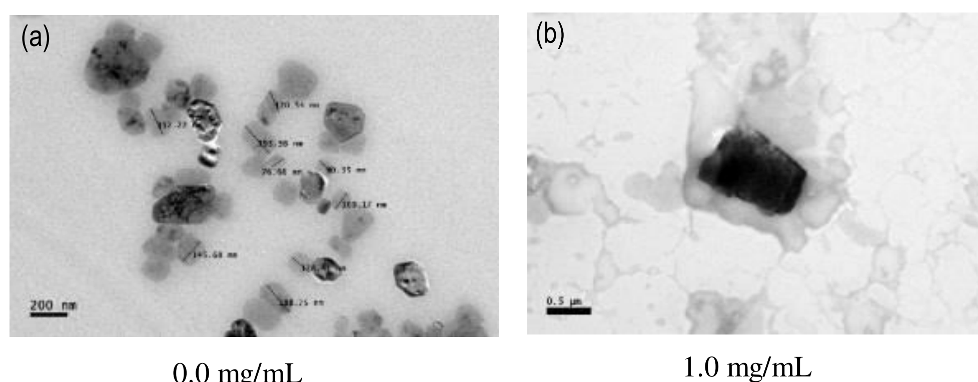


Figure 2: FTIR Spectra of (a) chitosan and (b) chitosan nanoparticles (2.4 0.7% chitosan: 1 0.5% TPP).

The TEM images of the chitosan nanoparticles with and without protein loading are shown in Figure 4. The chitosan nanoparticles have nearly spherical shape, smooth surface and size range about 70-200 nm. Compared to chitosan nanoparticles without protein, chitosan nanoparticles loaded with selected recombinant protein have a darker inner structure, which may suggest that the protein has successfully been encapsulated inside the chitosan nanoparticle.



0.0 mg/mL 1.0 mg/mL

Figure 3: TEM of (a) chitosan nanoparticle and (b) chitosan nanoparticles loaded with recombinant protein (1 mg/mL).

Conclusions

In this study, chitosan, a natural non-toxic biopolymer was chosen to encapsulate a protein, hrpS, which was produced through recombinant technology to enhance the efficacy and stability of the developed protein formulation in eliciting systemic acquired resistance (SAR) in papaya plant as a defence strategy against papaya dieback. The chitosan nanoparticles loaded with the recombinant protein hrpS (CNP-P) was prepared through ionic gelation and it was determined that the optimum ratio for chitosan:TPP:protein is 2.4:1:1 with 0.5% chitosan (pH5), 0.7% TPP (pH2) and protein concentration 1 mg/mL. This ratio allows for the size of the chitosan nanoparticles with encapsulated protein to be below 150 nm with a low dispersity index and an encapsulation efficiency of 80%. FTIR analysis and TEM images suggest the formation of chitosan nanoparticles and the incorporation of protein hrpS into the chitosan nanoparticles were successful. This study provides preliminary results in developing a CNP-P formulation to induce SAR in papaya plants that is protected against degradation, leading to enhance efficacy and effective duration in field conditions.

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Determination of Immunosensor Parameters for a Sensitive and Rapid Detection of *Xanthomonas oryzae* pv. *oryzae*

Abd Rahman, R.[1], Razali, H.[2], Mohd Said, N.A.[2], Masdor, N.A.[1] and Salam, F.[2,*]
[1]Agri-Nanotechnology Programme, Biotechnology and Nanotechnology Research Centre, MARDI, 43400 Serdang, Selangor, Malaysia.
[2]Biodiagnostic-Biosensor Programme, Biotechnology and Nanotechnology Research Centre, MARDI, 43400 Serdang, Selangor, Malaysia.
**E-mail: faridahs@mardi.gov.my*

Introduction

Rice is among the oldest crops of the world which feeds about half of the world's population (Molina et al., 2011). It is the third most important crop in Malaysia after rubber and palm oil, being a staple food for Malaysians. Rice is grown both in the Peninsular (300,500 hectares) and Borneo Island (190,000 hectares). There are 70 different microbial diseases documented and bacterial leaf blight (BLB) is the most devastating disease contributing to high yield loss of rice across the globe (Das et al., 2014). The causal agent of BLB is the bacterium *Xanthomonas oryzae* pv. *oryzae* (Xoo). Xoo infected both through wounds or hydathodes, multiplies in the epitheme and transmitted to the xylem vessels where active multiplication results in blight disease symptoms on rice leaves (Shen and Ronald, 2002). Different chemical treatments and cultural approaches have been used to combat BLB but the outcome were ineffective due to variability of pathogen, lack of durable resistance, insensitivity to different antibiotics or many other environmental factors (Ji et al., 2008).

Due to high damage caused by BLB to the rice field, a lot of studies have already been done for detection and controlling of this disease. The rapid diagnosis of BLB and timely initiation of appropriate treatment are critical determinants that ascertain the sustainability of the produce. Biosensors are now being applied for rapid diagnostics due to their capacity for point-of-care use with minimum need for operator input. Antibody-based biosensors or immunosensors are analytical devices that identify the formation of an antigen–antibody complex and convert it to a conclusive read-out. Antibodies are ideal biorecognition elements that provide sensors with high specificity and sensitivity (Sharma et al., 2016). As the measuring platform for the immunosensor, the screen-printed electrodes (SPCEs) are widely applied due to easy and reproducible fabrication at both laboratory and mass production scales (Hart et al., 2004).

Classical methods for measurement and characterization of antibodies include immunological assays such as agglutination tests, enzyme-linked immunosorbent assay (ELISA), western blot, immunofluorescence and flow cytometry (Özcürümez et al., 2004). From the immunosensor context, immobilization of antibodies onto the solid surface of the electrode is a key step that determines the stability, reproducibility and sensibility of the measured signal.

In this paper, we describe the optimal antibody concentration, set potential and calibration curve determination for Xoo detection using chronoamperometry. The aims were to select the best antibody concentration and the right constant current value of set potential for the development of Xoo immunosensor.

Materials and Methods

Apparatus

The Dropsens screen printed carbon electrodes, SPCEs (Metrohm Malaysia) consisted of a three-electrode configuration (10 mm × 34 mm) viz; round-ended working electrode (4 mm in diameter), counter electrode and silver pseudo-reference electrode printed on a ceramic support.

Chronoamperometric studies were performed with an Autolab PGSTAT III potentiostat/galvanostat (Eco Chemie, Utrecht, The Netherlands) and interfaced to Nova 1.10 software.

Antibody immobilization

0.05 mg/mL multiwalled carbon nanotube were added with 0.075 M pyrrole and mixed well. After that, 20 µL distilled water was dropped and spread out over the electrode surface. The antibody was electro-deposited on SPCE at 1.0v for 15 minutes using the Autolab PGSTAT III potentiostat/galvanostat. The modified SPCE was washed with distilled water and dried with nitrogen gas (N_2) prior to usage.

Antibody optimization

Purified Xoo antibody was diluted with phosphate buffered saline (PBS) into various concentrations: 0 mg/mL, 0.03 mg/mL, 0.05 mg/mL, 0.07 mg/mL and 0.1 mg/mL.

After the immobilization of antibody on the SPCEs, a 10 µL of freshly prepared EDC-NHS was dropped on the electrode surface and incubated for 15 minutes. The electrodes were then washed three times with PBS and distilled water before drying with nitrogen gas. The antibodies were added and incubated at room temperature for 1 hour. The SPCEs were then washed with PBS and distilled water before drying with N_2. Any unbound antibodies on the surface of the electrodes were blocked for 30 min using 0.1% Ethanolamine. Subsequently, Xoo bacterial suspension (10^9 CFU/mL) was dropped and incubated for 1 hour. This was followed with the addition of polyclonal antibody-HRP conjugate and incubated for 30 minutes. An electron transfer mediator, 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB) with hydrogen peroxide (H_2O_2) was used as the substrate system. Electrochemical measurements were performed by placing 50 µL TMB/H_2O_2 solution onto the electrode, covering the three electrodes' area. Each measurement was carried out in triplicates.

Electrochemical measurements

Selection of optimal potential for the immunosensor and chronoamperometry measurements were conducted using a range of Xoo bacterial suspensions from 10^2-10^9 CFU/mL diluted in carbonate buffer. Measurements were performed using the Autolab and analyzed using the Nova 1.10 software. Set potential measurements were carried out by scanning at 50mVs−1 and potential ranging from −0.6v to +0.6v within 1300 seconds.

