TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 28

Emerging Trends of Plant Physiology in Changing Environment

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http://www.mspp.org.my
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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

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30th Malaysian Society of Plant Physiology Conference (MSPPC 2020)

Webinar (17-18 November 2020)

Nor Mayati Che Husin
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Roohaida Othman
Normaniza Osman
Noorliana Mohd. Zan
Siti Aishah Hassan
Siti Hajar Ahmad
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Lok Eng Hai
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Tsan Fui Ying

Organised by

Malaysian Society of Plant Physiology

Publisher
Malaysian Society of Plant Physiology
Locked Bag No. 282, UPM Post Office,
43409 UPM, Serdang, Selangor, Malaysia
URL: http//mspp.org.my

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

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

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Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Growth Assessment of Mesta (*Garcinia mangostana* L.) Seedlings on Different Growing Media

Mohd Ridzuan, M.D.[1,*], Ab Kahar, S.[2], Puteri Aminatulhawa, M.A.[2] and Salehudin, M.R.[1]

[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

Mesta is a mangosteen variety and has been registered as GA2 by the Department of Agriculture, DOA (Jabatan Pertanian Semenanjung Malaysia, 1999). Mesta fruit shape is ovate, the white flesh is sweet and not very juicy as compared to ordinary mangosteen. It is a perennial plant with a slightly smaller canopy than mangosteen. According to the crop statistics book (food crops sub-sector) 2019, the area planted with mangosteen in Malaysia was 3,717 ha with a production yield of 26,170 mt. The main states for mangosteen production were Johor (747 ha), Kedah (584 ha), and Kelantan (535 ha). Recently, there are requests for exporting Mesta planting material to East Malaysia (Sarawak and Sabah), since the planting material is not readily available in those areas. Exporting planting material to those states required proper documentation and treatment as specified such as the planting material should be bare rooted (Wan Mahfuzah et al., 2016). This is to comply with the Malaysian Quarantine and Inspection Services Act 2011 [Act 728]. However, seedlings raise in nurseries often use soil mixing media. Hence there will be a difficult job to prepare the bare rooted seedlings to meet exporting plant material regulation. Seedling Mesta is a fragile plant due to its weak root system. Therefore, soilless media can be an alternative way to reduce damage to the roots during the cleaning process. Thus, the objective of this study was to evaluate the performance of Mesta seedlings on different types of soilless media.

Materials and Methods

The experiment was conducted in nursery with 70% shade level and irrigated by an overhead sprinkle system. Three months old uniform seedlings were selected and planted onto polybags containing different mixtures of soil and non-soil media. The media were T1: the standard soil mixture (sand + soil + organic matter, 3:2:1), T2: Peat moss + perlite (1:1), T3: Cocopeat + perlite (1:1), and T4: Peat moss + coco-peat + perlite (1:1:1). All combinations in T2, T3, and T4 were using the non-soil media. The treatments were arranged in RCBD and replicated four times, which each plot consists of 10 plants. Non-destructive growth measurements including plant height and stem diameter were taken at 2 weeks interval until the 16th week. At 16 weeks, 5 plants were sampled from each plot for destructive growth measurements.

Results and Discussion

Bi-weekly measurements on plant height and stem diameter are shown in Figure 1. Generally, seedlings grown in standard soil mixture (T1) had faster growth in terms of height and stem diameter as compared to all non-soil media. Among the non-soil media, a mixture of cocopeat and perlite gave better growth. Poorest growth was obtained in the media containing a large portion of cocopeat (T3). Result on dry weight of seedling is summarized in Table 1. There was a great difference in total dry weight between the soil media and non-soil media. The most affected component was the leaf. Stem dry weight was lower in the non-soil but the differences in the root dry weight was not significant among the treatment.

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

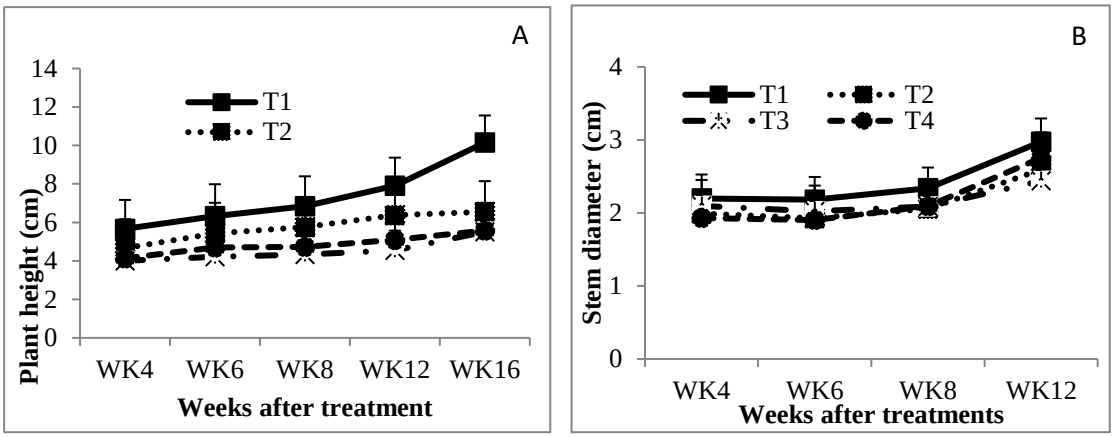

Figure 1: Plant height (A) and stem diameter (B) of Mesta seedlings grown on different media. Vertical bars represent the standard error. T1 - Sand: soil: peat (3:2:1), T2 - Peat moss: perlite (1:1), T3 - Coco peat: perlite (1:1) and T4 - Peat moss: coco-peat: perlite (1:1:1).

Table 1: Dry weight of Mesta seedling grown on different growing media after 16 weeks of transplanting.

Treatments	Dry weight/plant (g)			
	Leaf	Stem	Root	Total
T1 - Sand: soil: peat (3:2:1)	1.6[a]	0.5[a]	0.4[a]	2.4[a]
T2 - Peat moss: perlite (1:1)	0.5[b]	0.3[b]	0.4[a]	1.2[b]
T3 - Coco peat: perlite (1:1)	0.4[b]	0.2[c]	0.3[a]	0.9[b]
T4 - Peat moss: coco-peat: perlite (1:1:1)	0.4[b]	0.3[b]	0.4[a]	1.1[b]

Means followed by similar latter within column are not significantly different at p<0.05 by DMRT test.

Conclusion

The use of appropriate growing media is vital for good seedling establishment in the nursery (Rukayah and Zabedah, 1992; Wieble et al., 1992). The results of the present study affirmed these findings. The use of non-soil media seems to be not very promising. The shoots get stunted, although the root part was less affected. The effect of non-soil media on Mesta seedling growth is not fully known and requires detail investigation. Based on the handling operation procedure requirement for exporting mangosteen planting materials, cleaning of soil from the root cannot be avoided. From observations made during the export trial, if the cleaning process is handled carefully, the survival of mangosteen materials at the destination was high, but cleaning work was a bit tedious.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

The Effect of Different Water Levels on the Growth and Yield of Rice (*Oryza sativa*)

Norfatin Atikah, J., Noraida, M.R.* and Heary Azween, A.
Faculty of Plantation and Agrotechnology, Universiti Teknologi Mara (Melaka) Jasin Campus, 77300 Merlimau, Melaka, Malaysia.
**E-mail: noraida96@melaka.uitm.edu.my*

Introduction

Oryza sativa L. is one of the main crops after rubber and oil palm in Malaysia. Mainly, rice is grown in eight granaries covering about 205,548 hectares in Peninsular Malaysia (MOA, 2008). However, the country still has to import rice to meet the demand for insufficient production and supply. Malaysia will continue to dependent on imported rice as the country's production of the grain is nearly 30% short from the three million metric tonnes (MT) self-sufficiency level (SSL), therefore local rice production should be improved to ensure the country's demand.

In Malaysia, the agriculture irrigation sector is the largest water consumer with more than 70%, and is mostly used for rice irrigation. Irrigated rice is usually grown in a flooded environment that requires large amount of water throughout its growing period. However, rice water efficiency is relatively low and it is estimated that about 3000 L of water are used to produce 1kg of rice, given rice productivity index (WPI) at 0.3 kg grain/m^3 water.

The present global water crisis had threatened the sustainability of irrigated rice production. Climate variability and water resources facing intense competition from the domestic and industrial, that have resulted in water scarcity. Water demand from the domestic and industrial sectors is expected to increase and is likely to receive priority over-irrigation (FAO, 2011).

This scenario may require the adoption of rice production practices that reduce water inputs without lowering yields. Irrigation water use efficiency should play a more significant role in meeting future rice demands (Traore et al., 2014). Occasionally, a few scientific research on water saving practices on field to enhance the crop growing conditions are established (Jahan, 2013). Hence, this study aimed to investigate the effect of different water level on rice growth and yield.

Materials and Methods

The commercialized variety of MR220CL was used in this experiment. Twenty containers were filled up with rice soil at 13 cm depth. The treatments i.e. water at different levels (see below) was added to investigate its effects on growth and yield performance of rice. The water levels in each treatment were maintained throughout the experimental period. Fertilizer and agronomic practices were applied according to MARDI (2001). Fertilizer was applied until the rice mature on DAT 15, 35, 50, and 65.

Treatment and experimental design

This experiment was carried out in the greenhouse to study the impact of different water levels close to soil saturation for rice growth and yield to produce the same rice yield with low water used compared to standard agricultural practices. The experiment was conducted with four treatments and five replications where the experimental outline used completely randomized design (CRD). The treatments comprised of; T0 (control) = water level at 1 cm above the soil surface, T1= water level at 3 cm above soil surface, T2 = water level at 6 cm above soil surface, and T3 = water level at 9 cm above the soil surface (a common water level used by the farmers). The parameters recorded were plant height, number of leaves, number of tillers, number of panicles, fresh and dry weight, and yield (weight) for 1000 grains. The relative chlorophyll content in the leave of the rice plant was measured by Chlorophyll Meter Spad-502PLUS (KONIKA MINOLTA, Europe). All the parameters were measured, and data were collected every week after transplanting.

Results and Discussion

During this study, the variation of water level showed no practical impact on the growth and productivity of the rice. At the growth stage there was no significant difference (P>0.05) in the height of the plants as the different water level was applied in all containers after transplanting until the final week (Figure 1). It was observed that all treatment reaches almost the same height as their height increased during the mid-stage until the ripening.

There is no significant difference in tiller numbers of rice grew in different water level, where it did not affect the tillering ability (Figure 2). A study by Sarwar et al. (2005) also showed there was no significant effect of different flooding regimes on tiller numbers and stated that tiller production was found to be significantly lower under field capacity than flooded and saturated conditions. In this study, all treatments were above saturation level, and hence did not affect tiller production. The tiller numbers were in the range of 29 to 42 and reached its maximum potential at 75 DAS and at 90 DAS. The tillering process started to slow down in most of the treatments when the rice plants reach maturity and only a few small tillers were produced (Juraimil, 2009).

Generally, the rice panicle number were not affected by the flooding treatments. There was no significant difference for number of panicles observed among different water level treatments (Figure 3) when water level is in saturated condition, same result in Sarwar et al. (2005) study. Result showed that 1cm water level had produced panicles as much as other treatments while increasing water efficiency. Juraimi et al. (2009) had stated that the production of panicles was not significantly reduced when rice was grown in saturated soil, which was in line with the research done by Sariam (2004).

Relative chlorophyll content of rice leaves was measured to justify whether the different water level could affect chlorophyll related parameters. Chlorophyll content in leaves of rice gradually increased with increasing plant age regardless of soil water conditions (Figure 4). Different treatments did not significantly affect the relative chlorophyll content in leaves (P>0.05). This was supported by the previous study (Jahan, 2014), that suggested the plant in a saturated level might not affect chlorophyll-related plant growth and development.

The weight of 1000-grain showed there is no significant difference (Figure 5). Soil water level at saturated or above (1 cm water depth) did not affect the individual grain weight. During ripening, grain growth is characterized by an increased kernels' size and weight as starch and sugars are translocated from culms and leaves. Grain dry weight was increased despite of the decreased in fresh weight was possibly due to water loss from 58% to 20%. All plant parts, including grain, also undergo a colour changes from green at early stages to brownish at maturity stage (Smith and Dilday, 2003). The previous study by Jahan (2013) suggested that maintaining the water level at 1cm did not affected yield. Khairi et al. (2011) found that rice could be grown on saturated soil condition without affecting rice yield. However, if the plants were under alternate wetting and drying (AWD) condition, plants might suffer severe water stress when the water level was below the saturated level, and hence could reduce rice yield (Sariam et al., 2002; Khairi et al., 2015).

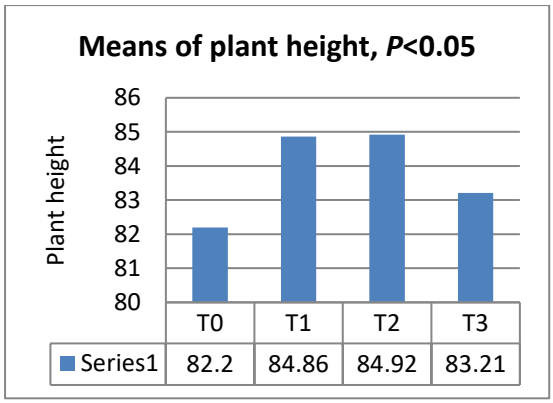

Figure 1: Effect of different water levels on plant height surface.

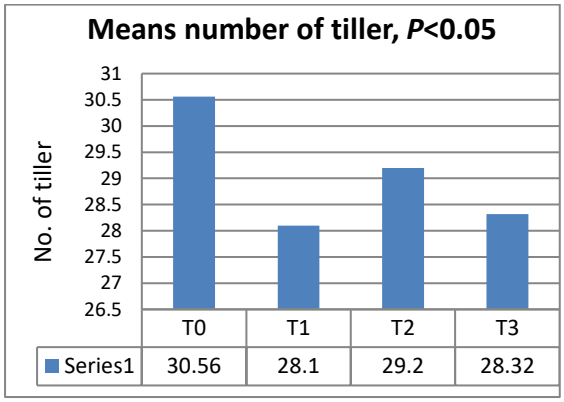

Figure 2: Effect of different water levels on number of tillers.

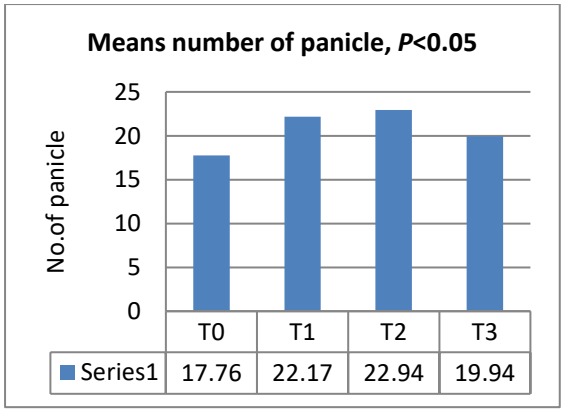

Figure 3: Effect of different water levels on number of panicles.

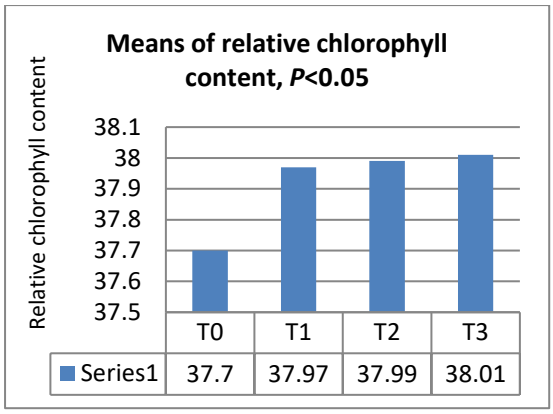

Figure 4: Effect of different water levels on relative chlorophyll content.

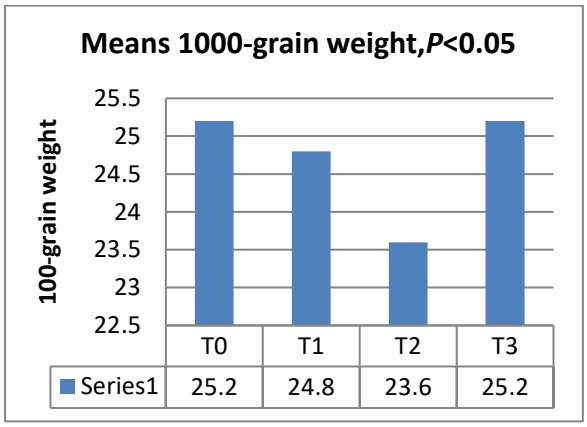

Figure 5: Effect of different water levels on grain weight.

Conclusion

Continuous flooding at 1 cm, 3 cm, 6 cm, and 9 cm did not significantly affect rice growth and yield. Therefore, low water input in rice production could be implemented to save fresh water to be used for other sectors. Farmers could practice low water input at saturation to 1 cm in their field for rice cultivation. Further studies focusing on different water levels on different growth stages and several cycles of rice planting shall be conducted to see the effect of different water levels under a longer period of time.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Shoot Vigour of 'Harumanis' Mango Grown under Rain-shelter and Greenhouse Structures

Abdullah, F.[1,*] and Tengku Maamun, T.A.M.[2]

[1]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia.
[2]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarters, P.O. Box 12301, General Post Office, 50774 Kuala Lumpur, Malaysia.
**E-mail: fadhil@mardi.gov.my*

Introduction

'Harumanis' is one of the popular mango cultivars in Malaysia. It is believed that this type of tropical mango was originated from Indonesia and being domesticated in Malaysia for more than 40 years ago. In Perlis state, 'Harumanis' is considered as an 'Iconic Fruit' which has economic benefit for the local growers (Sani et al., 2018). This mango fruit type is very sweet, juicy and has a fine texture (Zainal Abidin and Tengku Ab. Malik, 1996). For the past 15 years, new mango orchard management has been introduced by planting 'Harumanis' cultivar under greenhouse or rain-shelter structures to improve flowering and fruit setting (Jaafar, 2017). Besides that, unpredictable rains during pre-flowering and flowering season are becoming a major limitation to sustain the production of 'Harumanis' mango in Northern region of Malaysia especially in Perlis state.