Calibration curve determination

Current measurements of Xoo bacterial suspensions from 10^2-10^9 CFU/mL were performed using the Autolab and analyzed using the software.

Results and Discussion

Antibody optimization

The analytical performance of an immunosensor is dependent on various parameters of the assay procedures, including washing frequency, incubation time and optimum concentration of capture antibody and detection antibody (Bhattacharya et al., 2011). The effect of antibody concentration on the immunosensor response was studied in the concentration ranging from 0 mg/mL to 0.1 mg/mL (Figure 1). It was demonstrated in this study that the use of 0.03 mg/mL antibody gave the best current response, suggesting that at this concentration there is maximum availability of antibody recognition sites on the electrode (Salam and Tothill, 2009). On the contrary, concentration above or below 0.03 mg/mL resulted in poor detection signals, most probably due to a lack of antibody recognition sites

and saturated antibody, respectively. Thus, 0.03 mg/mL was selected as the optimal concentration for immobilization of antibody.

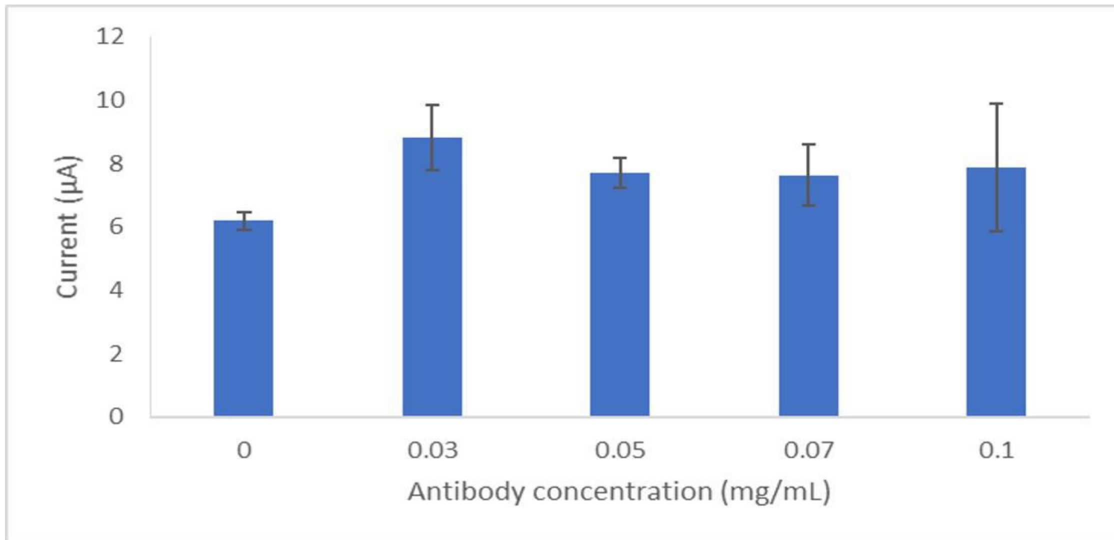

Figure 1: The effect of antibody concentration on the immunosensor response with Xoo.

Set potential determination

In order to study the optimal potential for the detection system, the current signals generated from TMB/H_2O_2 with HRP-antibody conjugate was analyzed using chronoamperometry. The technique was evaluated by the ratio of the signal (S) current to background (B) at a constant set potential range from −0.6 to 0.6v. The results (Figure 2) indicated that the maximum value of signal/background ratio for the best potential was at −0.2v. Therefore, this potential was selected for the immunosensor detection system.

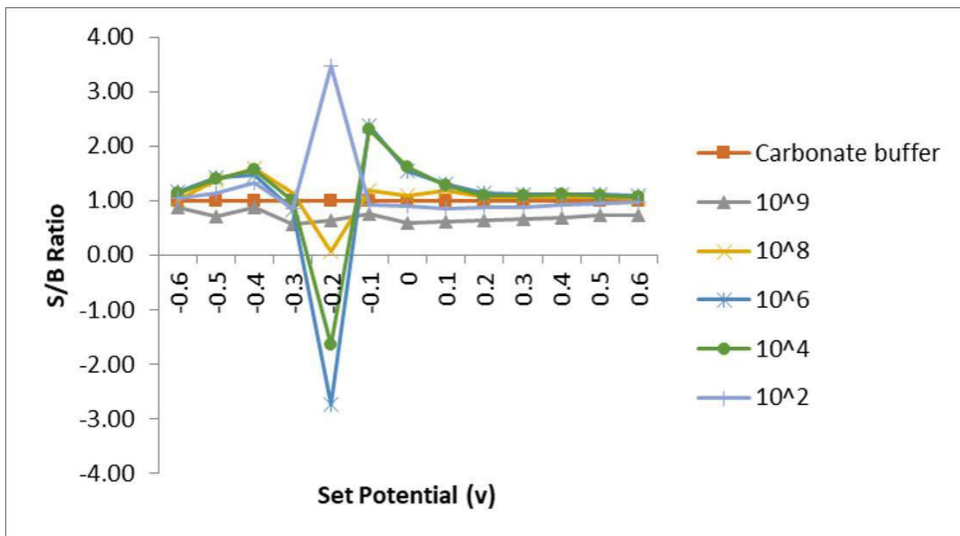

Figure 2: A signal over background graph versus set potential for all samples.

Calibration curve

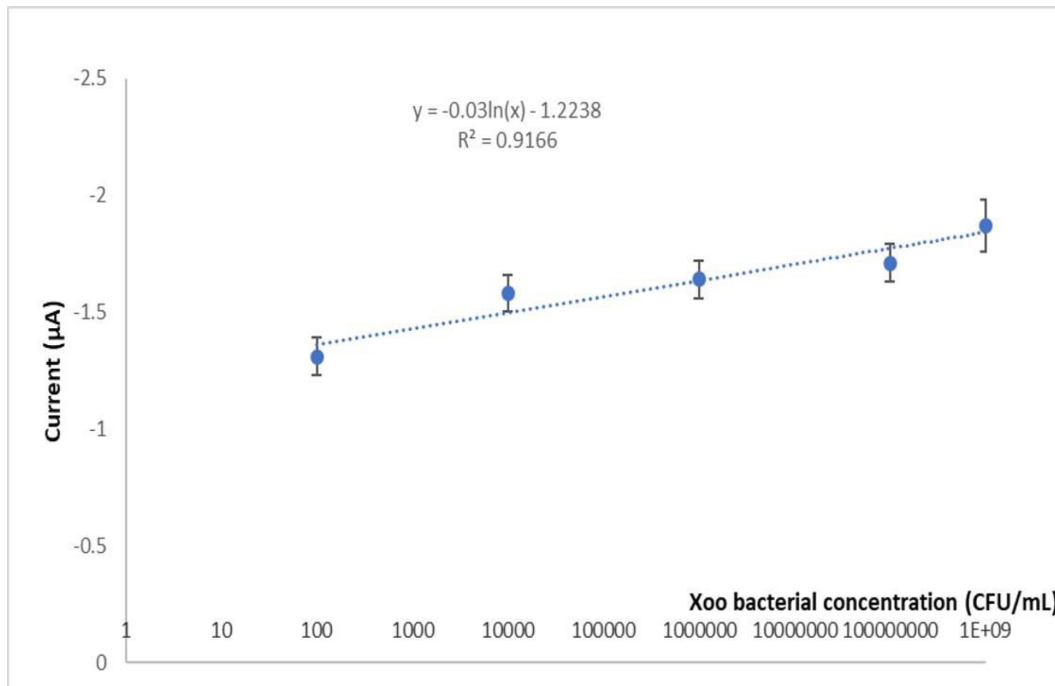

Figure 3: Calibration curve for Xoo determination.

The potential value of -0.2v for Xoo concentrations of 10^2-10^9 CFU/mL, showed the following regression equation, y=-0.03ln(x)-1.2238.

The results showed a linear range for the electrode response and the concentration of Xoo (Figure 3). The correlation coefficient for calibration curve was equal to 0.9166 while the detection limit is 9.0 x 10^4 CFU/mL.

Conclusions

Antibodies play a crucial role in determining the sensitivity and specificity of an immunosensor. We have demonstrated that the concentration of antibody influences the immunosensor response. Other important parameters, the set potential and calibration curve were also determined for the most sensitive reactions.

Acknowledgements

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Screening for Antimicrobial Activity of Essential Oils against *Xanthomonas oryzae* pv. *oryzae*

Noor Azlina, M.[1,*], Hazalina, Z.[1], Faridah, S.[1], Mohd Shahrin, G.[1], Siti Nadzirah, P.[1], Muhamad Shafiq, A.K.[1] and Kogeethavani, R.[2]
[1]Biotechnology and Nanotechnology Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Paddy and Rice Research Centre, MARDI Seberang Perai, 13200 Seberang Perai, Penang, Malaysia.
**E-mail: azlina@mardi.gov.my*

Introduction

Rice is an important staple food crop. The current global production of rice paddy is 746.9 million tons and 496 million tons of milled rice. Rice is used as a staple food in different areas of the world, especially in Asia (Savary et al., 2012). However, the susceptibility of this variety to different diseases is a major problem. Among these, *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of Bacterial Leaf Blight (BLB) is considered to cause severe yield losses. This disease is widely prevalent among various rice varieties worldwide. Bacterial blight is a vascular disease starting with *Xanthomonas oryzae* pv. *oryzae* (Xoo) invasion of rice leaves through wounds, opening and hydathodes at the leaf tip and margin (Niño-Liu et al., 2006). After multiplying in the intercellular spaces of underlying epitheme, Xoo enters and spreads into the rice plant through the xylem, causing long, grey to white, opaque necrotic lesions. The lesion length and bacterial growth rate can be taken as a measure of the progression of blight disease (Niño-Liu et al., 2006).

For over fifty years, control measures such as the use of chemical pesticides, antibiotics, resistant cultivars, disease-free seeds, quarantine regulations and cultural measures have been used. However, these measures have not proven to be effective, due to the ban on some chemical pesticides such as organochlorines in developing countries, excessive residues and wastage of chemicals in the environment, the emergence of virulent strains that overcome resistance of commercial cultivars, lack of reliable and sensitive methods for the detection of pathogens from seed, and cost-effective antibiotics for poor farmers in developing countries. Therefore, there is a need to find less hazardous, sustainable, safe, environmental, and toxicological benign control methods. The practical use of natural compounds as control agents is receiving increased attention, and this is partly due to their non-toxicity to humans, their sustainability and biodegradability. Volatile compounds from plants, especially essential oils, have been demonstrated to possess potent antifungal, antibacterial, insecticidal and nematocidal activity (Singh et al., 2015).

Essential oils (EOs) are promising alternative compounds which have an inhibitory activity on the growth of pathogens. EOs are synthesized naturally in different plant parts during the process of secondary metabolism in secretary opening of the cell wall of plants or glandular hairs and these survive as fluid droplets in roots, stems, leaves, bark, flowers, and fruits in various plants (Rehman et al., 2016). In nature, EOs play an important role in the protection of plants as anti-bacterial, anti-viral, anti-fungal, insecticides and against herbivores by reducing their appetite for such plants. Consist of volatile compounds, the reactivity of EOs is depended upon the nature, composition, and orientation of its functional groups (Wazir et al., 2014). The presence of different types of aldehydes, phenolics, terpenes, and other antimicrobial compounds means that the EOs are effective against a diverse range of pathogenic bacteria. EOs contains a wide range of secondary metabolites that are capable of inhibiting or slowing the growth of bacteria against a variety target, particularly the membrane and cytoplasm, and in some cases, they completely change the morphology of the cells (Nazzaro et al., 2013).

The project aims to assess the outcomes of plant essential oils (EOs) on the growth of Xoo. EOs from kaffir lime (*Citrus hystrix*), cinnamon (*Cinnamomum zeylanicum*), tea tree (*Melaleuca alternifolia*),

lemongrass (*Cymbopogon citratus*), lemongrass (*Cymbopogon naradus*), paper bark (*Melaleuca cajaputi*), garlic (*Allium sativum*), lemon myrtle (*Backhousia citriodora*) and neelam (*Pogostemon cablin*) were evaluated *in vitro* using disc diffusion method for inhibitory activity testing.

Materials and Methods

Essential oils

Essential oils from kaffir lime (*Citrus hystrix*), cinnamon (*Cinnamomum zeylanicum*), tea tree (*Melaleuca alternifolia*), lemongrass (*Cymbopogon citratus*), lemongrass (*Cymbopogon naradus*), paper bark (*Melaleuca cajaputi*), lemon myrtle (*Backhousia citriodora*) and neelam (*Pogostemon cablin*) were obtained from MARDI Kuala Linggi, Negeri Sembilan while garlic (*Allium sativum*) was bought from the local supermarket.

Bacteria

The strain of *X. oryzae* pv. *oryzae* was obtained from the culture collection of the National Collection of Plant Pathogenic Bacteria. The strain was cultured on potato sucrose agar (PSA) at 30°C for 48 hrs. Large quantities of the mass-produced bacterium on PSA agar were collected by washing the colony surface on the agar plate with 1 mL of ultra-pure water. Cells were harvested by centrifugation at 5,000 × g for 15 min at 4°C on a benchtop centrifuge. The pelleted cells were then washed with 0.01 M phosphate buffered saline (PBS) at pH 7.4 and the procedures were repeated three times. The bacterial cells were then re-suspended in PBS and the bacterial suspensions were adjusted to optical densities (OD) at 600 nm of between 1.0 and 1.3 to obtain bacterial concentrations at 1×10^9 CFU mL^{-1} on a UV/VIS spectrophotometer. The bacterial concentrations were confirmed by a spread plate method on PSA agar.

Antimicrobial activity

The antimicrobial study involved the paper disc diffusion assay method. The agar plates were inoculated with 100 µL of a suspension containing 10^9 CFU mL^{-1} of Xoo spread on PSA agar. Then, 6 mm diameter of filter paper disc was individually impregnated with 5 µL of essential oils and placed in the centre of the inoculated agar. Negative controls samples were prepared by replacing essential oils with mineral oil while positive controls were prepared using the streptomycin at the concentration of 0.5 mg mL^{-1}. Petri dishes were then incubated at 30°C for 48 hrs. After incubation, the essential oils diffuse into the agar and inhibits the germination and growth of Xoo. The antimicrobial activities of the essential oils were evaluated by measuring the zone of inhibition in diameter (mm) around the discs against Xoo. Each test assays were repeated in triplicate. The inhibition zones developed in and around sample indicate the antimicrobial activity. The experiment was repeated five times.

Statistical analysis

Results are given as mean ± standard deviation of triplicates. Differences were considered statistically significant at $p<0.05$ using Student's t-test with a Tukey post Hoc test through GraphPad Prism software (v 5.0) available from www.graphpad.com.

Results and Discussion

The antimicrobial activities of crude EOs against Xoo are summarized in Table 1. The results show the diameter of the inhibition zone, including the diameter of the paper disk (6 mm) after 48 hours. A broad variation in antimicrobial properties of the analysed EOs was observed in this study. The EO of kaffir lime (*Citrus hystrix*) show strong antimicrobial activity against Xoo with the highest inhibition zones of 23.8±0.7 mm followed by lemon myrtle (*Backhousia citriodora*) and cinnamon (*Cinnamomum zeylanicum*) with an inhibition zone of 20.6±1.9 and 18.2±0.40 mm, respectively. On

the other hand, the EOs of nelam (*Pogostemon cablin*), paper bark (*Melaleuca cajaputi*) and garlic (*Allium sativum*) were found to be weak or failed to inhibit the growth of Xoo.

Table 1: Antimicrobial activity of EOs against Xoo using paper disk diffusion method.