In other countries such as Japan and Egypt, greenhouse cultivation is widely used for the production of commercial mango cultivars (Yamashita, 1999; Akinaga and Hasbullah, 2000; Medany et al., 2009). Popular mango cultivars such as 'Irwin', 'Keitt', 'Sensation' and 'Haden' have been successfully planted and producing high-quality fruits (Saúco, 2000). By planting mango under rain-shelter or greenhouse structures, some of the problem like pests and diseases, and unpredictable rains during pre-flowering and flowering periods that can cause low fruit set can be solved. However, it is believed that by modifying the growth condition may alter the morphology (i.e. growth) and/or physiology of the mango trees (Whiley, 1992), because generally 'Harumanis' mango is grown in the open area.

Therefore, this preliminary study was conducted to evaluate the response of vegetative growth or shoot vigour of 'Harumanis' mango trees planted under different growing conditions (i.e. rain-shelter and greenhouse).

Materials and Methods

This study was conducted at 'Harumanis' Mango Multi-purpose Plot, MARDI Sintok Research Station, Kedah. The grafted 'Harumanis' mango trees managed under standard agronomic practices were planted under three different growing conditions (i.e. open area, rain-shelter, and greenhouse).

The 'Harumanis' mango trees planted in the open area represented as control trees. Each treatment was replicated with three (3) single tree replicate. After harvesting season, selected shoots were tip-pruned at the intercalation unit or ring of buds according to the methods described by Davenport (2006). Ten newly emerged primary shoots were randomly selected and tagged for each treatment.

Data of shoot morphology such as length (cm), shoot diameter (mm) and leaves number per shoot, including fresh (g) and dry weight (g) of shoots were taken and recorded at the full stage of shoot development. The length of primary shoots (cm) was measured using ruler from the shoot base until the tip of primary shoots. The stem diameter of primary shoots was measured at shoot base using a digital calliper (Mitotuyo, Japan) and converted to stem cross-sectional area (mm^2). The total number of shoots including primary shoots was manually counted. The fresh and dry weight (g) were destructively

measured using digital balancing (Shimadzu TX3202L). The shoot dry weight was obtained by placing the shoots in a drying oven at 70 °C until the weight was constant.

Before analysis, data were checked for normality. Due to highly skewed, data were transformed to square root transformation before analysis (SAS 9.3) using Least Significant Differences (LSD) at $P \leq 0.05$.

Results and Discussion

The objective of this study was to evaluate the shoot vigour of 'Harumanis' mango trees when planted under different growing environment. We strongly believe that any changes of the growing condition or growth environment may affect the physiological processes of the trees. We suggest that the interaction of multiple factors such as light quality (i.e. the amount of light diffused by the plastic roof), endogenous hormones (possibly gibberellins, GA) and temperature inside the greenhouse may have an influence on the vegetative growth especially on the shoot development (Whiley, 1992; Bastias and Corelli-Grappadelli, 2012). In this study, the mean length of primary shoots of 'Harumanis' mango was significantly ($P<0.0001$) increased when planted under the greenhouse and rain-shelter structures compared to the open area (Table 1; Figure 1). Similar result was also found on the mean cross-sectional area of primary shoots of 'Harumanis' mango ($P<0.0001$). However, no significant difference ($P=0.17$) was observed on the number of leaves of 'Harumanis' mango between growing condition (Table 1).

Table 1: Shoot length (cm), shoot cross-sectional area (mm^2) and number of leaves per shoot of 'Harumanis' mango cultivar at fully development stage (n=30) planted under different environment condition.

	Shoot length (cm)		Shoot cross-sectional area (mm^2)		Number of leaves per shoot	
Open area	3.7^{c}	(14.6±1.3)*	4.1^{c}	(121.6±8.9)*	2.8^{a}	(8.00±0.4)*
Rain-shelter	4.1^{b}	(17.8±1.2)	6.1^{b}	(187.0±12.3)	2.6^{a}	(7.00±0.3)
Greenhouse	4.7^{a}	(22.0±0.8)	6.8^{a}	(228.1±9.3)	2.7^{a}	(7.60±0.3)
$LSD_{0.05}$	0.40		0.52		0.18	
P-value	*P*<0.0001		*P*<0.0001		*P*=0.17	

*Data were transformed means using square root transformation for analysis. Means followed by similar letter are not significantly different at P≤0.05 according to LSD test. *Means of raw data (±standard error of means).*

Figure 1: The morphology of the primary shoots of 'Harumanis' mango planted under different growing environment.

These results indicate that the growth of shoots in terms of elongation and size of 'Harumanis' trees was increased when planted under rain-shelter and greenhouse (Table 1). In this study, the enhancement of shoot growth (i.e. vegetative shoots) could be influenced by the light quality diffused through the plastic roof used for the rain-shelter and greenhouse. However, we did not measure the actual of light quality received inside for both growing conditions. According to Smith (1982), the growth of plants may be affected by the changes of the spectral distribution of the natural radiation especially Red (R) and Far-Red light (FR). Other studies also highlighted that the light quality in the growing environment can affect plant morphology by the influenced of the amount of Red and Far-Red light received by the plants (Rajapakse et al., 1993; Graham and Decoteau, 1997; Shumin et al., 2003). In our study, it is suggested that the elongation of primary shoots of 'Harumanis' mango planted under rain-shelter and greenhouse (Table 1; Figure 1) probably due to the exposure to Far-Red light, because high proportion of FR in relation to R light can increase shoot elongation (Bastías and Corelli-Grappadelli, 2012).

Destructive measurements in terms of fresh and dry weight (g) of primary shoots were conducted in order to evaluate whether differences in the growing conditions may affect the dry matter of vegetative growth. The mean fresh weight (g) of 'Harumanis' mango shoots under rain-shelter and greenhouse was significantly increased (P=0.0004) and this increment was 22% and 62% (respectively) higher compared to the open area (Table 2). Similarly, it was also found that the dry weight (g) of primary shoots was significantly greater (P=0.0002) when planted under rain-shelter and greenhouse compared to the open area (Table 2). It has been noted in this study that the increment of dry matter of shoots (Table 2) was associated with the increment of shoot length and shoot cross-sectional area of primary shoots (Table 1). Therefore, it seems that this effect was possibly due to responses of shoots to the light quality (especially FR light), similar to the study with sunflower (*Helianthus annuus*) plants reported by Graham and Decoteau (1997). Our results in Table 2 also revealing that the shoot vigour of 'Harumanis' mango trees have been affected by the growing environment.

Table 2: Shoot fresh weight and dry weight (g) of 'Harumanis' mango cultivar at fully development stage (n=30) planted under different environment condition.

	Fresh weight (g)	Dry weight (g)
Open area	4.4^{b} (20.7±2.4)*	3.5^{b} (13.6±1.6)*
Rain-shelter	4.9^{a} (25.3±2.2)	4.0^{a} (17.3±1.6)
Greenhouse	5.7^{a} (33.6±1.9)	4.7^{a} (23.1±1.4)
$LSD_{0.05}$	0.59	0.50
P-value	P=0.0004	P=0.0002

*Data were transformed means using square root transformation for analysis. Means followed by similar letter are not significantly different at P≤0.05 according to LSD test. *Means of raw data (±standard error of means).*

In our study, the modification of shoot vigour of 'Harumanis' mango (Tables 1 and 2) also indicates that other factors such as temperature and endogenous hormones may also involve in regulating vegetative growth when 'Harumanis' mango trees were planted under different growing environment. It was well documented that temperature had a direct effect on the vegetative growth of mango (Whiley et al., 1989; Whiley, 1992; Dambreville et al., 2013; Makhmale et al., 2016). Previous study under controlled environment, Whiley et al. (1992) reported that there was a positive correlation between vegetative growth and temperature of mango, as they found shoot growth and dry matter production were increased with increased temperature. Under non-limited resources (i.e. water and nutrients), it was also found that the growth rhythm of mango shoots was increased more rapidly with increasing temperature (Dambreville et al., 2013). Therefore, it can be suggested that the temperature may had a strong effect on vegetative growth of 'Harumanis' mango, as we found that the vigour of shoots was significantly affected by the growing environment (Tables 1 and 2; Figure 1).

It was also noted that the shoot growth of mango is also regulated by the endogenous hormones such as GA. Study on two different mango cultivars, 'Keitt' and 'Tommy Atkins' found that rapid shoot growth is associated with the synthesis of GA (Davenport et al., 2001) and really responsive when the mango

shoots were exposed to warm temperature (Whiley et al., 1989). Therefore, highly vigorous of shoots (i.e. vegetative growth) of 'Harumanis' trees, especially for those trees planted under the greenhouse condition (Tables 1 and 2; Figure 1), was possibly due to the interaction between light quality and temperature that may regulate endogenous hormones (e.g. GA). In the current situation of climate change, it is believed that the vegetative growth, phenological patterns and reproductive processes are likely to be affected by the changes of temperature. These effects may directly influence the growth, subsequently quality and quantity of production in mango (Bastías and Corelli-Grappadelli, 2012; Makhmale et al., 2016). Study on other mango cultivars reported that the growth of 'Keitt' mango trees planted in the greenhouse was significantly taller compared with the trees in the open area (Medany et al., 2009), indicating that the shoot growth in the greenhouse tends to be vigorous compared to the trees planted in the open area, similar to what have been found in our study (Tables 1 and 2). In other fruit tree such as sweet cherry (*Prunus avium* L.), it was also found that the trees planted in the greenhouse had a longer shoot growth and greater total leaf area with larger trunk cross-sectional area (Lang et al., 2011). Therefore, in order to increase our physiological understanding on this aspect, more information on the mechanisms of vigour manipulation of vegetative growth of 'Harumanis' trees under greenhouse are needed.

Conclusion

Our results indicate that the vegetative growth of 'Harumanis' mango under greenhouse and/or rain-shelter are highly vigorous compared with the open area, possibly due to light quality and temperature, or interaction between these factors that may regulate endogenous hormones. Further study is needed to evaluate the effect of light quality and temperature, as well as endogenous hormonal mechanisms on the overall growth, flowering, and quality as well as yield components of 'Harumanis' mango under these new growing conditions. It is believed that the growing condition (i.e. under greenhouse or rain-shelter) may have altered the shoot vigour and possibly overall tree architecture of 'Harumanis' mango.

Acknowledgements

The authors wish to express his appreciation to Tn. Hj. Ahmad Mahdzir Ahmad (Sintok Field Manager), Tn. Hj. Abdul Aziz Abu Seman, Mr. Hamdan Zainon, Mr. Ros Shahidan Abd Aziz, Mr. Hassan Abu and Mr. Burhan Taib of MARDI Sintok for their technical assistance. The project was funded by MARDI Research and Development Project (No: P-RH089).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Vigour and Morphological Characteristics of Mango Seedling Rootstock cv. 'Telor'

Abdullah, F.[1,*] and Tengku Maamun, T.A.M.[2]

[1]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia.
[2]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarters, P.O. Box 12301, General Post Office, 50774 Kuala Lumpur, Malaysia.
**E-mail: fadhil@mardi.gov.my*

Introduction

In tropical fruits such as mango, the physiological understanding of rootstock effect on grafted scion is not fully understood. Grafting onto seedling rootstocks is a common practice in propagating popular tropical mango cultivars such as 'Chok Anan' and 'Harumanis' (Tengku Maamun et al., 2000b). However, it should be noted that the use of seedlings as rootstocks might lead to the variation of scion growth and vigour. Mango seeds are either poly-embryonic (multiple seedlings) or mono-embryonic (single seedling) depending on the cultivars (Litz, 1997). In poly-embryonic, the seeds are characterised by the development of more than one embryo in the same seed, which can be nucellar or zygotic (Ochoa et al., 2012). However, it is relatively unknown whether germinated seedlings from a single mango seed can be either zygotic or nucellar in origin, because less studies in Malaysia have described the effect of nucellar or zygotic seedlings when used as rootstocks. According to Litz (1997), zygotic seedlings are sexual, whereas nucellar seedlings come from the maternal tissue, which are asexual seedlings. Generally, nucellar seedlings are preferred for grafting, because they maintain the same genetic background of the rootstock mother plant, which may present disease resistance or dwarfness. Moreover, nucellar seedlings used as rootstock may keep adequate orchard homogeneity (Degani et al., 1993). From observation, it is very common to find out vigour and yield dis-uniformities on mango trees in orchards, especially in Northern region of Malaysia (i.e. Perlis and Kedah). In order to gain as much as information about the mango rootstock, therefore, a study was conducted to examine the initial vigour and morphological characteristics of the tropical mango namely 'Telor' cultivar that commonly being used as standard seedling rootstocks.

Materials and Methods

Germination of mango seedling rootstock was conducted in the nursery, during the fruiting season of 2008-2009 at Malaysian Agricultural Research and Development Institute (MARDI) Sintok Research Station, Kedah. A total of 300 seeds of mango rootstock namely 'Telor' cultivar were collected from 4 to 6 years-old trees in Perlis state and Multi-Purpose Plot at MARDI Sintok Research Station. The seeds were germinated according to the standard nursery practices (Anonymous, 1995) following the method described by Zakaria et al. (2002). The skin and the flesh of ripe fruits were manually removed. The seeds were washed with clean water and then soaked with fungicide 'Antracol' (active ingredient-Propinep, 2 g/L water) to prevent fungus infection and air-dried (27-30 ºC) for one day. The seeds were sown in a sandy seedbed at 20 cm apart under 50% shade. The seeds received daily irrigation to a nursery capacity (3 L h^{-3} sprinkler per bay) and full germination was observed three weeks after sowing. The number of germinated seedlings per seed was manually counted and recorded. Mean germination (%) was calculated following the standard equation; germination percentage=no. of seeds germinated/total no. of seeds x 100%. Then, the emerged shoots were segregated manually according to the number of seedlings produced from a single seed. Thirty plants of each seedling types (n=30) were subjected to the growth measurements such as plant height (cm), stem diameter (mm) and number of leaves of each seedling were recorded at this stage for measuring the vigour of the seedlings. The seedling height was measured from the base of stem to the apex of the shoots. Stem diameter of the seedlings was measured at 5 cm above the root collar using digital calliper and number of leaves was

manually counted. The photograph of each type of seedlings was taken one month after full germination was achieved. Results on germination type, seedling number produced, height of seedling, stem diameter and number of leaves are presented in Table 1.

Results and Discussion

In this study, the seeds of mango rootstock cv. 'Telor' (*n*=300) can produce higher germination rate (%) when sown properly in the sandy seedbed (data not shown). The rate of germination of mango seedling rootstock cv. 'Telor' was ranged between 91-95% with mean of 93.34%. We believe this could be the reason why this cultivar had been selected as a rootstock due to availability and higher germination rate. However, approximately 5 to 9% (mean of 6.7%) of the seeds did not germinate, possibly due to immature seeds. According to Mitra and Bose (1986), the germination rate of mango seedling was higher when the seeds were selected from the fully ripening fruits. It should be noted that other factors may also influence the germination of mango seeds such as hormonal status (i.e. gibberellins), cellular activity of the seeds (Rajjou et al., 2012) and temperature (Corbineau et al., 1986). Figure 1 shows the proportion of emerged seedlings (%) from a single seed of mango 'Telor' rootstock. The highest percentage (40.1%) was recorded on the single shoot per seed (Figure 2A) and the lowest percentage was recorded on the seed that produced five shoots per seed with less than 2% (Figure 2E).

However, the proportion of multiple seedlings per seed contributed the highest percentage of germination (60%) compared to single seedling per seed (40%) (Figure 1). Similar germination pattern was also observed on other tropical mango cultivars such as *M. indica* cv. Sala, Tangkai Panjang, Chok Anan and Harumanis (Abdullah and Tengku Maamun, 2006; Zakaria et al., 2002) as well as other tropical *Mangifera* species such as *M. feotida* (Bacang) and *M. odorata* (Kuini) (Zakaria et al., 2002). It is therefore possible that mango 'Telor' rootstock could be classified as polyembryonic due to the seeds of this cultivar producing multiple shoots, which is an agreement with the study by Litz (1997) and Zakaria et al. (2002) found that most of the tropical mango could be classified as polyembryonic.

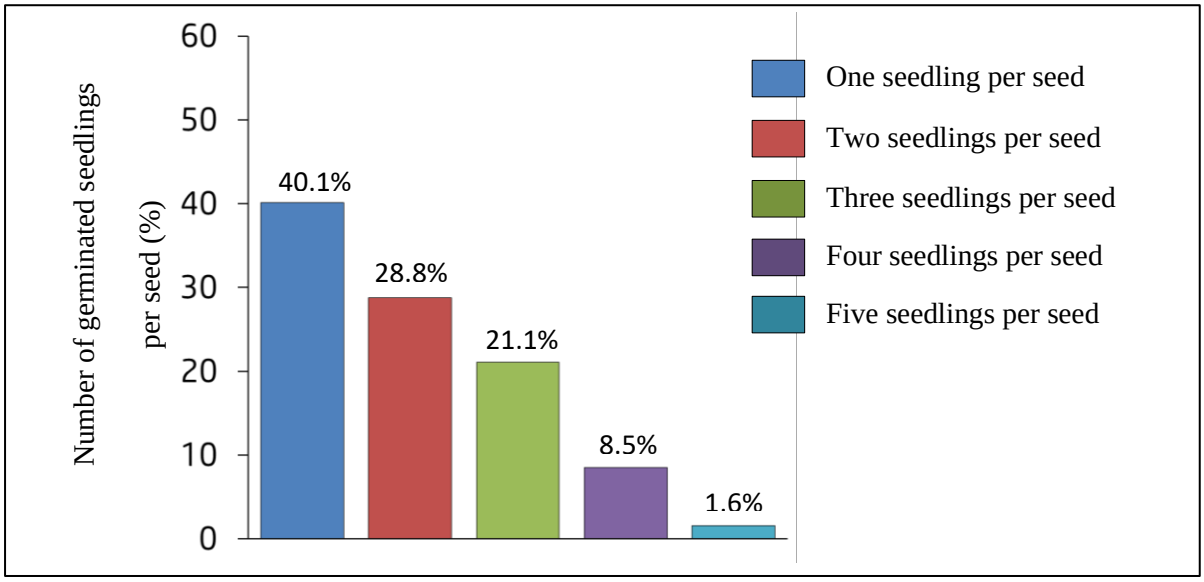

Figure 1: The proportion of seedlings (%) produced from one single seed of mango rootstock cv. 'Telor' (*n*=300).