Essential oils (EOs)	Diameter of inhibition zone (mm)
Positive control	8.5±0.4
Negative control	No inhibition
Kaffir lime (*Citrus hystrix*)	23.8±0.7
Cinnamon (*Cinnamomum zeylanicum*)	18.2±0.4
Tea tree (*Melaleuca alternifolia*)	7.6±0.2
Lemongrass (*Cymbopogon citratus*)	17.6±0.7
Lemongrass (*Cymbopogon naradus*)	12.4±0.8
Paper bark (*Melaleuca cajaputi*)	No inhibition
Lemon myrtle (*Backhousia citriodora*)	20.6±1.9
Neelam (*Pogostemon cablin*)	No inhibition
Garlic (*Allium sativum*)	No inhibition

** Values are the mean diameter of the inhibitory zone (mm), ±SD of five replicates*
** Significantly different ($p<0.05$)*
** The diameter is included of the paper disk (6 mm)*

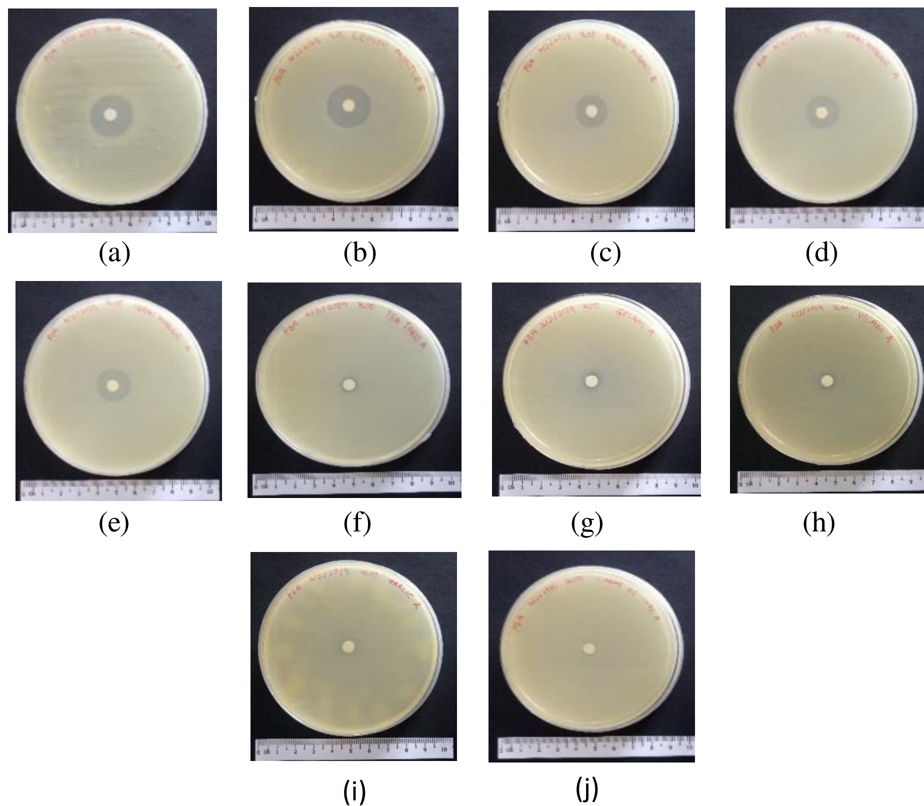

Figure 1: Inhibition diameter zones obtained by paper disc diffusion method for; (a) kaffir lime (*Citrus hystrix*), (b) lemon myrtle (*Backhousia citriodora*), (c) cinnamon (*Cinnamomum zeylanicum*), lemongrass, (d) (*Cymbopogon citratus*), (e) lemongrass (*Cymbopogon naradus*), (f) tea tree (*Melaleuca alternifolia*), (g) paper bark (*Melaleuca cajaputi*), (h) neelam *(Pogostemon cablin),* (i) garlic *(Allium sativum)* and (j) negative control (mineral oil).

The results of this study may be served as a guide for selecting EOs for future work. The data obtained in the present work suggest that EO of Kaffir lime (*Citrus hystrix*), lemon myrtle (*Backhousia citriodora*) and cinnamon (*Cinnamomum zeylanicum*) could be applied as an inhibitor to prevent the growth of Xoo in paddy field. Kaffir lime tree belongs to the plants of Ruta family (Rutaceae) while

cinnamon and lemon myrtle belong to the family of Lauraceae and Myrtaceae, respectively. In accordance to the previous report, 29 compounds were found in the essential oil of kaffir lime leaves with ß-citronellal is the major compound amounting to 66.85% of total oil (Loh et al., 2011).

According to Swamy et al. (2016), EOs are complex, volatile compounds, synthesized naturally in different plant parts during the process of secondary metabolism. Essential oils have great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens. The presence of different types of aldehydes, phenolics, terpenes, and other antimicrobial compounds means that the essential oils are effective against a diverse range of pathogens (Swamy et al., 2016).

Conclusions

Development of natural antimicrobial agents is a major step towards reducing the negative effects associated with chemicals and antibiotics such as toxic residues in agricultural products, resistance development in targeted microorganisms and general harmful effects on environment, humans and animal. *In vitro* studies revealed that the EOs of kaffir lime (*Citrus hystrix*) has remarkable antimicrobial activity against Xoo, and its potential in the management of pathogenic bacteria in agriculture will be validated in the future.

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A Preliminary Study on Optimization of Explants Surface Sterilization Condition for *in vitro* Propagation of *Horn-type Dendrobium* Orchid Hybrid MARDI

Halimah, H.[1,*], Rogayah, S.[2], Farah Zaidat, M.N.[3] and Nora'ini, A.[2]
[1]Industry Crop Centre, [2]Biotechnology Centre, [3]Horticulture Research Centre, Persiaran MARDI-UPM, MARDI Headquartes, 43400 Serdang, Selangor, Malaysia.
**E-mail: hally@mardi.gov.my*

Introduction

Orchidaceae, is the most species-rich plant family in the world, with estimated of 17,500 to 35,000 species (Maridass, 2010). There are many varieties of orchid like *Epidendrum, Dendrobium, Pleurothallis, Vanilla, Mokara,* and *Varda* were found in different part of the world. *Dendrobium* is the second-largest genus in the Orchidaceae, consisting of more than 1,100 natural species and a large number of hybrids (Wu et al., 2009). Many Dendrobium hybrids produce flowers multiple times a year, with myriad of shapes, vibrant colours, and textures. Dendrobium flowers gain high demand in cut-flower and pot plant markets (Khosrari et al., 2008). Production of high quality orchid hybrid is lucrative industry in Malaysia with export value of RM13.2 million in 2016 as compared to RM11.9 million in 2015 (Floradaily, 2017). Dendrobium is conventionally propagated by separating back bulbs or by vegetative cuttings, however these methods were progressed very slow and consumed laborious skills that resulted in the regeneration of only a few propagules in a year (Venturieri and Pickscius, 2013).

Tissue culture, on the other hand, provides an alternative solution for producing a large number of genetically similar, phytosanitarily and physiologically high quality plantlets within a limited time period. In this process, a high survival percentage, associated with a high standard of acclimatized plantlets, are desirable in commercial labs and companies involved with orchid micropropagation (Jaime et al., 2013*). In vitro* propagation not only accelerates plant production, but it also generates virus-free plantlets (Kozai et al., 1997).

In vitro propagation consists of various stages, including selection of explant, aseptic culture establishment, multiplication, rooting and acclimatization of plants. Since the plant tissue inherently have various pathogen on the surfaces and natural openings when sourced directly from the field grown plants, microbial contamination (either fungal or bacteria) presents a major challenge to the initiation and maintenance of viable *in vitro* culture. The most important step for aseptic culture establishment is sterilization of explants. Successful tissue culture of orchid depends on the removal of exogenous and endogenous contaminating microorganism (Bucket and Red, 1994). Therefore, the present study was aimed to standardize the sterilization method for shoot tips of *Horn-type Dendrobium* hybrid orchid for micropropragation using different concentration of bleach and duration of exposure.

Materials and Methods

Plant material collection

The experiment was conducted at plant tissue culture laboratory, Biotechnology Centre MARDI. The explants were taken from Horticulture Centre orchid green house.

Media preparation

The Murashige and Skoong (1962) media was used for the experiment. Media was prepared by dissolving the organic and inorganic components in distilled water. The solution was stirred until

dissolved and made up to final volume. The media pH was adjusted between 5.2 to 5.3 by using either 1 M (HCI) or 1M (NaOH) before the gelling agent was added. Media was then heated on microwave at 15 minutes until agar is dissolved and media dispensed in the culture flaks. The culture flaks were capped with aluminium foil and placed in basket and autoclaved. Autoclaving was at temperature of 121˚C and pressure of 15 psi for 20 minutes.

Aseptic techniques

The process of sterilization and dissection of plant materials were carried out under sterile conditions in laminar air flow cabinet. The cabinet was sterile with 70% ethanol using cotton wool or sterile towel and kept running for 15 minutes prior to routine procedures. All the plant materials were dissected under sterilization conditions in the sterile laminar air flow cabinet.

Surface sterilization of explant

The healthy, diseases-free and undamaged explant sources were washed under running tap water to remove the adhered soil particles and dirt from the explant surface and then were washed again thoroughly under running tap water using 2-3 drops of tween-20 detergent for about 1 hour. The explants were then treated with 80% ethanol for 1 minute under laminar air flow cabinet. The explants were then rinsed with distiller water three times to lower the toxic effect of ethanol. Explants were then treated with three concentration levels of sodium hypochlorite (NaOCl) (15, 30, 45% (v/v)) for various exposure time (5, 10, 15 min). After decanting the sterilizing solution under safe condition, the explants were washed three times with sterile distilled water to removed excess NaOCl.