According to Zakaria et al. (2002) only 2 to 3 seedlings per seed were produced from mango rootstock cv. Telor. However, our results showed that mango 'Telor' rootstock produced up to five seedlings per seed (Figures 1 and 2A-E). Litz (1997) reported that the seeds from polyembryonic cultivars produced

three to eight seedlings from a single seed. Even so, the number of seedlings of polyembryonic seeds may vary with species and cultivar, and could be influenced by the surrounding climate (Ram and Rajan 2003). Our result in this study supports the findings of Morton (1987) and Litz (1997) that most of the tropical mango cultivars are classified as polyembryonic which enable seeds to produce more than one seedling.

Variable in shoot size was found from the germinated seedlings of mango rootstock cv. 'Telor' (Figure 2 and Table 1). The growth of a single seedling (Figure 2A) germinated more rapidly compared to those seeds that produced multiple seedlings (Figures 2B, C, D, and E). Our results are consistent with Zakaria et al. (2002) on similar cultivar. It was notable that most of the first germinated seedling produced greater plant height, stem diameter and number of leaves compared to other seedlings (Table 1). These results indicated that the first germinated seedlings of mango 'Telor' rootstock might have greater vigour and plant size. Therefore, this type of seedlings may be advantageous to be selected for transplanting and grafting due to having greater growth rate. It should be noted that mango seeds can be classified into two groups, monoembryonic and polyembryonic based on their mode of reproduction (Litz, 1997). Polyembryonic seeds may contain one or more embryos, one of which usually, but not always zygotic. In contrast, monoembryonic seed contain a single zygotic embryo, and hence only one seedling per seed, that is of probable hybrid origin. It is important to note that mango 'Telor' was selected as a rootstock for tropical mango due to this type of rootstock induced the highest Calcium (Ca) and Kalium (K) uptake compared with other cultivars (Tengku Maamun, 2000a).

Figure 2: Morphological characteristics of mango rootstock seedlings cv. 'Telor' approximately one month after full germination stage; (A) One seedling per seed, (B) Two seedlings per seed, (C) Three seedlings per seed, (D) Four seedlings per seed and (E) Five seedlings per seed.

In this study, the classification whether the seedlings produced from this mango rootstock is nucellar or zygotic in origin will not be further discussed. In mango, polyembryonic cultivars are preferred as seedling rootstocks (Degani et al., 1993), however grafting of potential scions onto seedlings rootstocks showed some variation the orchards in terms of plant canopy and yield. For the solution, mango rootstock could be propagated through vegetative propagation to produce clonal rootstock on mango (Mitra et al., 1986; Abdullah et al., 2016). Additionally, identification of nucellar seedlings of mango rootstock is very important in order to facilitate the production of high quality planting materials. According to Schnell et al. (1994), early elimination of zygotic seedlings from seedbeds by using 'visual roguing' technique could avoid some variation of the seedlings, but we found this technique is to be labour intensive and time consuming.

Table 1: Height of seedling, stem diameter and number of leaves of mango seedling rootstock cv. 'Telor' at full germination stage (Means).

Germination type (Seedling per seed)	Seedling number produced per seedling[ǂ]	Height of seedling (cm)*	Stem diameter (mm)*	Number of leaves*	
				Range	Mean
Single seedling per seed	Single seedling	20.2 (± 3.3)	0.31 (± 0.1)	3-5	4.2 (± 0.9)
Two seedlings per seed	First seedling	20.7 (± 4.4)	0.33 (± 0.1)	4-5	4.0 (± 0.0)
	Second seedling	13.8 (± 4.4)	0.24 (± 0.1)	2-4	3.4 (± 0.9)
Three seedlings per seed	First seedling	18.0 (± 3.5)	0.27 (± 0.1)	3-5	4.0 (± 0.7)
	Second seedling	14.1 (± 4.1)	0.25 (± 0.1)	2-5	3.2 (± 1.6)
	Third seedling	9.6 (± 5.3)	0.13 (± 0.1)	2-3	2.0 (± 0.7)
Four seedlings per seed	First seedling	19.1 (± 5.5)	0.31 (± 0.1)	3-5	3.8 (± 0.8)
	Second seedling	13.7 (± 3.2)	0.27 (± 0.1)	2-4	3.4 (± 0.9)
	Third seedling	11.8 (± 3.9)	0.18 (± 0.1)	2-3	2.2 (± 0.4)
	Fourth seedling	7.3 (± 3.6)	0.14 (± 0.1)	2-3	2.4 (± 0.5)
Five seedlings per seed	First seedling	20.0 (± 3.2)	0.29 (± 0.1)	2-4	3.0 (± 1.0)
	Second seedling	15.0 (± 2.1)	0.23 (± 0.1)	2-4	3.4 (± 0.5)
	Third seedling	12.2 (± 1.8)	0.19 (± 0.1)	2-4	3.4 (± 0.9)
	Fourth seedling	9.0 (± 1.0)	0.25 (± 0.2)	1-4	2.6 (± 1.1)
	Fifth seedling	6.2 (± 1.4)	0.09 (± 0.1)	1-3	1.6 (± 0.9)

**Mean of 30 seedlings per germination type (n=30).*
Numbers in parentheses are standard error of means (±).
[ǂ]*Shoot number was sorted (in ascending order) according to the first until the last germinated seedlings (refer Figure 2).*

Conclusions

From this observation, mango rootstock cv. 'Telor' had a high percentage of germination rate and can easily be propagated from seedlings. In addition, this type of mango cultivar can be classified as polyembryonic, due to its characteristic of producing more than one seedling per seed. Each seedling of this type of rootstock showed a different growth pattern at the germination stage that contributed to the difference in the vigour of the seedlings at the nursery stage.

Acknowledgement

The authors express his appreciation to staff of the Horticulture Research Centre, MARDI for their technical assistance. The project was sponsored by MARDI Short Grant Project (Research Grant No. J-JH017).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Photosynthesis, Transpiration and Water Use Efficiency of Selected Mangrove Species at Larut Matang Mangrove Forest, Malaysia

Marryanna, L.*, Mohd Ghazali, H., Azian, M., Tariq Mubarak, H. and Hafizi, M.J.
Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.
**E-mail: marryanna@frim.gov.my*

Introduction

Photosynthesis (*A*) and transpiration (*E*) are the processes that control the water use efficiency (WUE) in plants. The rate of carbon dioxide (CO_2) assimilation to transpiration is termed as water use efficiency. According to UNEP (1994), the efficiency of mangrove water use will be enhanced, and there will be specific species variation in response to elevated CO_2. Due to the increase in WUE, mangroves may benefit because decreased water loss *via* transpiration will accompany CO_2 uptake (Ball and Munns, 1992). However, increases in CO_2 do not necessarily affect mangrove growth when salinity is too high for a species to maintain water uptake (UNEP, 1994).

Mangroves are a group of highly salt-tolerant woody plants that provide to a wide range of ecological services that protect the coast from erosion, buffer adjacent marine ecosystems (often coral reefs) from terrestrial inputs and are nursery grounds for important commercial fish species and habitats for migratory birds. The high water use efficiency of mangroves under saline conditions suggests that the regulation of water transport is a crucial component of their salinity tolerance (Ruth and Catherine, 2015). Since water acquisition is more energetically costly in saline than in non-saline soils, mangroves have evolved a range of adaptations that facilitate efficient water use during photosynthetic carbon gain during the day and reduce losses of water to saline soils at night.

Adaptations of mangrove species influence water uptake, transport and loss while maintaining photosynthetic carbon gain. The mangrove adaptation is important for salinity tolerance. Mangrove species is not only plays some important roles in saline water filtration but also supporting the water cycle through transpiration process. The ratio of carbon assimilation and water loss during the transpiration process reflect how much water have been recycled by the forest stand. Based on its important conservation features, we need to study the response of mangrove species WUE due to transpiration and photosynthetic activities for different species.

There is not much ecophysiology baseline data available for a mangrove forest in Malaysia. We need baseline data to clarify various aspect related to the mangrove forest. Therefore, the objective of this study is to measure the value of photosynthesis, transpiration and WUE rates for selected mangrove species at Larut Matang Mangrove Forest as baseline data for conservation. Ecophysiology study of this unique species would be beneficial to understand the physiological and hydrological aspect of mangrove species for research advancement.

Materials and Methods

Description of the study area

The Matang Mangrove Forest is located at latitude 4 ^{o}N – 5 ^{o}N and longitude 100^{o}2’E - 45’E. It covers of 40,466 ha of well manage forest areas comprises of three forest range; Kuala Sepetang Forest Range Forest Range, Kuala Trong Forest Range and Sg Kerang Forest Range. The *Rhizophora* forest is the major forest type in Matang Mangroves (85% of the total forested area), comprises predominantly of *R. apiculata* and *R. mucronata.*

Mangrove species selection

Two species were selected in this study; *Rhizophora apiculata* (Bakau minyak) and *Rhizophora mucronata* (Bakau kurap). The *Rhizophora* is a family from tropical and sub-tropical group. It has 16 genera with 120 species which consists of woody plant or shrubs. *Rhizophora* is a dominant genus of the most widespread mangrove family, the Rhizophoraceae. All *Rhizophora* taxa are characterized by large water-buoyant propagules with a remarkable ability for long-distance dispersal (Rabinowitz, 1978). Studies revealed that *Rhizophora apiculata* was effective in reducing both water depth and current velocity (Tanaka et al., 2007). Usually, the *R. apiculata* found in muddy firm substrates in mangrove areas while *R. mucronata* normally present along both sides of rivers and alongside streams and creeks in mangrove areas suggesting that *R. mucronata* needs more fresh water input as compared to *R. apiculata* (Nasir et al., 2016).

Leaf gas-exchange measurement

Measurements were carried out in the field using LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA) connected to a standard 6 cm^2 cuvette. Fully expanded, sunlight leaves were clamped in the sensor cuvette, maintaining their natural position. The leaves were flushed with ambient air (flow rate 500 mol $m^{-2}s^{-1}$), of which temperature and relative humidity were simultaneously recorded. Gas-exchange measurements, including CO_2 fixation rate (*A*), stomatal conductance to water vapour (*gs*), and transpiration rate (E), were logged after readings reached stable (1-3 min). Infra-red gas analyser was matched to reach equilibrium before every measurement. Measurements were conducted with ambient temperature, while CO_2 reference concentration was maintained at 400 µmol mol^{-1}. The photosynthetic photon flux density was set at 1600 µmol $m^{-2}s^{-1}$ to ensure that light-saturated photosynthetic rates were reached. The data were obtained from six plants of *R. apiculata* and *R. mucronata*, randomly selected within the forests. All measurements were performed in the morning (between 9:00 and 12:00 h) and afternoon (between 02:00 and 04:00 h). Regression analysis were done to determine the relationship between the morning and afternoon observations of the plants.

Results and Discussion

Photosynthesis, transpiration, and water use efficiency rates of R. mucronata and R. apiculata in the morning and afternoon observations

R. apiculata shows to have low average photosynthetic (*A*) rates during morning observation compared to *R. mucronata* (Figure 1a). The observation found that the rates of *A* in *R. apiculata* were ranges between 0.89 µmol CO_2 m^2 m^1 and 25.45 µmol CO_2 m^2 m^1 in the morning and between 0.79 µmol CO_2 m^2 m^1 and 33.11 µmol CO_2 m^2 m^1 in the afternoon with the average of 9.41 µmol CO_2 m^2 m^1 (SD±7.45) in the morning and 13.07 µmol CO_2 m^2 m^1 (SD±9.48) in the afternoon. However, the transpiration (*E*) rates were high at 0.83 mmol H_2O m^2 m^1 (SD±0.37) in the morning and 0.63 mmol H_2O m^2 m^1 (SD±0.26) in the afternoon (Figure 1b). The average ratio of assimilation rates to transpiration for *R. apiculata* for both observations was 12.82 µmol CO_2 $mmol^{-1}$ H_2O (SD±10.87) in the morning and 23.38 µmol CO_2 $mmol^{-1}$ H_2O (SD±19.28) in the afternoon (Figure 1c). In the *R. mucronata*, observation found that the rate of *A* is ranges between 0.37 µmol CO_2 m^2 m^1 to 30.26 µmol CO_2 m^2 m^1 with an average of 13.01 µmol CO_2 m^2 m^1 (SD±9.69) in the morning and slightly decrease to an average of 12.19 µmol CO_2 m^2 m^1 (SD±8.68) in the afternoon. The average rates of *E* were 0.69 mmol H_2O m^2 m^1 (SD±0.42) in the morning and 0.50 mmol H_2O m^2 m^1 (SD±0.23) in the afternoon. The average ratio of assimilation rates to transpiration in *R. mucronata* was 28.41 µmol CO_2 $mmol^{-1}$ H_2O (SD±28.86) in the morning and 29.84 µmol CO_2 $mmol^{-1}$ H_2O (SD±23.84) in the afternoon. Observation on the water use efficiency for both species shows that there is large variation between the photosynthetic and transpiration rates and thus the standard deviation. Overall, both species in the study area exhibit higher photosynthetic rate in the afternoon however, the trend in transpiration varied between morning and afternoon observation for

both species. *R. mucronata* has a higher average rate of photosynthetic and water use efficiency compared to *R. apiculata*.

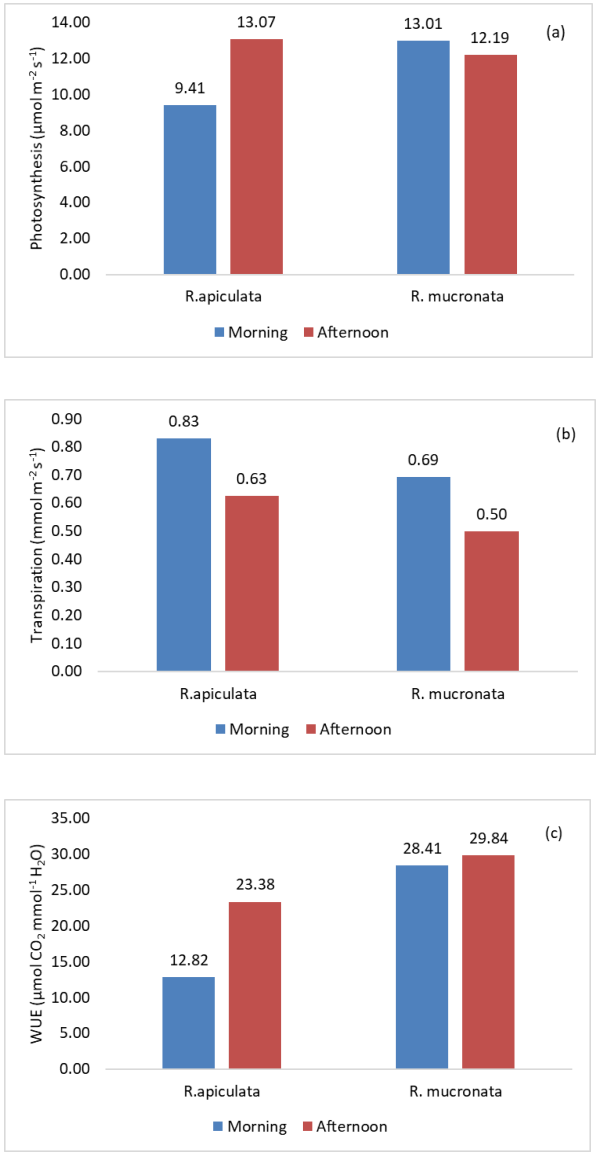

Figure 1: The rates of photosynthesis (a), transpiration (b) and water use efficiency, WUE (c) of the *Rhizophora apiculata* and *Rhizophora mucronata* in the morning and afternoon observations.

The relationship between transpiration and water use efficiency in R. apiculata and R. mucronata.

The *E* and WUE of the *R. apiculata* are negatively correlated (Figures 2a, b) however, the relationship was very weak at $R^2 = 0.09$ (morning) and $R^2 = 0.10$ (afternoon). The correlation between *E* and WUE in *R. mucronata* was slightly better compared to *R. apiculata* at $R^2 = 0.32$ (morning) and $R^2 = 0.28$ (afternoon) (Figures 3a, b). In general, increasing in *E* cause decreasing in WUE. The intercellular activities during the photosynthesis affect the WUE value. A lower intercellular CO_2 concentration during the photosynthesis will correspond to a higher WUE (Cernusak, 2018). This means that the WUE depends on the extent of drawdown in the CO_2 concentration from the atmosphere to leaf interior and also the dynamism of photosynthesis and transpiration rates in the leaf. The fluctuation in transpiration and WUE was also influenced by several factors such as stomatal conductance, stomatal density, and the vapour pressure deficit.