The wounded sites exposed sterilizing agent were trimmed properly under aseptic condition to provide a newly cut surface and to remove any cell damage by sterilant. Then, the shoot tips (0.5-1 cm) subsequently cultured on MS media supplemented with vitamin, sucrose (20 g/L), and 6-benzylamnopurine (BAP) (1.0 mg/L) solidified with gelrite (4.0 g/L). The test tubes with cultured explants were properly sealed with parafilm and labelled. The cultures were kept under a photoperiod of 16/8 h light/dark using fluorescent lamps at 25±2˚C.

Statistical analysis

The experiment was arranged in a Completely Randomized Design (CRD). Each treatment was replicated three times and each replication per treatment contained 5 explants. The percentages of survival, mortality and contamination rates were recorded after 4 weeks of culture. Data were analysed using analysis of variance (ANOVA) and Duncan New Multiple Range Test (DNMRT).

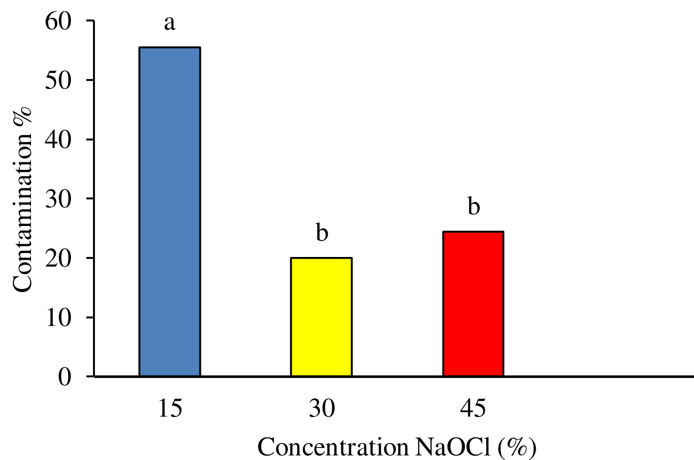
Results and Discussion

Effect of sterilization on percentages of survival, mortality and contamination rates (%)

The result of this study shown that main effects of concentration of NaOCl and exposure time had significant ($p<0.01$) affected percentages of survival and mortality, with significant ($p<0.01$) interaction recorded between them. Percentage of contamination was significantly affected only by main effect of percentage concentration NaOCl. Explant treated with 30% NaOCl had significant lower percentage of contamination as compared to 15% NaOCl (Figure 1). This might be due to weaker chemical reaction and shorter exposure duration that unable the pathogens to decimate from the explant surface (Thkchom and Maitra, 2016).

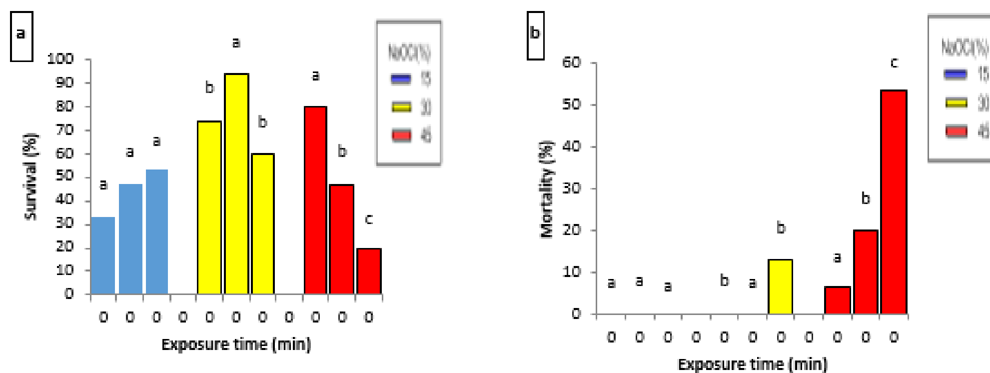
The highest explants survival rate (93.33%) were recorded when explants treated with 30% NaOCl for 10 min. Survival rate for explants treated with 15% NaOCl had no significantly difference as exposure time increased. For explants treated with 45% NaOCl, as the exposure time increased, the survival rate decreased significantly (Figure 2a). Mortality rate for explant treated with 15% and 30% had not

significant different as exposure time increased. The highest explant mortality (53.33%) were recorded when the explants treated with 45% NaOCl for 15 minutes (Figure 2b). This has determined that the exposure duration for 15 minutes has leds to the internal injury of the explant tissues that ultimately dried and died after becoming necrotic (Thkchom and Maitra, 2016). Excessive sterilization had increased the tissue mortality of explants (Majid et al., 2014). The results were found in close conformity with the finding of Asghar et al. (2011) and Maridass et al. (2010) that reported surface sterilized of explants by cleaning thoroughly under running tap water and treated different concentration NaOCl with specific period to control the microbial contamination in the explant of Dendrobium.



Means with different letters indicate significant differences at P<0.01% level according to DMRT.

Figure 1: Effect of concentration level of NaOCl (%) on contamination rate of *Horn-type Dendrobium* Orchid Hybrid MARDI explant.



Means in each column with the different letters within each factor indicate significant differences at P≤0.01% level according to DMRT.

Figure 2: (a) Effect of different concentrations of NaOCl (%) on survival rate (%) and (b) mortality rate (%) of *Horn-type Dendrobium* Orchid Hybrid MARDI explant.

Conclusions

NaOCl is widely applied to orchid explants and seed for disinfestations because of its oxidizing nature to kill the microorganisms. It is useful in reliable plant sterilization as it is inexpensive and easily

available chemical. Bacterial and fungal infections can be eliminated accurately by proper immersion time of explants in the sterilization agent. Therefore, it can be conclude that treatment of new emerging shoot explant of Dendrobium in 30% NaOCl for 10 min exposure time was found to be the best for explant survivability.

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Antifungal Effect of Cinnamon (*Cinnamomum verum* J.Presl) Essential Oil against Aflatoxigenic *Aspergillus flavus* Link

Padrilah, S.N.[1,2], Masdor, N.A.[1], Ghazali, M.S.[1], Khalid, K.[1] and Samsudin, N.I.P.[2,3,*]
[1]Biotechnology and Nanotechnology Research Centre, MARDI HQ, P.O. Box 12301, GPO, 50774 Kuala Lumpur, Malaysia.
[2]Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43000 UPM Serdang, Selangor, Malaysia.
[3]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43000 UPM Serdang, Selangor, Malaysia.
**E-mail: nikiskandar@upm.edu.my*

Introduction

Corn (*Zea mays* L.) is a cereal crop widely cultivated in a range of agro-ecological environments. Corn plants usually grow to 2.5 m (8 ft) in height and some cultivars may grow up to 12 m (40 ft). Corn seeds or kernels are botanically known as caryopsis (dry single-seeded fruit), while the endosperm of corn forms most of the volume and weight of the kernel. In Malaysia, corn is the third most important food crop in the world surpassed by two other grains which are rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). In 2015, Malaysian corn production was almost 62,459.85 metric ton (Department of Agriculture, Malaysia, 2015). However, various diseases involving a wide range of pathogens including fungal and bacterial have been reported thus limiting the production of corn.

According to Garuba et al. (2015), fungi were accounted for approximately 75% of seed-borne pathogens and cause infectious diseases such as discoloration, rot, necrosis and blight. The growth of fungi on corn usually occur during the storage period, rendering them unfit for human and livestock consumption on account of the reduction in their nutritive value (Kumar et al., 2007). This is due to the higher grain moisture content, higher temperature during storage and long storage period. One of the fungal genera commonly reported to infect corn is *Aspergillus* spp. Subedi, (2015) and Sweets and Wright, (2015) reported that corn infection by *Aspergillus* spp. would have symptoms such as leaf spot, common leaf rust, brown spot, grey leaf spot and eyespot. Furthermore, some of *Aspergillus* species could be mycotoxigenic in nature for example the *Aspergillus* section *Flavi* (*A. flavus, A. parasiticus*) that produce highly toxic and carcinogenic secondary metabolites known as aflatoxins. Aflatoxins can degrade seeds' quality, thus reducing their yield. The carcinogenicity of aflatoxins include hepatotoxicity, teratogenicity and immunotoxicity on both humans and animals (Kumar et al., 2017).

Due to these issues, various researches have been carried out on the development of antifungal agent against *A. flavus* such as chemical preservatives and synthetic fungicides (Samuel et al., 2013; Jalali and Avagyan, 2016). However, most of the clinically used antifungals have various drawbacks in terms of toxicity, efficacy and cost. Therefore, novel, efficient and safe remedies for controlling plant fungal diseases are a necessity. Plant-based products such essential oils are gaining increasing attention as a natural product in controlling fungal and bacterial diseases. Essential oils are aromatic and volatile liquids extracted from plants, which have a broad spectrum of antimicrobial activity. The presence of compounds including aldehydes, phenolics and terpenes has been shown to be responsible for the antimicrobial activity of essential oils.

The present work thus aimed to evaluate the antifungal activity of cinnamon essential oil against the growth of *A. flavus* through agar dilution method. The minimal inhibitory concentration (MIC) of cinnamon essential oil and its chemical composition was also evaluated.

Materials and Methods

Chemicals

Potato dextrose agar (PDA), Tween-20, Tween-80, and dimethyl sulphoxide (DMSO) were purchased from Sigma Aldrich and Systerm, USA.

Essential oil

Cinnamon essential oil used in the present work was extracted from cinnamon leaves using steam distillation conducted at Malaysian Agricultural Research and Development Institute (MARDI), Linggi, Negeri Sembilan, Malaysia. The steam distillation was employed in the present work due to its robustness and efficiency as compared to hydro distillation process.

Fungal strain and culture condition

Aspergillus flavus strain was obtained from the Faculty of Food Science and Technology, Universiti Putra Malaysia, and maintained on PDA. The strain was sub-cultured every two weeks and stored at 4°C until further use.

Determination of Minimal Inhibitory Concentration (MIC)

The minimum inhibitory concentration of cinnamon essential oil against *A. flavus* was measured through the agar dilution method according to Rana et al. (2011) with slight modifications. PDA plates were prepared with increasing concentrations of cinnamon essential oil from 0.1 µL/mL to 12 µL/mL (v/v), and 0.05 % Tween-20 was used to dissolve the essential oil in the medium. The plates were then centrally inoculated with mycelial disc (≈5 mm Ø) and incubated for seven days. Following incubation, the means of colony diameter were recorded. Three replicates (n = 3) were made for each of the treatment, and medium with no essential oil served as control. The plate with the lowest concentration of cinnamon then showing no visible growth was regarded as the MIC.

Determination of active compound in cinnamon oil using Gas Chromatography Mass Spectrometry (GC-MS)

The cinnamon essential oil was analysed by GC-MS for the identification of their active compounds. Gas Chromatography – Mass Spectrometer (Perkin Elmer) equipped with HP-5MS 5% Phenyl Methyl Silox column (30 m × 250 µm × 0.25 µm) was used. The oven temperature was programmed as isothermal at 60°C for 10 minutes, at 3°C for 1 minute and at 180°C for 15 minutes. Helium gas was used as carrier gas at the rate of 3 mL/min. Effluent of GC column was directly introduced into source of the MS via a transfer line with temperature program 280°C.

Statistical analysis

All the experimental results were statistically analysed by Analysis of Variance (ANOVA) followed by Tukey's multiple comparison tests at 95% confidence level and plotted using Graph pad prism 5.0 software. $p<0.05$ was considered statistically significant.

Results and Discussion

The scientific interest on biological sources as natural antifungal agents is growing. Plant-based essential oils contain active compounds responsible for inhibiting microbial growth. In the present work, cinnamon essential oil was used as a natural source for antifungal agent against *A. flavus*.

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The evaluation of minimum inhibitory concentration (MIC) has been carried out and the result shows that the cinnamon essential oil was capable of inhibiting the growth of *A. flavus* at the different concentrations of cinnamon essential oil tested (0.01% - 1.2%). Based on the result presented in Figure 1, the growth of *A. flavus* increased every 24 hours at the concentrations ranging from 0.01% to 0.04%. However, starting at the concentration of 0.08% to 1.2%, there was no significant growth observed until day 7. Therefore, 0.08% was taken as the MIC. The efficacy of cinnamon essential oil as an antifungal agent against *A. flavus* has also been reported by Hua et al. (2014) and Rahimifard et al. (2008).

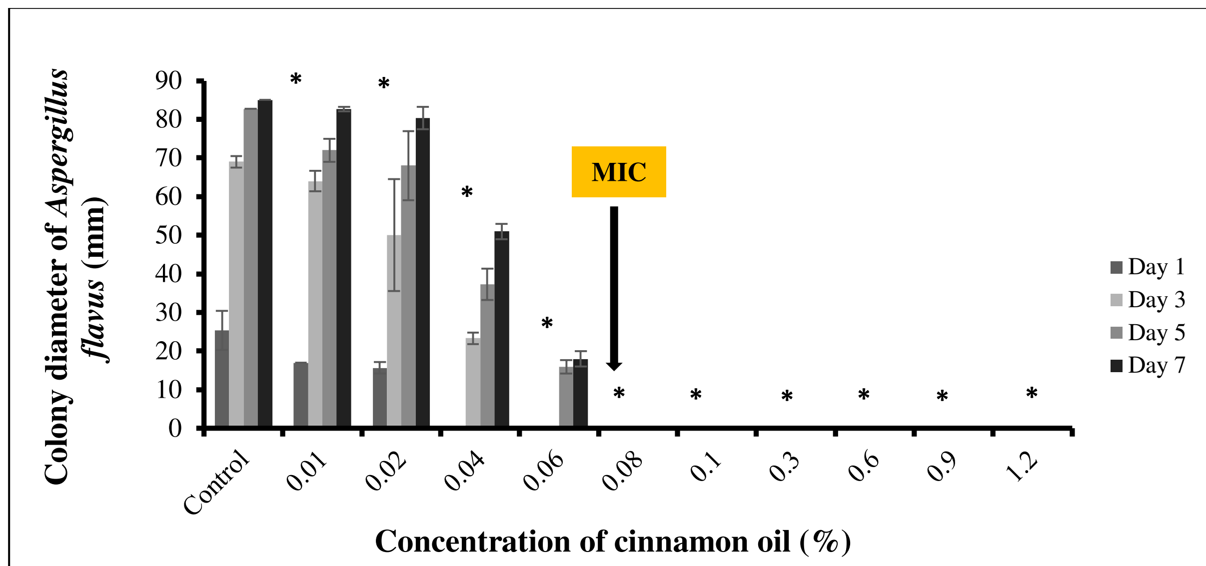

Figure 1: Minimum inhibitory concentration (MIC) of cinnamon essential oil against *Aspergillus flavus*. Data are means ± SD of triplicates (n = 3). Asterisks (*) indicate statistically significant difference as compared to control at $p<0.05$.

The effectiveness of cinnamon essential oil could be due to the presence of active components (Ghosh et al., 2013). According to Sukatta et al. (2008), the main constituent of cinnamon essential oil is cinnamaldehyde which contains aldehyde with conjugated double bonds outside the ring. This compound possesses much stronger antifungal activity (Wang et al., 2005), and it may be a potential lead compound for the development of antifungal drugs through the control β-(1,3)- glucan and chitin synthesis in yeasts and moulds (Bang et al., 2000).

Therefore, GC-MS analysis was carried out to identify the chemical composition present in the cinnamon essential oil. A total of 12 active compounds were identified with eugenol found to be the major compound (49.25%) while the minor compounds were benzyl benzoate, alpha-pinene, camphene, beta-pinene, linalool, E-cinnamaldehyde, ortho-cymene, sabinene, alpha-copaene, E-caryophyllene and alpha-humulene.