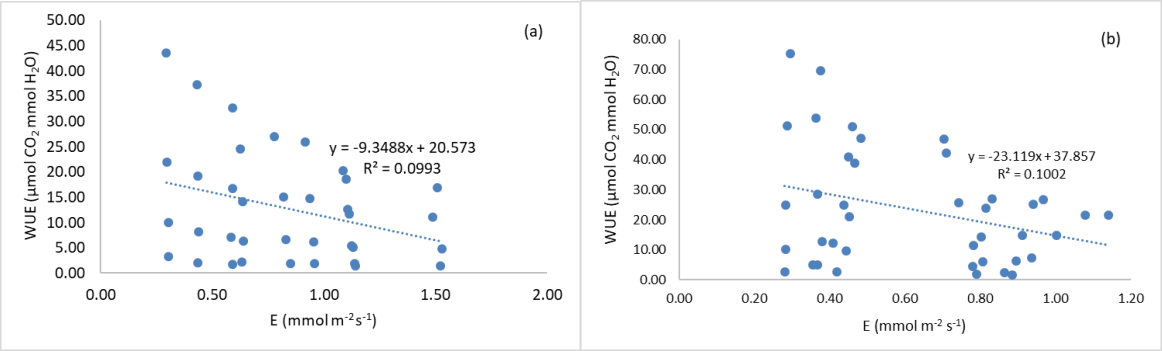

Figure 2: The relationship between transpiration (E) and water use efficiency (WUE) in *R. apiculata* during morning (a) and afternoon (b) observations at Matang Mangrove Forest.

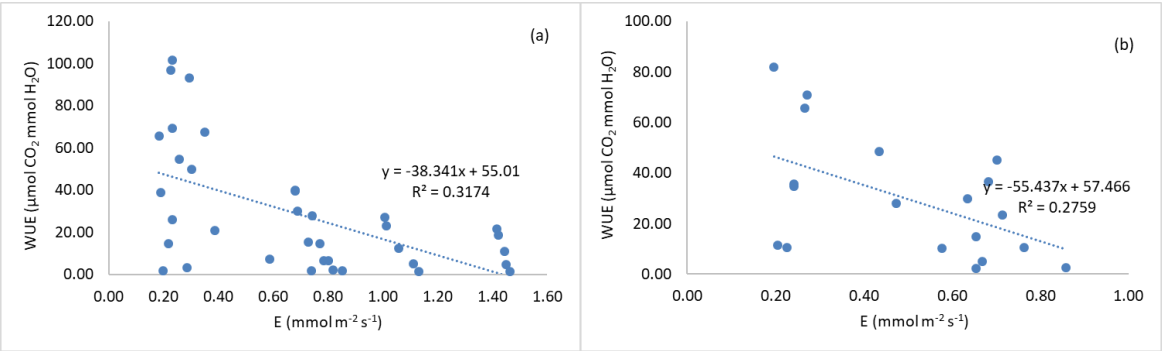

Figure 3: The correlation between transpiration (E) and water use efficiency (WUE) in *R. mucronata* during morning (a) and afternoon (b) observations at Matang Mangrove Forest.

Conclusion

Generally, the photosynthesis of *R. apiculata* is much lower in the morning compared to the afternoon. The photosynthesis in *R. mucronata* was much stable in the morning and afternoon. *R. apiculata* was having slightly higher transpiration rates compared *to R. mucronata* in the morning and afternoon. In general, the large variations between photosynthesis and transpiration rates in mangrove species influence the fluctuation in WUE rates. This data is of importance to understand the physiology processes in mangrove forest which have not been well explored in Malaysia. The present findings were based on the short-term observation, therefore, it is suggested a similar study to be conducted in longer-term and includes more variables to understand mangrove ecosystem and its roles in assessing and monitoring of climate change impact.

Acknowledgements

The authors wish to thank Forestry Department Peninsular Malaysia (FDPM) and Forest Research Institute Malaysia (FRIM) for funding and administration of the project.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Haze on Fruit Development, Pigmentation and Productivity of *Passiflora quadrangularis* L. (Giant Granadilla Passion Fruit)

Shahbani, N.S.[1], Ismail, H.A.[1], Ramaiya, S.D.[1,2,*], Saupi, N.[1,2], Zakaria, M.H.[3] and Awang, M.A.[4]

[1]Department of Crop Science, Faculty of Agriculture Science and Forestry, Universiti Putra Malaysia Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia.
[2]Institut Ekosains Borneo, Universiti Putra Malaysia Bintulu Campus, 97008 Sarawak, Malaysia.
[3]Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[4]Ganofarm R&D Sdn. Bhd., Sky-Pod Square, Persiaran Puchong Jaya Selatan, Bandar Puchong Jaya, 47100 Puchong, Selangor, Malaysia.
**E-mail: shiamala@upm.edu.my*

Introduction

The world today faces air pollution as a major environmental problem, as industrialisation and anthropogenic activity are growing rapidly. Agricultural fires in Indonesia and Borneo, along with forest and peatland fires, are responsible for transboundary haze that contributes to environmental degradation in Malaysia (Aziz et al., 2018). Haze occurrence in Malaysia has become a common feature over the last two decades. The most extreme haze phenomena were observed in April 1983, August 1990, June 1991, August 1994, and March 1998 (Jamal et al., 2014). Kuching's API reading in September 1997, spiked to 893 was the highest ever recorded in Malaysia (Ahmad et al., 2006). Most of these haze episodes occurred in conjunction with a period of prolonged drought associated with the El-Nino phenomenon. Haze events are projected to increase as forest and peat fields burning increases due to global warming and prolonged drought worsen (Hawa, 2008).

Haze is generally considered to be a product of high concentrations of fine particulate matter circulating in the atmosphere. As the numbers of these particles increase, their cumulative effect causes lower light intensity on Earth and results in reduced visibility (Philip, 2001). Haze development interrupts the natural air circulation, which decreases the dispersion and dilution of suspended contaminants and particles. Haze is often caused by an excessive amount of pollutants, i.e., particulate matter, sulphur dioxide (SO_2), carbon monoxide (CO), nitrogen dioxide (NO_2) and ozone (O_3). Increased aerosol loadings in the atmosphere are aided by burned biomass. Greenwald et al. (2006) showed that atmospheric haze resulting from distant forest fires reduced solar radiation at almost all spectra. About 73-92% of overall light extinction comes from organic carbon and sulfate particles trapped in haze (Yanhong et al., 1996). The field data collected in the Yangtze Delta region of China shows that aerosols reduce solar radiation by about 30% on clear days (Xu et al., 2003).

Studies have shown that haze has major effects on various ecosystems as its impact on solar radiation, temperature, and relative humidity resulting in reduced plant photosynthetic activity (Yanhong et al., 1996; Davies and Unam, 1999; Aziz et al., 2018). The photosynthetically active radiation (PAR), a solar radiation utilised by plants and ambient temperature, was reduced by the haze, which indicates a reduction in photosynthesis rate and stomatal conductance. Based on the experimental measurement of the light-CO_2 relationship, Fan et al. (1990) reported an increase in cloudiness might decrease net CO_2 uptake by the plants. Studies by Aziz et al. (2018) on the yield of Malaysian rice varieties during the haze event in March 2014 showed a major reduction in the net photosynthetic rate and stomatal conductivity due to the reduction of PAR solar radiation. The PAR level at which a plant is exposed is related positively to the photosynthetic and has a significant effect on plant development and growth. Lately, the occurrence of haze over the country has been a great concern. Understanding the plant behaviours and responses is very important to explain their physiological patterns and tolerance when exposed to haze conditions. Therefore, this study was carried out to determine the effects of haze on the

fruit development and productivity of *Passiflora quadrangularis* during the haze event in July to September 2019 at Bintulu due to forest fires in Indonesia and Borneo.

Materials and Methods

Study location and plant cultivation

The present study was conducted at a passion fruit farm, Universiti Putra Malaysia Bintulu (N 03° 12.45' and E 113° 4.68'), Sarawak. *P. quadrangularis* seeds were obtained from a commercial provider Trade Winds fruit, Windsor, and cultivated in a vertical trellis system with 20 rows of 25 m length. The trellis system consisted of 2.0 m tall posts set at 5.0 m intervals along the rows. Furthermore, five-month-old seedlings were transplanted with a planting distance of 2.0 x 1.5 m, while plant maintenance of weeding, fertilisation, and pruning was performed.

Climate data dan air pollution index

The climate variables, i.e., monthly rainfall, mean surface temperature, mean relative humidity and mean sunshine hours for Bintulu, were obtained from the Malaysian Meteorological Department, Sarawak Branch (Kuching, Sarawak) daily from January 2019 to March 2020. During the study period, the average annual temperature was 26.6-27.8 °C, while the average rainfall received was 268-619 mm (Figure 1). The air quality is reported as the Air Pollution Index (API).

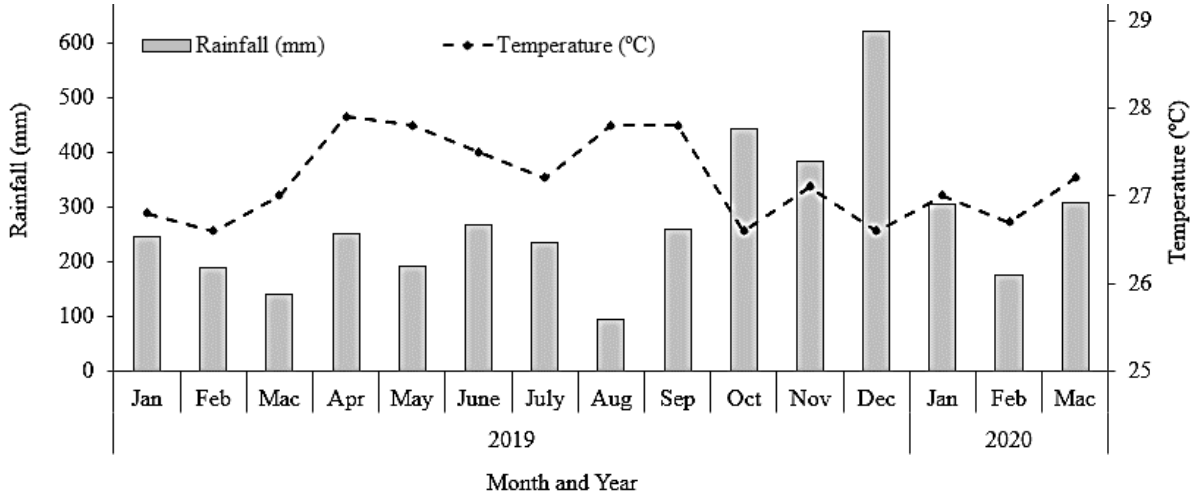

Figure 1: Meteorological data for monthly rainfall and temperature at Bintulu, January 2019 to March 2020 were obtained from the Malaysian Meteorological Department, Sarawak Branch (Kuching, Sarawak).

The Malaysian government uses API for health classification (Table 1). During the planting period of *P. quadrangularis*, the incident of haze occurred from July to September 2019. In the early haze phenomena (July 2019) the API values ranged from 43 (good API) to 74 (moderate API). However, this value increased to 52-81 (moderate API) in August 2019, coinciding with the first flowering season for *P. quadrangularis*.

Table 1: Air pollution index (API) for health classification from the Department of Environment.

API	Air pollution level
0-50	Good
51-100	Moderate
101-200	Unhealthy
201-300	Very unhealthy
>301	Hazardous

The API values continuously increased to a high of 110-146 API (unhealthy) prolong for 14 days in September 2019 was concurrent with the major blooming of this species and fruiting. However, this value decreased in October 2019 to 34-55 API (good to moderate API), which coincided with fruit development and ripening. Simultaneously, August to September 2019 was the driest months with temperature and rainfall ranging from 94.8-158.0 mm and 26.6-27.8 °C.

Plant development and production

Observations were carried out for a year starting June 2019 to March 2020. Vegetative shoots were tagged and observed for their developmental changes up to the fruiting stage. The phenological records were correlated with metrological data. The plant development phase recorded in the present study based on an extended Biologische Bundesanstalt, Bundessortenamt and CHemical Industry (BBCH) scale (Growth stages of plants, BBCH Monograph). The observations considered the occurrence, duration, and frequency of the vegetative growth, flowering, and fruiting. Data on flowering phenology were obtained to verify the blooming pattern. The major and minor blooms were worked out based on the number of flowers opened every day. Fruiting was also defined as the presence of young and ripe fruits. Detailed observations were also made on fruit development and fruit productivity.

Statistical analysis

The data recorded for fruit development and production were statistically analysed using SAS Window Programme 9.4. Independent t-test was used to detect significant differences among the mean comparison between the two treatments.

Results and Discussion

Phenological event of P. quadrangularis

The non-native *P. quadrangularis* well adapted to the local tropical climate. Based on the observation, the *P. quadrangularis* plant managed to survive and initiated good flowers and fruits during haze period. The success of this plant is reflected through its continuous flowers' initiation and fruit production. *Passiflora qudrangularis* seedlings have been transplanted into the field in June 2019 and continuously monitoring their plant development. The phenological activity that has been observed in this study was based on BBCH scale, and the data were presented in Table 2. Based on the continuous observation from January 2019 to March 2020, the plants continuously produced flowers and fruits throughout the year. It is contradicted to the finding by Ramaiya et al. (2016), where *P. quadrangularis* only possessed two peaks with a major peak in September to November 2010-2012 and a minor peak from February to March 2011-2012 at Bintulu (same study location).

Table 2: Phenological events of *Passiflora quadrangularis* from January 2019 to March 2020.

Months	2019												2020		
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3
Vegetative growth					■										
Flower blooming									○	○	○	○	○		
Fruiting and harvesting												♦	♦		

Note			
	Vegetative growth	○	Major flower blooming
■	Transplanting at field		Fruiting
	Flower blooming	♦	Major harvesting

The management practices for common passion fruit (*P. edulis*) are slightly different from the studied species as *P. quadrangularis* needs more energy and nutrients to produce heavier and bigger fruits than the other species of Passifloraceae family. It is synchronising with its phenological pattern in its origin country of South America (Ulmer and MacDougal, 2004). However, in Florida, this plant begins to flower in spring, and its fruits mature in summer. Recent research by Esashika et al. (2018) showed that this species does not produce fruits from August to November. During the first blooming of *P. quadrangularis* (July to September), the haze events occurred and completely ended during the first fruiting cycle (October to December). The haze incidents did not affect the blooming pattern of *P. quadrangularis* as more flower buds were observed during the haze phenomenon (July to September 2019). However, the success rate of fruit development was 68% (October to December) than during the second cycle of fruiting in January to March (85%).

Plant development of P. quadrangularis

The plant exhibited vegetative growth throughout the year, and the most vigorous growth was recorded during the wet seasons from November to December 2019. The haze level was mostly moderate from July to August 2019, with API values between 52 to 81 (Figure 2). Meanwhile, in September 2019, the haze condition turned to be heavy, with the maximum API value attained was 146 and prolonged unhealthy condition for two weeks in the month.

2020																														
Mar	45	43	43	38	39	38	40	52	52	53	52	56	49	55	37	43	51	56	58	58	49	44	52	46	38	54	54	38	33	49	50
Feb	42	47	54	50	44	43	43	48	54	51	42	45	54	57	54	55	41	28	44	45	47	48	52	57	68	62	42	44	37		
Jan	-	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	37	-	-	-	-	-	-	-	44	34	32	30	27
2019																														
Dec	41	47	27	21	27	32	27	39	37	32	35	39	32	43	39	33	36	43	36	36	36	36	36	36	36	36	36	36	36	36	-
Nov	54	54	56	57	51	51	54	56	61	54	51	55	56	59	61	60	56	53	47	46	39	52	32	41	31	33	46	48	45		
Oct	47	34	35	50	43	47	53	27	30	47	52	52	55	54	49	54	54	44	47	47	37	43	49	53	49	42	52	40	32	37	50
Sep	65	58	61	58	69	78	80	86	90	89	72	82	72	108	110	145	146	126	127	144	115	138	120	138	112	63	57	50	47	33	
Aug	56	70	71	73	67	65	76	77	64	66	74	74	81	81	72	66	59	59	58	56	54	52	59	65	67	64	57	53	52	52	60
Jul	57	57	54	66	56	59	62	62	63	64	56	60	43	56	58	62	65	66	69	74	74	64	57	68	59	59	44	45	54	56	53
Jun																													8	58	

Air Pollution Index (API)	Air Quality Category	Color Code
0 – 50	Good	
51 – 100	Moderate	
101 – 200	Unhealthy	
201 – 300	Very Unhealthy	
>300	Hazardous	

Figure 2: Daily data on the Air Pollution Index (API) in Bintulu, Sarawak, June 2019 to March 2020 was obtained from the Malaysian Meteorological Department, Sarawak Branch (Kuching, Sarawak).

In October 2019, the API was recovered and get back to health conditions. During the heavy haze events in September 2019, the successfully pollinated flowers resulted in an irregular fruit shape considered to be "dumbbell" compared with the usual fruit shape (oblong-ovoid) (Figure 3). The abnormality could be haze pollutants and concurrent with the prolonged driest season (mean temperature 27.8 °C and rainfall 94.8 mm) during the flowering. However, this abnormality was not recorded during the following fruiting cycles from January 2020 onward till the present. Knight and Sauls (1994) from South Florida observed a similar abnormality and claimed that it was attributed to high temperature, which interfered with regular fertilisation after pollination. Joy (2010) has explained the species required a mild temperature (23-28 °C) for normal fruiting, and when the temperature falls ~33 °C, the plant bore abnormal fruit shape. The shape of the fruit can be determined by temperature during ovarian development, and it is an important quantitative attribute closely to the quality of the fruit (Wien and Stutzel, 2020). Commonly, the ripened fruits of *P. quadranguaris* fruits were yellowish-green. Unlike other passion fruit, this species does not produce pigmentation of purple, yellow, or orange upon ripening. However, the fruits that were produced during these haze events induced purple pigmentation on their exocarp. The purple pigmentation is probably due to anthocyanin content development in plants (Zhao et al., 2014). Anthocyanins are a group of water-soluble pigments in a large variety of plant tissues that develop reddish, bluish, and purple shades (Schrader et al., 2006). It also has critical biological functions as protective compounds against abiotic stresses such as UVB radiation, cold temperatures, water stress, and plant protection against herbivores and pathogens (Goncalves et al., 2005).