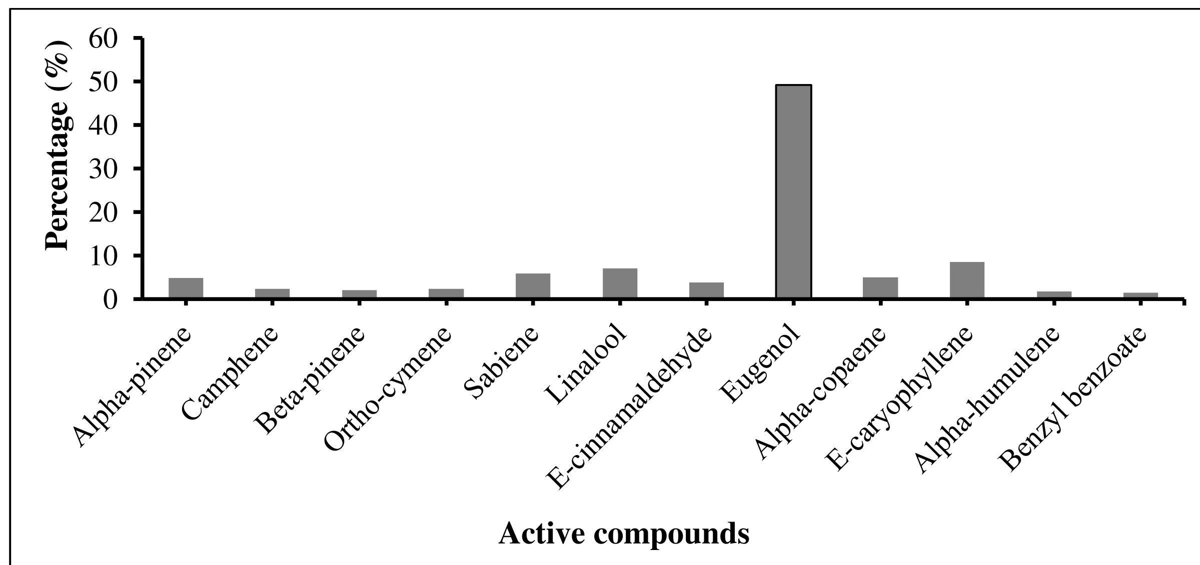

Figure 2: The percentage of active compounds in cinnamon essential oil as analysed by GC-MS. Eugenol was the highest active compound present (49.25%).

According to Gotmare, (2019), in most of the researches conducted on cinnamon, eugenol was commonly found as the main component of cinnamon leaf essential oil while cinnamaldehyde as the major component of cinnamon bark essential oil. In the present work, the essential oil was extracted from leaves, thereby yielding eugenol as the highest component. Ghosh et al. (2013) and Rangel et al. (2018) found that cinnamon leaf essential oil to be rich with eugenol up to 59.92% and 68.96%, respectively, and inhibited *Bacillus cereus* and *Candida* spp., respectively.

For the antifungal property, eugenol, cinnamaldehyde, linalool and any other active components could be involved in the inhibition of extracellular enzyme synthesis and disrupt the cell wall structure of the fungi which result in damage of integrity, leakage of cytoplasm and ultimately the mycelial death (Carmo et al., 2008). Velluti, (2003) and Carmo et al. (2008) described that the phenolic compound eugenol exerts its antimicrobial activity through the presence of an aromatic nucleus and phenolic OH group known to be reactive and able to form hydrogen bonds with active sites of target enzyme. Besides the major compounds, the synergistic or antagonistic effects of the minor compounds present must also be considered (Dafereraet al., 2003; Souza et al., 2007).

Conclusion

In conclusion, the development of natural antifungal agents is a major step towards reducing the negative effects associated with chemicals and antibiotics such as toxic residues in agricultural products, resistance development in targeted microorganisms and general harmful effects on the environment, humans and animals. The present work demonstrated that the essential oil of cinnamon had a significant inhibitory effect on the tested *A. flavus*. Therefore, cinnamon essential oil could be further explored as an alternative to be used as a biological control agent against fungal diseases in corn.

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Development of Electrochemical Biosensor for the Detection of *Xanthomonas oryzae* pv. *oryzae* in Bacterial Leaf Blight Disease and its Cross-reactivity Study

Husin, N.H.[1], Mohd Said, N.A.[1], Awaludin, N.[1], Abd Rahman, R.[1], Ramachandran, K.[2] and Salam, F.[1,*]
[1]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarter, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Rice and Industrial Crops Research Centre, MARDI Seberang Perai, 13200 Seberang Perai, Penang, Malaysia.
**E-mail: faridahs@mardi.gov.my*

Introduction

Rice is used as a basic food in different areas of world especially in Asia. *Xanthomonas oryzae* pv. *oryzae* invades the plant through wound or water pores. It was estimated that the demand for food will continue to increase for another 40 years due to the continuous increase in human population. Although decrease in agricultural productivity can be attributed to a variety of reasons, damage caused by pest and pathogens plays a significant role in crop losses throughout the world. The losses in crop yield due to pathogen infections range between 20% and 70% (Savory et al., 2012). In the past, various disease management strategies have been employed to reduce the yield losses, and to avoid disease epidemics but use of chemicals has not been successful due to variation in sensitivity of pathogenic races toward applied chemicals. As a result, biological control seems to be cost effective and environmentally friendly way to manage this serious threat (Gnanamanickam, 2009).

Antibodies are multipurpose and are suitable for varied immunosensing applications. Antibody-based biosensor allows rapid and sensitive detection of a range of pathogens especially for foodborne diseases and this technique has already been developed for food safety monitoring. The antibody-based biosensors offer several advantages such as fast detection, improved sensitivity, real-time analysis and potential for quantification. Antibody-based biosensors hold great value for agricultural plant pathogen detection. The principle of establishing antibody-based immunosensors lies in the coupling of specific antibody with a transducer, which converts the binding event (the specific binding of antibody modified on the biosensor with the antigen, e.g., pathogen of interest) to a signal that can be analysed. Depending on the operating principle of the sensor, the analytes could be detected using a sensor based on electrical, chemical, electrochemical, optical, magnetic or vibrational signals. The limit of detection could be enhanced by the use of nanomaterial matrices as transducers and the specificity could be enhanced by the use of bio-recognition elements such as DNA, antibody, enzymes etc.

Despite the economic importance of BLB, development of immunosensor were conducted for early detection *Xanthomonas* pv. *oryzae* in rice. The detection was improved from sandwich enzyme-linked immunoabsorbent assay (ELISA) and employing chronoamperometry (CA) technique. The parameters of this study including standard curve and cross-reactivity as a result of specific and fast detection.

Materials and Methods

Chemicals and reagents

Polyclonal antibody against Xoo was developed in-house at Animal Complex, MARDI using cultures isolates from Mardi Seberang Perai (P0.0, P1.0 and P7.3 strains). Pyrrole, 3,3’, 5,5’-tetramethylbenzidine (TMB) substrate solution, bovine serum albumin (BSA), ethanolamine, sodium

bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3) and phosphate buffer saline (PBS) tablets were purchased from Sigma-Aldrich, USA. *N*-(3-Dimethylaminopropyl) - *N*'- ethylcarbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were from Sigma-Aldrich, Japan. Functionalized multi-walled carbon nanotubes (MWCNT-COOH) and ceramic-based screen-printed carbon electrodes (SPCE) were from Dropsens, Spain. Blotto Non-Fat Dry Milk and EZ-Link^{TM} Plus Activated Peroxidase Kit were supplied by Santa Cruz and Thermo-Scientific, USA, respectively. Phosphate buffered saline (PBS) solution was prepared by dissolving one PBS tablet in 200 mL deionized (DI) water yielding 0.01 M phosphate buffer, pH 7.4 at 25°C. All solution were prepared using DI water from a Milli-Q Ultrapure water system with a resistivity of 18.2 MΩcm.

Bio-functionalization of carbon surface

Electrodeposition of polypyrrole (Ppy)/functionalized-MWCNT was performed by electropolymerization of 0.075 M pyrrole in the presence of 0.1 mg/mL functionalized-MWCNT (MWCNT-COOH) onto Dropsens by chronoamperometry technique. Electropolymerization potential of 1.0 V was applied and the mixtures were deposited for 900 s in PBS as electrolyte. Dropsens was then rinsed with DI water and dried under nitrogen (N_2) flow. The carboxylic acid groups of MWCNT-COOH was activated in 10 µL mixture of 0.4 M EDC and 0.1M NHS (1:1) for 15 min. The excess was washed out using PBS, N_2 dried and Dropsens was immediately incubated with 5 µL of anti-Xoo antibody for 1 hour. The SPCE surface was then washed with PBS and blocked for 30 min using different types of blocking agent, i.e. bovine serum albumin (BSA) and ethanolamine. Xoo cells at concentration 10^0 to10^9 CFUs/mL was placed on the SPCE's WE and incubated for 1 hour. The antigen was diluted in 0.1 M carbonate-bicarbonate, pH 9.6. The unbound antigen was washed away with PBS and 5 µL of purified anti-Xoo antibody conjugated with HRP was dropped onto working electrode and incubated for 30 min, washed again with PBS. All modification steps were performed at room temperature.

Electrochemical set-up and measurements

Autolab PGSTAT 20 potentiostat (Eco Chemie, Netherlands) was used for the sensor analysis. Electrochemical measurements for the modified Dropsens with bounded antibody and Xoo cells were carried out by placing 50 µL of TMB solution onto Dropsens covering all three-electrode and measured using chronoamperometry at optimized potential for 300 s. All electrochemical measurements were carried out at room temperature using NOVA 1.10 software. Calibration curve was fitted with non-linear regression using 4-parameter logistic equation and the detection limit (LOD) was then calculated based on the following equation as described by Tijssen:

$$\text{LOD} = x\left(\frac{a-d}{(a-d)-3s} - 1\right)^{-1/k}$$

where *s* is for standard deviation of the zero value.