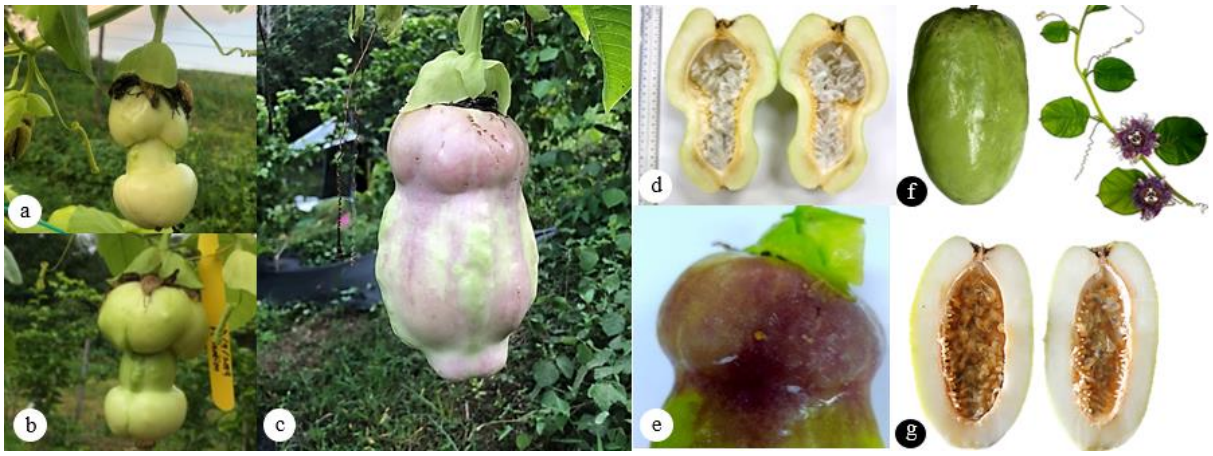

Figure 3: Fruit development of *P. quadrangularis.* (a)-(e) Development of abnormal fruit and pigmentation on fruit exocarp produced during haze events, and (f)-(g) the normal fruit shape produced during normal days.

The subsequent production and localisation of anthocyanins in *P. quadrangularis* fruit's exocarp may allow the plant to develop resistance to environmental stresses during the haze events. During the haze period, the environmental stress leads to the disappearance of chlorophyll on the exocarp of *P. quadrangularis* fruit, with consequent emergence of the purple pigments obscured. According to Schrader et al. (2006) stress caused by the environment, particularly light and drought, mediates various regulatory responses in plants, such as enhancing reactive oxygen species (ROS) and enzymes that restore DNA damage, with effects depending on dosage and genotype.

Yield performance of P. quadrangularis

The yield performance of *P. quadrangularis* affected by haze incident is presented in Table 3. The yield of the *P. quadrangularis* also showed a 42.9% reduction (October to December 2019), which was (4871.10 kg ha^{-1}) compared to the potential yield (January-March 2020), which was 8530.80 kg ha^{-1}. Haze events occurred during the flower blooming of *P. quadrangularis* and ended at the early fruiting stage. Although a good number of fruit sets were recorded during the haze period, the fruits were

significantly smaller in size (770.0-1352.0 g) compared to the heavier fruit size (1224.0-2760.0 g) obtained during the clear days. Generally, the passion fruit species are very exigent in light conditions, only flowering with full light and producing the highest yield.

Table 3: Effect of haze on the yield of *Passiflora quadrangularis* per hectare.

Haze events (July to September)	Fruiting months	No. of fruits (n)	Yield (kg ha^{-1})	Average fruit size (g)
Effect of haze	October	819	655.20	
	November	1711	1539.90	1007.58±32.15[b]
	December	2230	2676.00	(770.0-1352.0)
	Total	4760[b]	4871.10[b]	
Normal condition	January	2338	3273.20	
	February	1804	2886.40	1908.11±43.82[b]
	March	1482	2371.20	1224.0-2760.0[a]
	Total	5624[a]	8530.80[a]	

Mean values in the same column (hazy days versus clear days) are significantly different at $p<0.05$ (Independent t-test). Values are given in means±standard deviation.

Passion fruit requires higher solar radiation, at least 60%, for leaf area growth and floral bud production (Sanchez et al., 2013). The present finding agrees with the studies by Aziz et al. (2018) by which the yield of Malaysian rice varieties reduced 10-19% compared to their potential yield due to moderate haze. The authors have claimed the reduction of yield was mainly due to the lower solar radiation/PAR that resulted in a reduction of photosynthetic rate throughout the haze phenomena.

Conclusions

The finding showed that plants exposed to haze have a significant impact on growth and development. A longer duration of severe haze level at flowering, fruiting, and ripening stages will potentially have greater effects on plant physiological performance and yield of the fruits. *P. quadrangularis* plants grown under environmental stress conditions shown abnormal fruit structure and developed anthocyanin pigmentation as a protective function to cope and survive in the harsh environment.

Acknowledgments

This research was granted with funding support by UPM under GP-IPM/2018 [Biology, Cultivation, and Potential Uses of *Passiflora quadrangularis* L. (Giant Granadilla Passion Fruit)].

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Evaluation of Growth and Quality of Purple Red *Brassica* Influenced by Different LED Wavelengths in Indoor Vertical Farming

Rosniza, K.[1,*], Winnie, Y.S.P.[1], Ajit, S.[1], Joshua, Y.L.[1], Farahzety, A.M.[1,2], Zulhazmi, S.[2] and Mohd Abid, A.[2]

[1]School of Biosciences, University of Nottingham Malaysia Campus, 43500 Semenyih, Selangor, Malaysia.
[2]Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail*: *hbxrk1@nottingham.edu.my*

Introduction

Vertical farming is one the latest technology to produce efficient agro-crops promoted by the government. This cultivation system is a sunrise technology in Malaysia as a high potential problem solver in local produce industry. The advanced controlled-environment system able to solve issues involving land-scarcity, labour intensive, pest and disease, and unpredictable weather condition. It is expected that the Malaysian population will increase to 41.2 million by 2040, thus an effective vertical farming technology for sustainable vegetables production is needed, thus could reduce the country's dependence on imports, and thereby ensure our national food security. The potential use of LED has nowadays linked with controlled-environment agriculture, such as growth chambers, greenhouses, and latest technology of the vertical farming system. Recently, LEDs incorporation with newly built vertical farms has gaining increasing demand and popularity(Mickens et al., 2019).

Vegetables is one of the significant group of food crops that provides essential sources of nutrients in daily diet (Tee, 1979). In Malaysia, vegetables commonly consumed both cooked and raw as ulam or salad. *Brassica rapa chineenis* (purple red *brassica*) is one of the vegetable species that are widely consumed by the nation. In the present study, purple red brassica growth and quality were evaluated under LED light incorporated with hydroponic indoor vertical farming system. Different proportion of LED light spectrum were used to grow the plants. Purple red *brassica* was selected as a model crops because it has rapid growth rate and enriched with anthocyanin an important antioxidant for dietary consumption (Neugart et al., 2018). In this study, we will determine the effect of red, blue, and green wavelengths on purple red *brassica* growth and quality. Optimal wavelength proportion for cultivation of purple red *brassica* growth in indoor vertical farming will also be identified.

Materials and Methods

The experiment conducted in LED light lab in MARDI, Serdang. Seeds of Purple Red Brassica (*Brassica rapa chineensis*) were sown in wet urethane foam containing 117 cells. The seeds were grown in the hydroponic germination rack with controlled environment condition using white light, photosynthetic photon flux (PPF) 150, for 8 hours photoperiod in 7 days cycles. The seedlings grown in the urethane foam cube cells were then filled into hydroponic pots before transplanted to multilayers hydroponic system. The temperature was at 21.6 °C to 23.4 °C, CO_2 at 497 mg/L and relative humidity (RH) at 78% using Temp/RH/CO_2 Meters (Spectrum Technologies, Inc., IL, USA).

The plants were sub-irrigated as needed with nutrient solution containing (150 mL/30 L). Nutrient set A: Ca, NO_3, NH_4, Fe, and K, nutrient set B: H_2PO_4, SO_4, K, Mg, B, Cu, and Mo. The nutrient solution was supplied using pump for 24 hours until the experiment completed. The study was in a Complete Randomized Design (CRD) with lighting environment as the main factor. The light source used in this experiment were LED lights consisted of white light (WL): blue light (BL): red light (RL): green light (GL) LEDs (Impressive Edge Inc, Malaysia).

The experimental conditions were set up with three treatments; full spectrum, which is white light (WL) as a control (the proportions of RL, BL, and GL wavelengths were 42%, 15% and 43%, respectively), the combination $RL_{81}BL_{19}$ (the proportions of RL and BL wavelengths were 81% and 19%, respectively) and $RL_{70}BL_{21}GL_{9}$ (the proportions of RL, BL, and GL wavelengths were 70%, 21%, and 9%, respectively). There were seven replicates for each light treatment at each harvest, 17, 27, and 37 days after sowing (DAS). A total of 21 experimental plants were subjected to each light treatment. The photoperiod was 12 hours' day/12 hours' night for 37 days for each study. The spectral distribution scans of all treatments were measured at five locations in the plant-growth area using a spectrometer (LI-180; LI-COR Biosciences, Lincoln, USA).

Growth characteristics included plant width, plant height, fresh and dry weights of shoots and roots, and total leaf area. The shoots and roots were dried in a drying oven (UN 30; Memmert, Schwabach, Germany) for 72 hours at 70 °C. The fresh and dry weights from each harvest before and after drying were obtained using a scale (ME 3002; Mettler Toledo, Ohio, USA). Total leaf areas were measured using a leaf area meter (LI-3100; LI-COR Biosciences, Lincoln, USA). A portable chlorophyll meter (SPAD-502DL; Konika Minolta Sensing, Osaka, Japan) was used to measure relative chlorophyll content in dimension less SPAD units. Anthocyanin levels were estimated non-destructively from leaves directly exposed to the light, using an anthocyanin content meter (ACM-200 plus; Opti-Sciences. Inc., USA).

Statistical analysis was performed using the SPSS 23.0 (IBM, Inc., Chicago, IL, USA). All data were analysed using analysis of variance (ANOVA) followed by Tukeys test for mean separation at the $P<0.05$ ($n=7$). Data was tested for normality of residuals and homogeneity of variance prior to carrying out ANOVA.

Results and Discussion

Table 1 shows the plant height and plant width for purple red *brassica* are significantly higher under treatment $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_{9}$. Whereas the, the largest plant width and height are the plants that exposed under $RL_{81}BL_{19}$. In addition, $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_{9}$ significantly induced a higher leaf area compared with WL (control). The highest increased of leaf area was observed in $RL_{81}BL_{19}$ at every DAS examined. While in Table 2, the fresh and dry weight of shoots significantly increased with exposure under $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_{9}$. The purple red *brassica* shoots fresh weight of $RL_{81}BL_{19}$ at 37 DAS was 88% higher, while $RL_{70}BL_{21}GL_{9}$ was 83% higher than that of WL (control). It shows that $RL_{81}BL_{19}$ had the greatest fresh for all harvest and dry weight except during 17 DAS. The range of BL added to RL from 2 to 15% have a positive effects on the growth of lettuce (Hogewoning et al., 2010; Chen et al., 2016;). While in this study, the positive impact can be seen for the percentage of BL at 19% and 21%.

Estimated chlorophyll content (SPAD measurement) on purple red brassica is shown in Figure 1. The chlorophyll content was significantly higher in treatment $RL_{81}BL_{19}$ at each harvest. Chlorophyll has maximum energy absorption with RL and BL, with RL having the highest quantum yield (Hogewoning et al., 2012). This may indicate that spectra enriched with GL tend to reduce leaf chlorophyll levels in purple red *brassica*. Chlorophyll can move and concentrations can fluctuate based on duration and light quality (Lefsrud et al., 2008).

Table 1: Influenced of LED light recipes on purple red brassica plant width, plant height and leaf area (days after sowing-DAS).

Parameters	Plant width (cm)			Plant height (cm)			Leaf area (cm)		
Treatment	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS
WL (control)	7.78^{a}	21.85^{c}	31.73^{c}	4.22^{c}	9.92^{c}	13.38^{c}	3.795^{c}	8.05^{c}	27.96^{c}
$RL_{81}BL_{19}$	10.93^{a}	33.5^{a}	40.32^{a}	7.23^{a}	17.05^{a}	20.53^{a}	8.12^{a}	42.85^{a}	107.045^{a}
$RL_{70}BL_{21}GL_{9}$	10.52^{a}	30.95^{ab}	38.17^{ab}	6.85^{ab}	14.22^{b}	18.95^{ab}	6.37^{ab}	18.56^{bc}	92.32^{ab}
SEM	1.266	1.759	1.227	0.594	0.697	0.506	1.127	5.977	6.162
$P_{0.05}$	0.195	0.001	0.000	0.005	0.000	0.000	0.049	0.003	0.000

Means with different letters are significantly at the P=0.05 level by Tukey's multiple comparisons test. SEM=Standard error mean.

Table 2: Influence of LED light recipes on purple red brassica shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight over time (days after sowing-DAS).

Parameters	Shoot fresh weight (g)			Shoot dry weight (g)			Root fresh weight (g)			Root dry weight (g)		
Treatment	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS
WL (control)	0.271^{c}	1.47^{c}	7.94^{c}	0.032	0.23^{c}	0.62^{c}	0.105^{c}	0.975	1.63^{c}	0.022^{c}	0.253	0.163
$RL_{81}BL_{19}$	0.85^{a}	7.62^{a}	71.40^{a}	0.070	1.14^{a}	5.055^{a}	0.37^{ab}	0.968	3.68^{a}	0.065^{a}	0.633	0.523
$RL_{70}BL_{21}GL_{9}$	0.63^{ab}	4.44^{abc}	48.96^{ab}	0.058	0.71^{abc}	3.38^{ab}	0.415^{a}	1.298	3.435^{ab}	0.062^{ab}	0.380	0.580
SEM	0.139	1.469	8.043	0.012	0.196	0.523	0.052	0.166	0.490	0.007	0.163	0.167
$P_{0.05}$	0.031	0.032	0.000	0.114	0.017	0.000	0.001	0.303	0.019	0.001	0.276	0.194

Means with different letters are significantly at the P=0.05 level by Tukey's multiple comparisons test. SEM=Standard error mean.

Figure 2 shows anthocyanin content in purple red brassica measured over time. The plants exposed under $RL_{81}BL_{19}$ showed the highest anthocyanin levels at each harvest compared to other treatments. $RL_{81}BL_{19}$ treatment has increased the chlorophyll content in most of the harvest of purple red *brassica.* The anthocyanin in the plants under $RL_{81}BL_{19}$ was increased between 73-88% as compared to WL (control). The results had suggested BL to increase the anthocyanin content in purple red *brassica*, similar to those reported of increased carotenoid and anthocyanin in lettuce and tomato with BL (Giliberto et al., 2005).

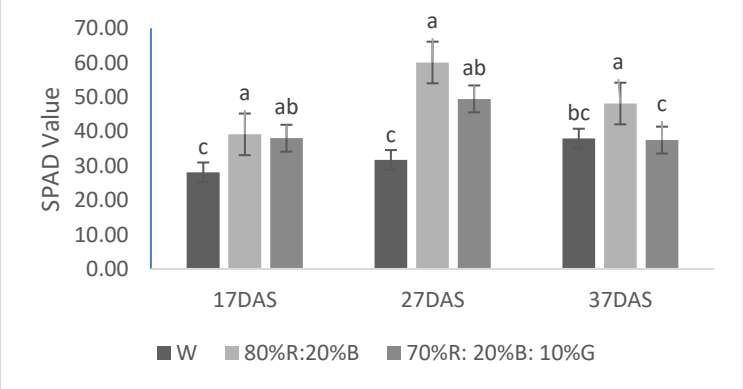

Figure 1: Estimated relative chlorophyll content in purple red brassica plants over times (days after sowing-DAS) for all light treatments. Means with different letters are significantly different at the P=0.05 level by Tukey's multiple comparisons test.

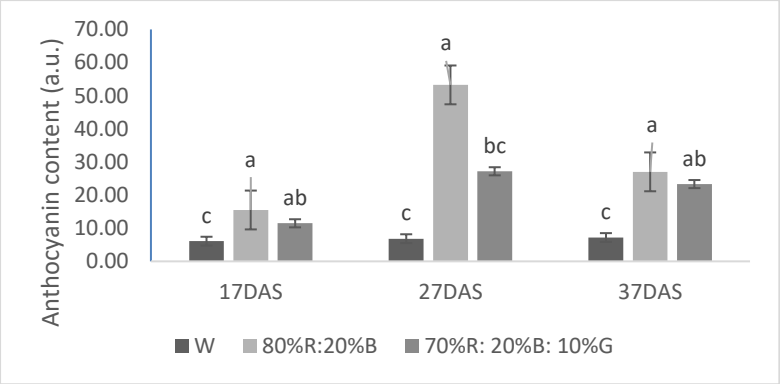

Figure 2: Estimated relative anthocyanin content in purple red brassica plants over times (days after sowing-DAS) for all light treatments. Means with different letters are significantly different at the P=0.05 level by Tukey's multiple comparisons test.

Conclusion

The result of this study has suggested that treatment with $RL_{81}BL_{19}$ is the best light proportion for purple red *brassica* production in indoor vertical farming. RL and BL showed a positive impact towards growth and accumulation of chlorophyll and anthocyanin content of purple red *brassica*. GL therefore, did not contributed to the overall growth and accumulation of chlorophyll and anthocyanin content. This study supports that different light spectrum is useful in enhancing crop morphology and nutritional attributes. However, there is no light spectrum at a specific combination is optimum for all crops as each crop requires its own lighting recipe. Therefore, more studies on the LEDs wavelength combination in different crops need to be carried out in the future.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Variations in Banana Growth Development and Soil Nutrients Status During Vegetative Stages

Deniel, S.[1], Izzah, A.H.[1], Phebe, D.[2], Nurulhikma, M.I.[3], Norliza, A.B.[4] and Wan Asrina, W.Y.[1,*]

[1]Department of Crop Science, Faculty of Agriculture Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia.
[2]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3]School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), 43400 Bangi, Selangor, Malaysia.
[4]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: asrina@upm.edu.my*

Introduction

Sustainable banana production is indispensable and plays a significant role in food security. Great challenges involved in banana production consequently affecting banana yield production and quality. Vegetative stage is one of the crucial parts in banana production which is associated with the yield production and productivity. Fluctuations of environmental condition have attributed stressful effect on banana growth performance. To overcome these problems, strategies for better management of banana production and environmental resources are recommended. This study aimed to investigate the banana (*Musa acuminata* cv. Berangan and *Musa acuminata* cv. Tanduk) growth development and soil nutrients status during vegetative stages.

Materials and Methods

The study was carried out at Share Farm 2, Universiti Putra Malaysia, Bintulu Campus, Sarawak from March to September 2020. The field study was conducted using Randomized Complete Block Design (RCBD) with 48 replications of two banana variety. Banana varieties *Musa acuminata* cv. Berangan and *Musa acuminata* cv. Tanduk were used in this study. Banana sucker were planted at 3 m x 3 m apart and two sword suckers per hole. The NPK fertilizer was applied as according to recommended rate by Department of Agriculture (DOA), Malaysia. A composite soil samples were collected at 0 to 20 cm depth before planting and after 6 months of cultivation. Selected soil physicochemical properties were determined, including bulk density, soil pH, total nitrogen (N), available phosphorus (P) and exchangeable potassium (K). The growth of 48 suckers from both varieties were monitored: the observation of vegetative characteristics was done monthly by determining the pseudostem height and size, leaf area, chlorophyll content, and number of functional leaves. The chlorophyll content was analyzed by SPAD meter. Leaf area measurement was calculated using equation from Obiefuna (1979): Leaf area = leaf length x leaf width x 0.8 (coefficient). The significance differences of banana varieties were determined by LSD test at $P \leq 0.05$ using the SAS 9.0.

Results and Discussion

The means of selected soil physicochemical properties are shown in Table 1. During the first 6 months, a significant increase in soil pH was observed, which might in relative to liming effect application before planting (Li et al., 2018). A significant response was also noticeable in soil available P, total N and exchangeable K after 6 months of cultivation. A two-fold increase in soil total N and exchangeable K after 6 months of growing period might be attributed by application of NPK fertilizer. The result of soil available P was consistent with the previous studied which reported increased due to increasing soil N availability as well as altering soil ion balance and phosphate production (Olander et al., 2000; Treseder et al., 2001).

Table 1: Selected soil physicochemical properties used in banana cultivation.

Period	Bulk density (gm^{-3})	Soil pH	Total N	Available P	Exchangeable K
				mg/kg	
Before planting	1.41	4.66±0.07	6.25±0.53	22.12±0.53	68.8±5.85
6 months after planting	-	5.07±0.06	12.8±1.09	26.34±0.32	153.3±8.11
t-test	-	**	***	**	***

*Asterisk symbols are significant at **P≤0.01, ***P≤0.001, respectively.*

Chlorophyll content was significantly difference only at 90 and 150 days after planting (DAP) between banana varieties (Table 2). The chlorophyll content ranged in berangan and tanduk were recorded from 42.3 to 52.5 and from 40.6 to 51.9, respectively. The result showed that number of functional leaves was found statistically significant only at 60 and 150 DAP, the range number of functional leaves in two banana varieties were recorded from 3 to 11.

As the DAP increased, banana pseudostem size was markedly increased from 30 to 150 DAP, while pseudostem height was only found significantly difference at 90 DAP (Figure 1). In addition, chlorophyll content and leaf area were observed higher in Berangan starting at 120 DAP in comparison to Tanduk. The leaf area between banana varieties was observed significantly difference at 30, 60, 150, and 180 DAP, while no significant difference was detected at 90 and 120 DAP. As shown in Figure 1, the leaf area of Berangan started to increase and recorded higher than Tanduk after 120 days upward after planting. Further research up to reproductive stage is needed to explore the optimal management practices for improving banana yield production in respective of Malaysia agroclimatic condition.

Table 2: Chlorophyll and number of functional leaves in banana variety up to 6 months cultivation.

Varieties	Chlorophyll content					
	DAP					
	30	60	90	120	150	180
Berangan	49.8±1.2	52.5±1.4	47.0±0.7	50.0±1.2	51.3±1.2	42.3±2.2
Tanduk	50.5±1.3	51.9±1.3	49.1±0.7	51.2±1.3	48.0±0.8	40.6±1.5
t-test	ns	ns	*	ns	***	ns
Varieties	No. of functional leaves					
	DAP					
	30	60	90	120	150	180
Berangan	3±0.1	8±0.2	9±0.2	11±0.2	5±0.2	9±0.3
Tanduk	3±0.1	7±0.3	9±0.3	10±0.3	8±0.4	8±0.5
t-test	ns	**	ns	ns	***	ns

*Asterisk symbols are significant at *P≤0.05, **P≤0.01, ***P≤0.001, respectively. ns: not significant.*

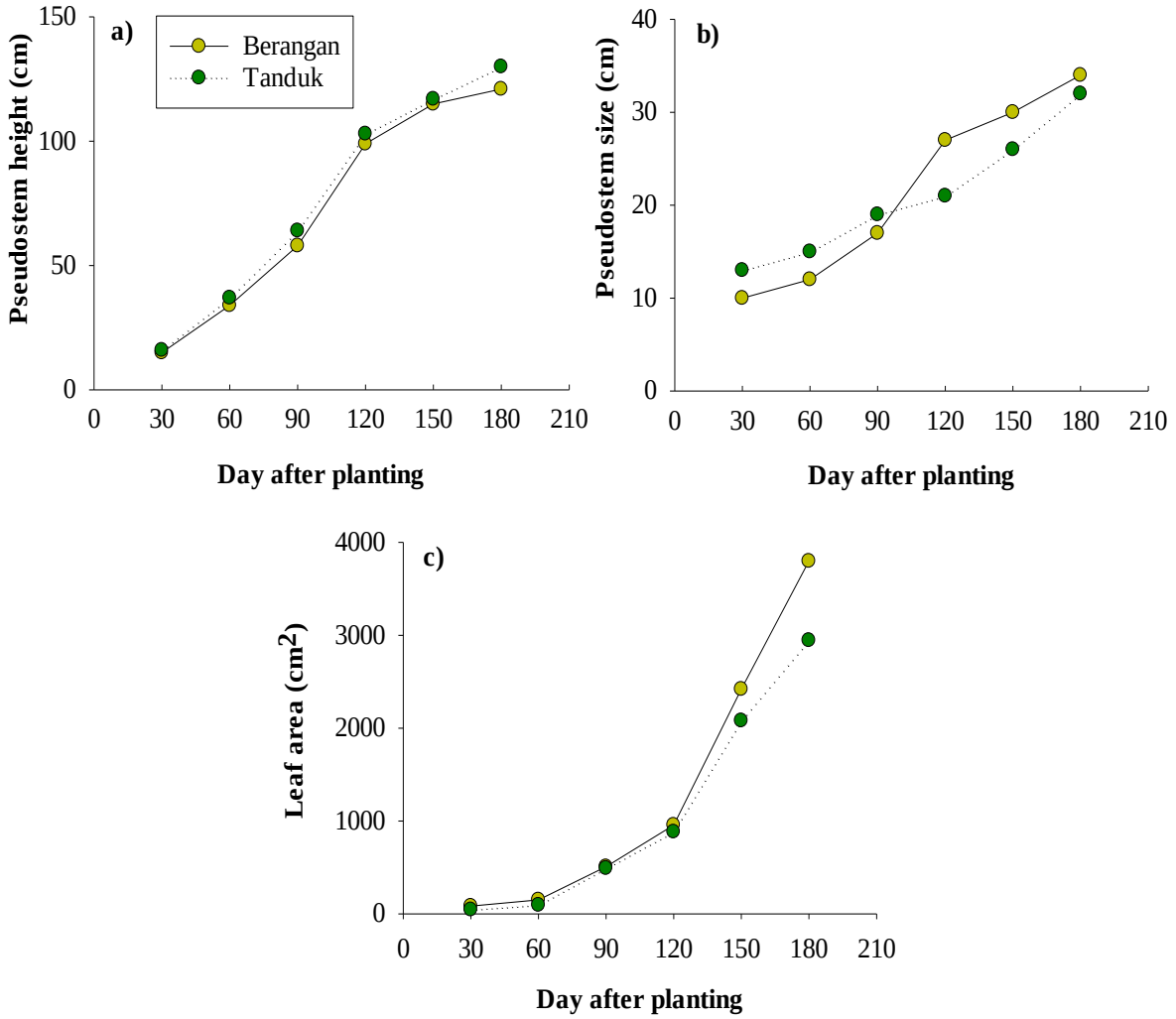

Figure 1: a) Pseudostem height, b) pseudostem size and c) leaf area of the banana plant at different day after planting in different banana varieties.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Preliminary Observation on Effect of LED Lighting on Growth and Physiological Characteristics of Sweet Basil (*Ocimum basilicum*)

Izyani, R.[1,*], Halimah, H.[1] and Mohamad Abid, A.[2]

[1]Industrial Crop Research Centre, MARDI, 43400 Serdang, Selangor, Malaysia.
[2]Horticulture Research Centre, MARDI, 43400 Serdang, Selangor, Malaysia.
**E-mail: izyani@mardi.gov.my*

Introduction

Light is one of the main factors that provides energy for plant photosynthesis. Light acts as a signal that can stimulates various physiological responses in plants, which determined by the light quality, light intensity, and photoperiod (Jiao et al., 2007). Nowadays, plant production under closed environment such as plant factory is a popular method used by growers. It tends to produce high quality plant with fast production by using light emitting diode (LED) as an alternative light source.

Herbs plantation in plant factory using LED light was important to produce bioactive compounds, nutrients, and antioxidant as well the quality of crops such as shapes, colors, and textures of leaves. In addition, production of herbs in plant factory will also produce herbs that are safe to eat (pesticide free) as compared to the conventional method.

Therefore, this study aimed to evaluate the effect of light emitting diode (LEDs) lighting on growth, biomass, and physiological characteristics of sweet basil (*Ocimum basilicum).*

Materials and Methods

This experiment was conducted in Plant Factory LED laboratory at Malaysian Agricultural Research and Development Institute (MARDI) Serdang, and was laid out using split plot arrangement. Sweet basil Genovese was used in this study. Seeds were sown in trays with germination sponge for 21 days. Germinated seedlings were then transplanted in nutrient film technique system equipped with three LED light treatments for 45 days. The three LED light treatments tested were; (i) full spectrum (control), (ii) Red Blue (RB; 80% Red: 20% Blue), and (iii) Red Blue Green (RBG; 70% Red: 15% Blue: 15% Green) under 10 hours photoperiod. The nutrient solution in container tank was monitored and adjusted accordingly when necessary, to maintain an electrical conductivity (EC) at 1.6-2.2 dSm^{-1} and a pH of 6.5.

The morphological parameters of plants including plant height, stem diameter, number of leaves, leaf length, leaf width, internode length, and number of internodes were measured at final harvest. Plant height was manually measured from the ground level to the shoot tips using a measuring tape. Stem girth was measured at the level of 1cm from the base level by using a Vernier callipers. The number of internodes and leaves for each plant were counted manually. Leaf area was measured using a leaf area meter (LI-3100; LI- COR, Inc., Lincoln, Nebraska, USA).

Plant harvests were carried out at 45 days after treatments. Plant samples from each treatment were harvested and partitioned into roots, stems, fruits, and leaves to determine the dry weights of individual plant parts. Leaf fresh weight was measured before oven-drying and then, the dry weights of the plant parts were measured using a digital balance (QC 35EDE-S Sartorius, Germany) after oven-drying at 60 ^{o}C for 48 hours. Total dry weight was obtained by combining the weight of all plant parts (Hunt, 2003).

Net photosynthetic rate, stomatal conductance, and transpiration rate were determined using a LI-COR 6400 Portable Photosynthesis System (Lincoln, Nebraska, USA). The measurements were carried out on matured, fully expanded leaves for each treatment. For this measurement, light intensity

(Photosynthetically Active Radiation, PAR) of the leaf chamber was set at 200 µmolm^{-2}s^{-1} (full spectrum LED), 400 µmolm^{-2}s^{-1} (RB LED) and 450 µmolm^{-2}s^{-1} (RBG LED). This light intensity was setup higher than the actual light based on PAR measured for each LED treatments. The chamber temperature was maintained at 28 ^{o}C and the reference CO_2 concentration was at 400 mgL^{-1}. The relative humidity was controlled between 50-70% with humidity flow into the chamber was fixed at 500 µmol s^{-1}, and desiccant mid-range between scrubs and by passes. All readings were taken between 8.00 am to 11.00 am at harvest, which was presumed that photosynthetic rates would be maximal (DiCristina and Germino, 2006).

The Soil Plant Analyzer Development (SPAD) for relative chlorophyll content was measured on the same leaves as photosynthetic rate using SPAD-502 Chlorophyll Meter (SPAD 502, Minolta-Camera Co., Osaka, Japan). Data points were recorded at five locations of the leaf blade and an averaged was determined. The measurements were taken between 9.30 am to 10.30 am. The SPAD value was used preferentially because of the strong relationship between portable chlorophyll meter readings and actual leaf chlorophyll content as acknowledged by several authors (Jangpromma et al., 2010; Gholizadeh et al., 2011; Garousi et al., 2015).

Results and Discussion

The RB and RBG LEDs showed great influence on sweet basil vegetative growth (Table 1). Overall, there was no significant different in term of vegetative growth of sweet basil between RB and RBG LED treatments. It was observed that plant height of basil was significantly increased by 76% for RBG and 69% for RB as compared to full spectrum LED.

Table 1: Effects of various LED treatment on vegetative growth of sweet basil.

Spectrum	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Stem diameter (mm)	Internode length (cm)	Internode number	Leaf fresh weight (g)	Total leaf area (cm^2)
Full spectrum	5.93^{b}	6.53^{b}	3.75^{b}	2.50^{b}	1.22^{a}	2.20^{a}	2.19^{b}	1.96^{b}	85.10^{b}
R:B	10.03^{a}	10.07^{a}	6.58^{a}	4.26^{a}	3.13^{a}	4.22^{b}	3.17^{a}	15.93^{a}	536.80^{a}
R:B:G	10.48^{a}	9.61^{a}	6.60^{a}	4.37^{a}	3.15^{a}	4.36^{a}	3.29^{a}	16.24^{a}	493.00^{a}

Means followed with the same letter in the same column and source are not significantly different at P≤0.05 by Least Significant Difference (LSD) test.

In this study, a significantly highest leaf number was observed for sweet basil plant under RB treatment (10.67) compared to control, but not significant with RBG treatments. The effects of light intensity of 80% Red and 20% Blue LEDs in this study is similar to the finding of a previous study by Naznin et al. (2019) that used a combination of 91% Red: 9% Blue LEDs.

Observation of leaf length showed an increase by 76% for basil under RBG treatment followed by RB (75.4%) as compared to control. Leaf width also showed highest result for basil under RBG treatment (74.8 %). RB LEDs also showed an increasing in internode length of sweet basil when compared to RBG and full spectrum treatments. The highest number of internodes was observed for the RBG treatment (Table 1). Leaf fresh weight was highest for basil under RBG treatment with an increment by 728% as compared to full spectrum followed by RB treatment with an increment by 712.7%. There were no significant different between RB and RBG treatment for total leaf area of basil plant, but both RB and RBG treatment showed significant result when compared with full spectrum treatment (Table 1).

The effect of various LED lighting on dry matter yield and partitioning are shown in Table 2. Significant increase in all parameters measured were observed for RB and RBG LED when compared with full spectrum treatment.

Table 2: Effects of various LED treatment on biomass partitioning of sweet basil.

Spectrum	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Total dry weight (g)
Full spectrum	0.16[b]	0.22[b]	0.12[b]	0.50[b]
R:B	2.18[a]	1.23[a]	1.01[a]	4.42[a]
R:B:G	2.10[a]	1.02[a]	1.15[a]	4.37[a]

Means followed with the same letter in the same column and source are not significantly different at P≤0.05 by Least Significant Difference (LSD) test.

Sweet basil plants under RBG gave significant result with highest net photosynthesis rate (5.44 $\mu molCO_2m^{-2}s^{-1}$), stomatal conductance (0.15 $mmolm^{-2}s^{-1}$) and transpiration rate (2.47 $mmolm^{-2}s^{-1}$) when compared to RB and full spectrum treatment (Table 3). Meanwhile, there is no significant different in term of physiological characteristic for RB when compared to full spectrum LED treatments. Relative chlorophyll content was significantly different among all the LED treatments, with RBG treatments showed greatest relative chlorophyll content (SPAD meter reading) by 31.54 followed by RB (27.25) and the least value was recorded for full spectrum treatment (20.93).

Table 3: Effects of various LED treatment on physiological characteristic and relative chlorophyll content of sweet basil.

Spectrum	Net photosynthetic rate ($\mu molm^{-2}\ s^{-1}$)	Stomatal conductance ($mmolm^{-2}\ s^{-1}$)	Transpiration rate ($mmolm^{-2}s^{-1}$)	Relative chlorophyll content (SPAD value)
Full spectrum	1.36[b]	0.02[b]	0.50[b]	20.93[c]
R:B	2.20[b]	0.05[b]	1.22[b]	27.25[b]
R:B:G	5.44[a]	0.15[a]	2.47[a]	31.54[a]

Means followed with the same letter in the same column and source are not significantly different at P≤0.05 by Least Significant Difference (LSD) test.