Cross reactivity studies

The sensitivity and specificity of the sensor was investigated in relation to other bacteria such as Pseudomonas sp., *Xanthomonas oryzae* pv. *oryzicola* 1585, *Xanthomonas oryzae* pv. *oryzicola* 2921 and Pantoea amantis to examine the specificity of the immunosensor. Each bacterial solution (1.0×10^9 CFU ml^{-1}) was used as the sample and incubated for 2 h at 37°C. Polyclonal antibody–HRP was used as the detection antibody.

Results and Discussion

The electrochemical immunosensor system developed in this work for Bacterial Leaf Blight detection was based on direct competitive and sandwich immunoassay format with HRP used as the enzyme

label. Standard curve for Xoo detection was performed via chronoamperometry (CA) analysis using mix culture of P0.0, P1.0 and P7.3 Xoo strains in carbonate buffer pH 9.6. After Xoo cells concentrations of 10^0 to 10^9 were added, conjugated anti Xoo antibody-enzyme (HRP) were drop to allow binding onto the Xoo cells. TMB was finally dropped onto electrode and the current was recorded. Figure 1 shows the standard curve plot of Xoo in the mixed culture. The chronoamperometry result for standard curve development studies shows a limit of detection 9.0 x 10^4 CFU mL^{-1} was obtained. The absolute current values increased accordingly with Xoo concentrations in both linear regression graph with R^2 value 0.9166. The higher Xoo cell number in the sample the higher signal is achieved in the electrochemical measurement. These results emphasized the success and sensitivity of the developed immunosensor.

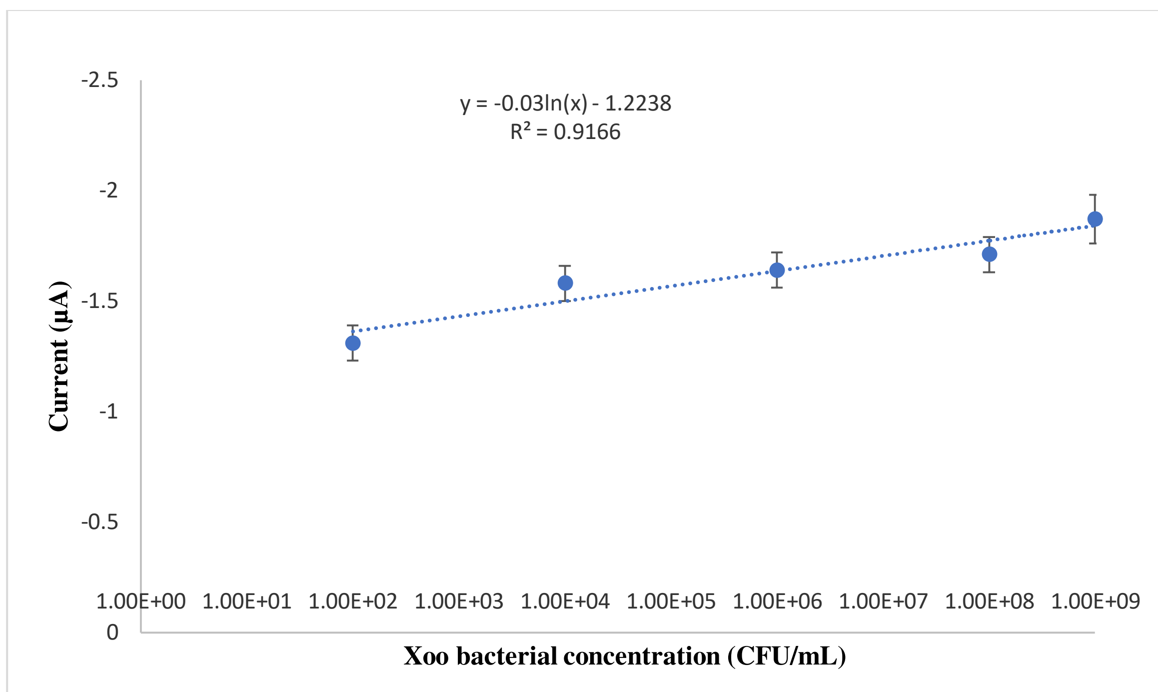

Figure 1: Standard curve plot Linear Regression graph; Xoo mixed culture of P0.0, P1.0 and P7.3.

The sensitivity and specificity of the sensor was investigated in relation to other bacteria such Pseudomonas sp., *Xanthomonas oryzae* pv. *oryzicola* 1585, *Xanthomonas oryzae* pv. *oryzicola* 2921 and Pantoea amantis. The results showed that there are no cross- reactivity with Xoo value greater than 20%.

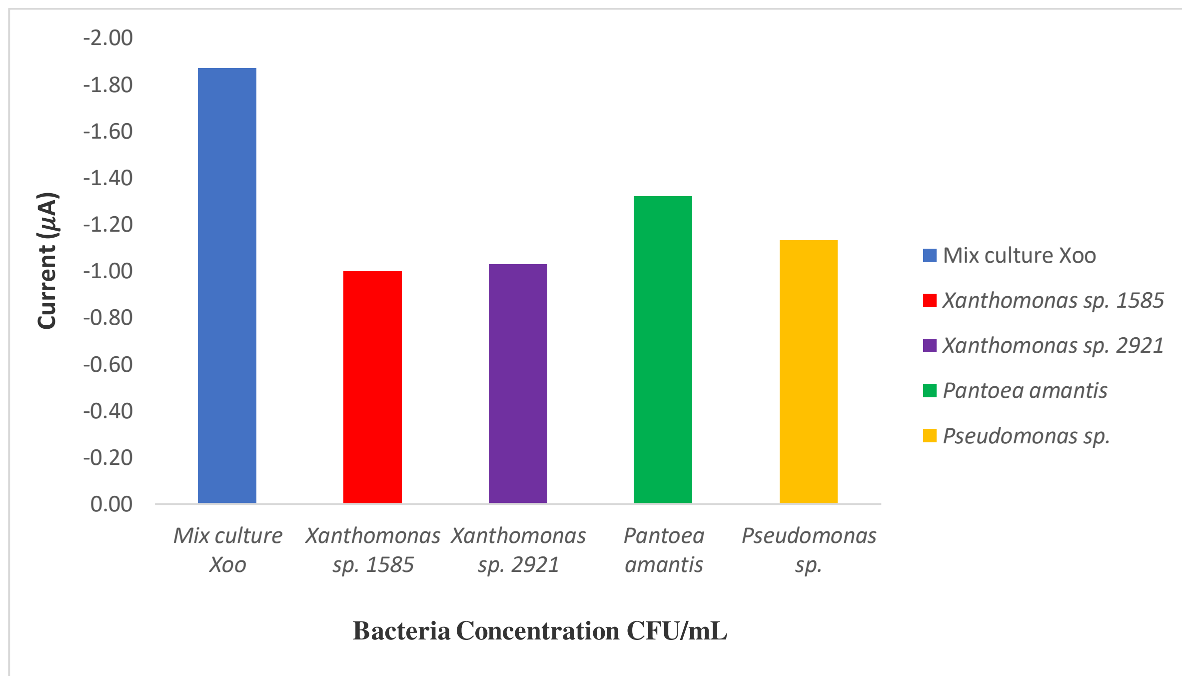

Figure 2: Cross-reactivity Xoo with Pseudomonas sp., *Xanthomonas oryzae* pv. *Oryzicola* 1585, *Xanthomonas oryzae* pv. *oryzicola* 2921 and Pantoea amantis.

Conclusion

The development of electrochemical immunosensor for the detection of Xoo in Bacterial Leaf Blight disease was successfully achieved. The high signal were obtained accordingly with the concentration of the bacteria. Specificity and sensitivity of the polyclonal antibody ensure the accurate detection. The advantages of this immunosensor studies can be packed with customized portable digital reader and bio-reagent for easy use and handling in field. Further, the present invention can provide for very rapid test in real time. Electrochemical biosensors have attracted considerable attention due to the advantages of robustness, low cost, low power consumption, simplicity, high sensitivity, compatibility with mass manufacturing using existing micro-fabrication technologies, and portability; therefore they are excellent candidates for easy-to-use immunosensors.

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