Conclusions

In conclusions, the RB and RBG LED treatment was effective in enhancing and stimulating vegetative growth, leaf area, chlorophyll content, and physiological characteristics of the basil plant.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effects of Foliar Fertilizers on the Growth and Yield of Roselle (*Hibiscus sabdariffa* L.) on Bris Soil

Norhayati, Y.[1,2,*] and Goo Chian, Y.[1]

[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
[2]Biological Security and Sustainability Research Interest Group (BioSes), Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
**E-mail: yatiyusuf@umt.edu.my*

Introduction

Roselle is cultivated for its leaves, calyces, and seeds. These parts can be used as sauces, jams, juices, jellies, syrups, flavoring, and coloring agent for food and drinks (Suresh and Ammaan, 2017). Roselle has a high content of nutrients, antioxidants including ascorbic acid, carotenoids, and anthocyanin. With the highly adaptable with all ranges of soils; Roselle can be potentially planted at Beach Ridges Interspersed with Swales (BRIS) soil which is poor in water holding capacity and nutrient availability.

Fertilizers are substances containing nutrients to support plant growth. Crops use nitrogen, phosphorous, and potassium at different rates and different soils contain/lack those essential materials. Land scarcity is compounded by low soil nutrients and it can be solved by fertilizer application or other soil management practice (Mwangi, 1997). Application of foliar fertilizer can help to maintain a nutrient balance within the plant, which may not occur strictly with soil uptake. Thus, the objective of this research is to determine the time interval for foliar application in order to obtain best growth, yield and its effect on anthocyanin, carotenoids, chlorophyll, and ascorbic acid contents of Roselle (UKMR-2).

Materials and Methods

The seeds of variety UKMR-2 were obtained from Faculty of Fisheries and Food Science (FFFS), Universiti Malaysia Terengganu and were planted in Green House of FFFS, UMT. Thirty seeds were sown in polystyrenes cups with a dimension of 8.0 cm (top diameter) x 5.6 cm (bottom diameter) x 9.3 cm (height). The peat moss was used as the growing medium and soil amendment. To ensure an optimum plant growth, basal fertilizer was added according to the standard amount needed by Roselle in farm as stated by Adzemi et al. (2010). After two weeks of seeding, the seedlings were transplanted into polybags contained 30 kg of BRIS soils obtained from Rhu Tapai, Terengganu.

About 4.5 mL of foliar fertilizer (VITA-GROW) was diluted with 900 mL of water and was applied on the surface and underneath of the leaves. The time interval of foliar application (Treatments, T) was divided into five groups including T1 (control), T2 (a two-week interval), T3 (a three-week interval), T4 (a six-week interval), and T5 (an eight-week interval).

Growth parameters were measured every 3 weeks starting from 2 weeks of transplanting. The parameters were the number of leaves, leaves area, stem diameter, and plant height. Plant yields i.e. number of calyces, diameter and length of calyces, and fresh and dry weights of calyxes. Ascorbic acid, anthocyanin, and carotenoids were measured in the harvested calyxes whereas leaf was taken to determine the chlorophyll content. Ascorbic acid was assayed based on the method of Jagota and Dani (1982). Carotenoids were determined following the method of Lichtenthaler (1987) and anthocyanin content in calyx was assayed according to the method of Wrostald (1976). Leaf chlorophyll content was extracted based on the method of Harborne and Williams (2001). The data taken was analysed using a one-way ANOVA with multiple comparison, Duncan's Multiple Range Test at a significance level of α=0.05.

Results and Discussion

Roselle plant showed a gradual increase in height from 3 to 15 weeks in all treated plants. After 15 weeks, the rate of plant height become lower (Figure 1). No significant difference ($P>0.05$) on the plant height were observed in all treatments. Similar results were observed in the stem diameter (Figure 2). The results obtained in this study correlates well with the responses in cotton (Yalappa, 2006). Abdelgadir and Johnson (2009) also found that foliar application has no effect on plant height and stem diameter of biofuel crop *Jatropha curcas* L. Number of leaves slowly increased up to 12 weeks treatment and gradually decreased thereafter (Figure 3). A similar trend was noticed in leaves area, but it started to decrease from week 15 as shown in Figure 4. The T3 plant produced the biggest leaf area (79.33±6.39 cm^2) on week 18 whereas the smallest leaf area was observed in T5 (33.50±1.44 cm^2) on week 3. The T3 plants which has a smaller number of leaves need the bigger leaf area for photosynthesis. Large leaf area is important to absorb large amount of nutrients (Knoche et al., 1994). Leaf area increased with the age of the Roselle up to week 15 and decreased due to ageing and senescence of leaves. Higher leaf area correlates with higher photosynthetic surface area resulting in better growth at early stages (Takashi and Gaku, 2010).

Figure 5 shows the number of harvested calyxes from week 12 until week 24. The number of calyxes significantly increased ($P<0.05$) in T1 and T2 plants from week 12 to week 18 and decreased afterward. The T1 plants produced the highest yield in week 15 (9.33±4.18). However, the highest number of calyxes in T2 (9.00±2.00), T3 (6.33±1.86), T4 (9.00±3.00) and T5 (7.00±0.58) were recorded on week 18. This indicated that the mature calyxes of T1 was harvested earlier than the other treatments. On the other hand, all treatments had the lowest yield on week 12. Figure 6 shows the total harvested calyxes in T1 to T5. The T1 plants had the highest yield with 79 calyxes, followed by T2 of 68 calyxes, T5 (57 calyxes) and T4 with 56 calyxes. Whilst the T3 plants produced the lowest yield with 55 calyxes. The results revealed that application of foliar fertilizer at different time interval did not give any impact on the Roselle yield. Highest number of calyxes in control plant may be due to efficient translocation of sugar within the plants and production of growth promoting substances, carbohydrates, and synthesis of nucleic acid. Such an effect on early flowering was also noticed in cotton by Silva et al. (1982) and Ullagaddi (2000).

Figures 7 and 8 show the calyxes' fresh and dry weights after the treatments. Both the fresh and dry weights generally increased in all plants from week 12 to week 18 and decreased thereafter. The highest fresh weight was obtained in T1 of 117.78±52.39 g in week 15. A similar trend was spotted in calyx's fresh weight. The highest dry weight was also obtained in T1 (96.92±3.09 g). However, no significant difference ($P>0.05$) was observed for calyxes' fresh and dry weights in all treatments. The length and diameter of the calyxes increased from week 12 to week 15 and then decreased thereafter. The highest calyx's length was found in T4 (week 18) followed by T1 and T5. Similarly, T4 calyxes was significantly higher in calyxes' diameter ($P<0.05$) compared to that of T1.

Table 1 shows the anthocyanin, ascorbic acid, carotenoids, and chlorophyll contents in foliar fertilizer treatments. No significant difference was noticed in anthocyanin and ascorbic acid content in all treatments. These results were consistent with the results of Hassanein et al. (2005) in *Hibiscus sabdariffa* L. In contrast, significantly higher carotenoids were found in T4 (6.35±0.46 mg/L) whereas the lowest concentration was observed in T1 (3.96±0.45 mg/L). The T4 plants also exhibited greatest chlorophyll content whilst the least was observed in T2. Total chlorophyll content determines the photosynthetic capacity and influences the rate of photosynthesis, dry matter production, and yield (Krasichkova et al., 1989).

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

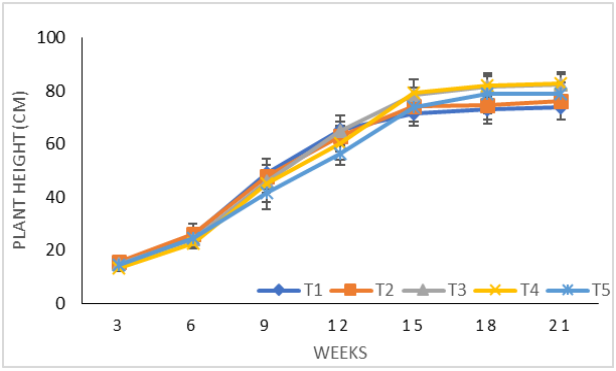

Figure 1: Effect of different treatments on the plant height of Roselle. Data are mean ± standard error (n=3).

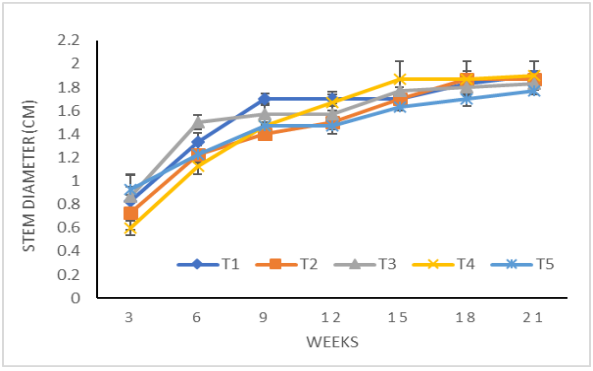

Figure 2: Effect of different treatments on the stem diameter of Roselle. Data are mean ± standard error (n=3).

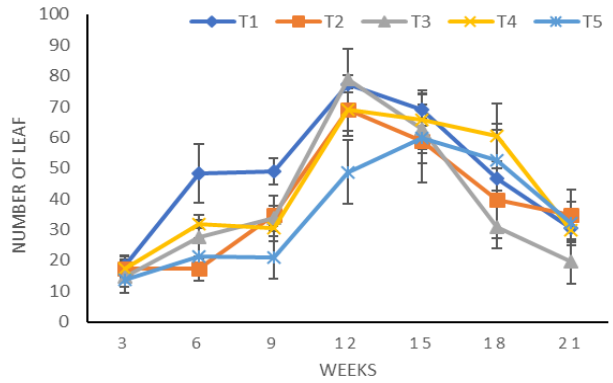

Figure 3: Effect of different treatments on the number of leaves in Roselle. Data are mean ± standard error (n=3).

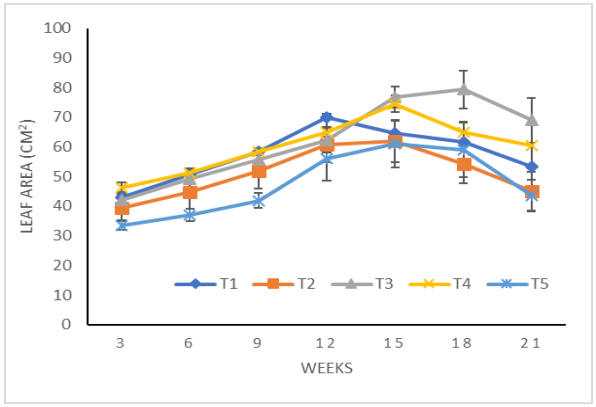

Figure 4: Effect of different treatments on the leaf area of Roselle. Data are mean ± standard error (n=3).

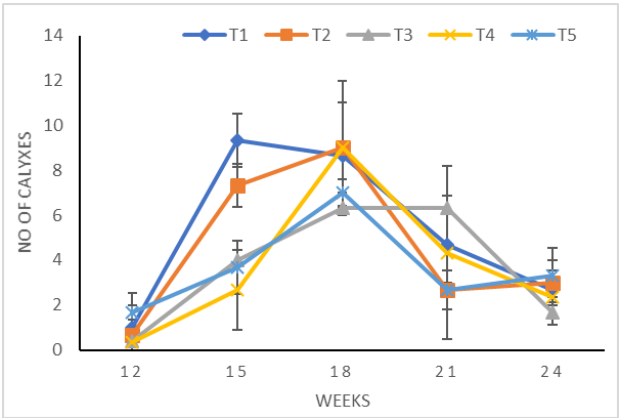

Figure 5: Effect of different treatments on the number of calyxes in Roselle. Data are mean ± standard error (n=3).

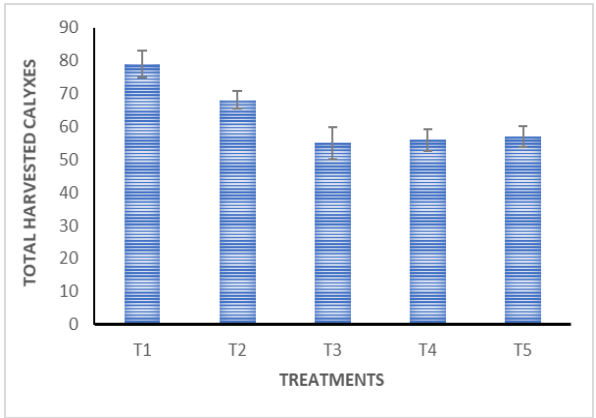

Figure 6: Effect of different treatments on the total harvested calyxes of Roselle. Data are mean ± standard error (n=3).

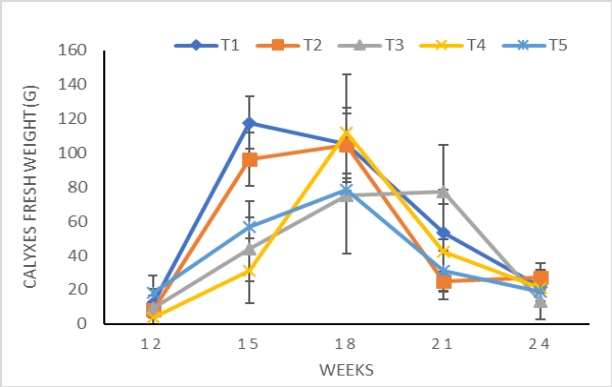

Figure 7: Effect of different treatments on the fresh weight of calyxes in Roselle. Data are mean ± standard error (n=3).

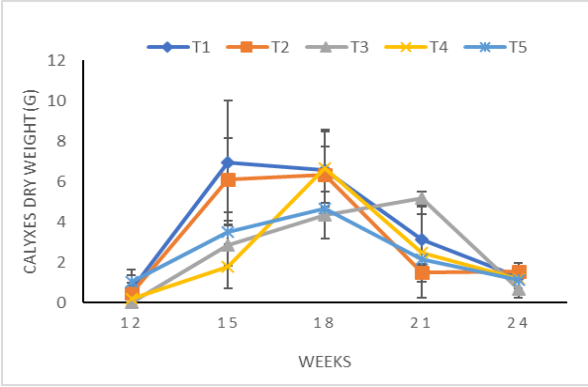

Figure 8: Effect of different treatments on the dry weight of calyxes in Roselle. Data are mean ± standard error (n=3).

Table 1: Effect of different treatment on anthocyanin, ascorbic acid, carotenoids, and chlorophyllcontent of Roselle.

Treatments	Anthocyanin (mg/L)	Ascorbic acid (µg/g Fwt)	Carotenoids (mg/L)	Chlorophyll (mg/g FWT)
T1	126.64±3.52^{a}	22.71±1.59^{a}	3.96±0.45^{a}	0.79±0.11^{a}
T2	104.91±15.65^{a}	21.44 ± 0.90^{a}	4.23±0.06^{ab}	0.72±0.04^{a}
T3	121.73±21.89^{a}	20.50±1.17^{a}	5.40±1.17^{ab}	0.79±0.06^{a}
T4	124.10±24.96^{a}	23.63±2.38^{a}	6.35±0.46^{b}	0.88±0.04^{a}
T5	121.55±2.52^{a}	21.37±0.68^{a}	5.55±0.56^{ab}	0.77±0.09^{a}

Data are mean ± standard error (n=3). Means with different letters within a column indicates that there was a significant difference at P<0.05.

Conclusion

The results revealed that the highest plant height, stem diameter, calyxes' size, ascorbic acid, carotenoids, and chlorophyll content were observed in plants treated with foliar application at 6 weeks interval, T4. Control plant had the highest number of calyxes, calyx's fresh and dry weights as well as anthocyanin content. However, no significant different was observed in most of the treatments except for T1 and T2 in the number of calyxes and calyx's fresh and dry weights. It was noticed that the plant height, stem diameter and leaf areas for all treatments, and control increased throughout the experiments. Whereas the number of leaves, number of calyxes, and fresh and dry weights initially increased and maximum at week 15 and week 18 and decreased thereafter. The finding suggested that application of foliar fertilizer used in this study, at different time intervals may significantly enhance the growth especially on T1 and T2 plants compared to other treated plants.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Growth Variations in the First-generation of *Tectona grandis* (Teak) Progeny Test at Papulut Forest Reserve, Gerik, Perak, Malaysia

Nor Fadilah, W.[1,*], Mohd Zaki, A.[1], Keeren, S.R.[1], Mohamad Lokmal, N.[1] and Muhammad Asri, L. [1.2]

[1]Plant Improvement Programme, Forest Biotechnology Division, Forest Research Institute Malaysia (FRIM), Kepong 52109, Selangor, Malaysia.
[2]Forest Plantation Programme, Forest Biotechnology Division, Forest Research Institute Malaysia (FRIM), Kepong 52109, Selangor, Malaysia.
**E-mail: norfadilah@frim.gov.my*

Introduction

Tectona grandis, commonly known as teak, is a large tree species that can grow up to heights of 30 m. It grows naturally in tropical monsoon climates, such as India, Myanmar, Laos, and Northern Thailand. Introduced to Southeast Asia by Dutch colonists, it has proven to be a versatile species that can grow under a wide range of climatic conditions. *Tectona grandis* is highly valued for its wood, often used for furniture and other high values products. Furthermore, this species is also highly resistant against pests and disease attacks, due to certain chemical compounds present in the wood (Akram and Aftab, 2016).

Tectona grandis has been introduced in Malaysia since the 1950s. During the early introduction, *T. grandis* has been planted at Northern states of Malaysia and Sabah. Among early resource stand of teak for seed production has been established at FRIM Research Station Mata Ayer, Perlis. The resource stand has been established with provenances from Thailand, Indonesia, India, Papua New Guinea, Trinidad, and Sabah. Selected mother trees were then identified and the planting materials for this progeny trial were obtained from this resource stand.

The current trend of plantations in Malaysia is focusing on palm oil and rubber tree which promising early cash returns as compared to forest tree species. Based on a report by Ahmad-Zuhaidi et al. (2011), Malaysia has only within 2000-3500 ha of teak plantations. Planting materials mostly imported from Indonesia, Myanmar, and Thailand. Among the challenges of establishing teak plantations in Malaysia, aside from the plantation cost is the limited presence of growth and yield data. The previous study has not been able to answer in conclusive the growth, yield, and modelling information.

Therefore, to provide high-quality planting materials of teak that can grow optimally, the breeding strategy of this species must be planned carefully. This study, in a long run, would be able to provide information on the growth, yield and selected quality planting materials of teak. Conceptually, in progeny trial, seedlings are planted in the replicated field trial. Growth performance of the trial is evaluated regularly. The established trial plots also can be converted into a seedlings seed orchard in the future. Seedlings for progeny trial study were obtained from selected mother tree or called as the superior plus tree. The superior plus tree is defined as the selected tree that has been graded for the sources on production for further breeding study (Hettasch et al., 2002). However, the genetic superiority of the selected plus tree is still needed to be tested. The probabilities of the progenies from selected plus tree to have good genotype is high due to reasonable heritability.

Materials and Methods

Plant materials

Seeds of *T. grandis* were obtained from 29 mother trees planted in a provenance resource stand which was established in 1974, located at FRIM's Research Station Mata Ayer, Perlis. The provenance resource stand was planted with teak provenances from Thailand, Indonesia, India, Papua New Guinea,

Trinidad, and Sabah. The mother trees were selected based on the plus tree's characteristic such as height, diameter at breast height, straightness, and stem form.

Progeny trial plot establishment

The progeny trial plot of *T. grandis* was established in February 2002 at Papulut Forest Reserve, Gerik, Perak. The trial plot was laid out in Randomised Complete Block Design (RCBD) with eight blocks and four progenies per family. Thus, making a total of 928 progenies were planted with the distances of 4 m × 3 m, making the total plot areas amounting to 1.2 ha. The trial plots were cleaned and maintained every 3 months during the first 2 years, every 6 months during the age of 3 to 5 years, and once per year from the age of six years old. Growth data such as total height (HT), clear bole height (CBH) and diameter at breast height (DBH) were collected every 3 months during the first 2 years, every 6 months at the age of 3 to 5 years old and once per year from the age of 6 years old onwards. This paper will be discussing the variations in the growth performances at the age of 14 years old.

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA) and followed by a Tukey post-hoc test using Statistical Package for the Social Sciences (IBM SPSS Statistics 22).

Results and Discussion

Survival rate

The survival rate of the 29 half-sib families at the age of 14 years old was varied from as low as 34.4% (T9) to as high as 75.0% (B78, R5 and C1). There were 20 families recorded survival rate above 50.0% (Figure 1). The overall survival rate of the whole research plot was 55.3%.

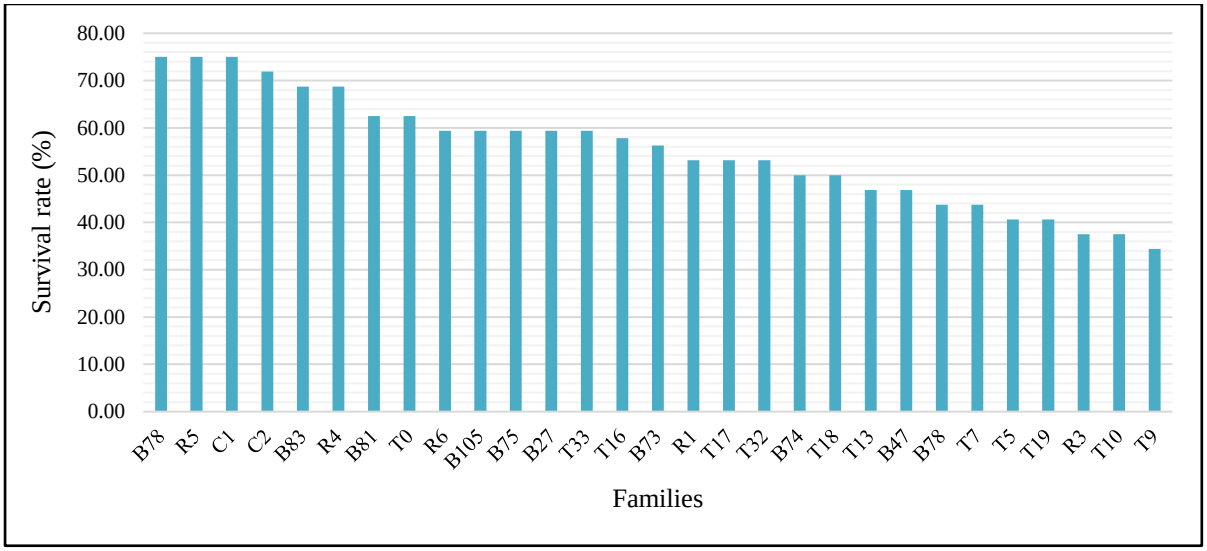

Figure 1: Survival rate (%) of the 29 half-sib families of *Tectona grandis* at 14 years old.

Growth variations

Test of homogeneity of variances (data not shown) indicated that the data has complied with the requirements of ANOVA. HT and CBH data showed that the test was not significant in which supporting the hypothesis whereby the variance is equal across groups. ANOVA test revealed that the growth traits of DBH and CBH were highly significant with $P<0.05$, indicating that there were high

variations within the growth traits among the 29 half-sib families. However, HT trait showed no significant differences among the 29 half-sib families (Table 1).

Table 1: Analysis of Variances (ANOVA).

		Sum of Squares	df	Mean Square	F	Sig.
Diameter at breast height (DBH)	Between groups	3107.864	28	110.995	1.948	0.004
	Within groups	28601.076	502	56.974		
	Total	31708.94	530			
Height (HT)	Between groups	1381.58	28	49.34	1.37	0.129
	Within groups	18127.25	504	35.97		
	Total	19508.82	532			
Clear bole height (CBH)	Between groups	810.531	28	28.948	2.448	0.000
	Within groups	5250.079	444	11.825		
	Total	6060.61	472			

Further analysis by Tukey Post Hoc test showed that there were six families significantly have the highest CBH (R5, B83, R3, B74, T0, and B78), whereas no distinct variations observed from the DBH and HT traits. The highest DBH was recorded by family T5 (25.98 cm). Family T5 also recorded the highest HT (21.27 m) but only ranked the 8th for CBH (11.9 m). Then, family R3 recorded the second highest DBH (24.55 cm) and ranked the third for CBH (12.56 m) even though the HT trait only ranked at the fifth (20.15 m). Family R5 recorded the highest CBH (13.52 m) but only ranked twelfth for HT (18.77 m) (Table 2).

Even though standard deviation (STDEV) of mean for each growth trait seems high, but none of the coefficients of variation (STDEV/mean) was more than 1.0, indicating that the CV can be considered as low. Besides, the test of homogeneity of variances showed that the data is normally distributed. In addition, in the case of progenies, high variations within a family are expected due to a reasonable heritability (Hettasch et al., 2002).

Mean annual increment for each trait were as followed; DBH: 1.2-1.9 cm/yr, CBH: 0.5-0.97 m/yr and HT: 1.1-1.5 m/yr. These values were corresponding to the teak plantations of smallholder in Southwest China; DBH: 1.9-2.4 cm/yr and HT: 1.8-2.3 m/yr. These values derived from 18 teak plantations with the age varied from 7 to 9 years old. In addition, the planting distance was varied from 3 m × 2 m, 5 m × 3 m, and 6 m × 4 m. Growth performance of trees is significantly affected by genetic, environment, genetic by environment (G × E) factors and silvicultural treatments. The study also reported on the quality features of the teak trees. Almost 50% of all the observed trees were forked and the CBH was recorded from as low as 2.9 m (Langenberger and Liu, 2013). From our observation, almost none of the teak trees in our research plot has forking characteristic and the lowest CBH was recorded at 7.3 m. Quality features of a tree such as forking mostly contributed by genetic factor.

In the Tanzanian progeny trial, 6% genetic gain in HT was reported. Albeit the figure cannot be applied directly, it showed an increment of at least 2% from the assumption of the genetic gain obtained from the first superior plus trees selection of a native stand (Kjaer and Foster, 1996). According to Hashim et al. (2015), the new forest plantation model proposed *T. grandis* to be classified under the Medium Rotation Plantation (MRP). MRP is a strategic plan focusing on high-quality timber species. Suggested rotation age is within 45 to 60 years whereas thinning is recommended to be carried out at age 5-7 years and 15-18 years and 25-30 years by removing 40%, 30%, and 20% of the growing stock respectively. This model is appropriate for large and medium scale reforestation model (Hashim et al., 2015).

Table 2: Mean for diameter at breast height (DBH), total height (HT), clear bole height (CBH) and standard deviation (STDEV) of the 29 half-sib families at the age of 14 years old.

No.	Family	DBH (cm) ± STDEV	Family	HT (m) ± STDEV	Family	CBH (m) ± STDEV
1	T5	25.98^{a} ± 7.28	T5	21.27^{a} ± 3.72	R5	13.52^{a} ± 3.39
2	R3	24.55^{a} ± 9.66	R4	20.31^{a} ± 5.96	B83	12.57^{a} ± 3.59
3	B78	23.71^{a} ± 8.07	T0	20.27^{a} ± 5.49	R3	12.56^{a} ± 3.27
4	T13	23.47^{a} ± 7.27	B47	20.25^{a} ± 4.33	B74	12.27^{a} ± 4.07
5	B73	23.33^{a} ± 5.68	R3	20.15^{a} ± 6.04	T0	12.08^{a} ± 3.36
6	R5	23.23^{a} ± 10.13	T13	20.04^{a} ± 4.09	B78	11.99^{a} ± 3.54
7	B83	23.16^{a} ± 6.21	B78	19.94^{a} ± 6.66	R6	11.94^{ab} ± 2.48
8	R6	22.58^{a} ± 9.15	B83	19.90^{a} ± 6.22	T5	11.90^{ab} ± 4.18
9	C2	22.55^{a} ± 7.53	B74	19.69^{a} ± 5.79	B105	11.25^{ab} ± 3.20
10	B81	22.24^{a} ± 8.79	B73	19.67^{a} ± 4.98	B73	11.21^{ab} ± 3.41
11	R4	22.24^{a} ± 7.99	C2	18.83^{a} ± 6.58	B81	11.17^{ab} ± 3.91
12	R1	22.14^{a} ± 6.49	R5	18.77^{a} ± 7.58	R4	11.12^{ab} ± 3.23
13	T0	21.96^{a} ± 5.57	R6	18.38^{a} ± 6.87	B47	11.00^{ab} ± 2.02
14	B74	21.33^{a} ± 7.60	T19	18.28^{a} ± 5.62	R1	10.68^{ab} ± 3.15
15	T17	21.13^{a} ± 8.64	R1	18.01^{a} ± 4.56	C2	10.65^{ab} ± 3.78
16	B105	20.85^{a} ± 7.04	B81	17.91^{a} ± 6.53	T13	10.64^{ab} ± 3.30
17	B47	20.71^{a} ± 7.68	T10	17.90^{a} ± 4.83	T17	10.54^{ab} ± 2.93
18	T32	19.71^{a} ± 8.85	T17	17.41^{a} ± 6.16	T10	10.43^{ab} ± 3.87
19	T19	19.41^{a} ± 6.53	C1	17.23^{a} ± 5.71	T19	10.42^{ab} ± 2.82
20	B29	19.14^{a} ± 6.32	B105	17.08^{a} ± 6.80	C1	10.35^{ab} ± 3.03
21	B75	18.66^{a} ± 8.24	B75	16.77^{a} ± 6.43	T33	10.11^{ab} ± 3.72
22	T16	18.42^{a} ± 7.78	T16	16.65^{a} ± 6.64	B75	10.01^{ab} ± 3.44
23	T9	18.36^{a} ± 8.30	T33	16.58^{a} ± 5.15	T16	9.53^{ab} ± 3.21
24	T10	18.14^{a} ± 4.85	T32	16.32^{a} ± 7.14	T32	9.48^{ab} ± 4.20
25	T18	17.98^{a} ± 8.25	B29	16.26^{a} ± 3.21	T18	9.16^{ab} ± 3.44
26	C1	17.83^{a} ± 5.54	T9	16.20^{a} ± 6.03	T7	9.14^{ab} ± 4.13
27	B27	17.52^{a} ± 6.99	T7	16.19^{a} ± 5.83	T9	9.04^{ab} ± 4.46
28	T7	17.38^{a} ± 7.78	T18	16.04^{a} ± 5.81	B27	8.90^{ab} ± 3.41
29	T33	16.48^{a} ± 4.80	B27	15.28^{a} ± 6.38	B29	7.27^{b} ± 2.49

**Mean with the same alphabet showed no significant different at 0.05*.

Ordinal ranking of the growth traits

Ordinal scale identifies the rank of the families. The overall rank (determined based on the mean ranking of all the growth traits) showed that the top five families were; T5, R3, B78, B83 and R5 (Table 3). If the selection of the progenies were based on the CBH trait alone, the selected families would be R5, B83, R3, B74, T0, and B78 (Table 3). Whereas if the selection were based on the DBH trait alone, the selected families would be T5, R3, B78, T13, and B73 (Table 3). However, considering the economic importance of all the growth traits represent, we decided to select based on the overall rank. Therefore, the selected promising growth' genotypes were T5, R3, B78, B83, and R5.

Table 3: Ordinal ranking of the growth traits showing differences among the 29 half-sib families progeny test of *T. grandis* at the age of 14 years old after planting at Papulut Forest Reserve, Gerik, Perak, Malaysia.

No.	Family	DBH	HT	CBH	Mean	Overall rank
1	**T5**	1	1	8	3.33	1
2	**R3**	2	5	3	3.33	1
3	**B78**	3	7	6	5.33	2
4	T13	4	6	16	8.67	7
5	B73	5	10	10	8.33	6
6	**R5**	6	12	1	6.33	4
7	**B83**	7	8	2	5.67	3
8	R6	8	13	7	9.33	9
9	C2	9	11	15	11.67	11
10	B81	10	16	11	12.33	12
11	R4	11	2	12	8.33	6
12	R1	12	15	14	13.67	13
13	T0	13	3	5	7.00	5
14	B74	14	9	4	9.00	8
15	T17	15	18	17	16.67	15
16	B105	16	20	9	15.00	14
17	B47	17	4	13	11.33	10
18	T32	18	24	24	22.00	20
19	T19	19	14	19	17.33	16
20	B29	20	25	29	24.67	23
21	B75	21	21	22	21.33	18
22	T16	22	22	23	22.33	21
23	T9	23	26	27	25.33	24
24	T10	24	17	18	19.67	17
25	T18	25	28	25	26.00	25
26	C1	26	19	20	21.67	19
27	B27	27	29	28	28.00	27
28	T7	28	27	26	27.00	26
29	T33	29	23	21	24.33	22

Conclusion

Findings from this progeny trial have led to the establishment of clonal trial research plots in 2014 using selected promising growth' genotypes. There were three research plots that have been established at Perlis, Kelantan, and Melaka. Currently, growth data are still being collected from the three clonal trial plots to assess the potential clones that can grow optimally at different environmental conditions. Future study should also include the assessment of the teak wood quality in order to provide thorough information on the potential clones. Selected clones from these trials would be the high-quality planting materials for teak plantation.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Size and Area of *Passiflora edulis* Sims. Mesocarp Cells During Fruit Growth and Development

Shahidah, M.N. and Ding, P.*
Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
**E-mail: phebe@upm.edu.my*

Introduction

Passion fruit is a semi-woody plant that belongs to the dicotyledonous family Passifloraceae. Purple passion fruit (*Passiflora edulis* Sims) is the most well-known cultivar after yellow passion fruit (*P. edulis* Sims *f. flavicarpa* Deg). It is cultivated commercially in tropical and subtropical countries such as Argentina, Brazil, and India (Pinzon et al., 2007). The plant can be self-fruitful with natural pollination, especially under humid condition. Research conducted by Shahidah et al. (2019) showed that the percentage of fruit formation was not affected by either natural or assisted pollination. Pollen viability of passion fruit that can last 24 h after anthesis makes it responsive for its viable reproductive capacity. Currently, passion fruit is grown mainly for fresh and processed fruit for both domestic consumption and export. The value of fresh passion fruit is higher than the processed fruit as it is legislated to a stricter requirement (Shahidah and Phebe, 2020). Codex Alimentarius decrees the fresh fruit should be consumed readily after preparation and packaging. Big fruit size is a significant contributor to achieve the perception of high-quality fruit and as a critical consideration for export grading. The fruit is graded into three classes (Class 1, 2, and 3) based on the external appearance such as fruit shape, colour and size. The size of fruit is determined by the maximum diameter of the equatorial region; segregated into A (>78 mm), B (67-78 mm), C (56-67 mm), and D (≤56 mm) (Codex, 2014).

The anatomical feature of passion fruit is a modified berry that evolves from a single ovary equipped with a leathery rind that appears in green when unripe, cream-yellow as it gets mature and dark purple when it achieves ripening (Pinzon et al., 2007). The rind composed of the pericarp layer (external part) and dominated by mesocarp, the spongy white area. The cell located in the mesocarp region is called mesocarp cell as it has been used widely in cellular studies. Mesocarp cells play a great role in external fruit quality; they can determine fruit size, shape, softening, and peel cracking development (Tee et al., 2011; Grimm et al., 2019). Fruit organ is built by the cellular process of cell division, cell enlargement and cell differentiation. Each step of the developmental stage is influenced by the cell number and cell size of fruit (Bünger-Kibler et al., 1982; Li et al., 2010). Investigating the mesocarp cell of passion fruit may drive knowledge into the underlying process of how cellular development influence the final fruit size. To our knowledge, there is no study have yet been conducted on the influence of cell number and cell size on the final fruit size of passion fruit. Thus, this research was conducted to study the changes in cell number, diameter, and area at the mesocarp region of purple passion fruit during fruit growth and development.

Materials and Methods

Plant materials

The experiment was conducted at Ladang 10B, Universiti Putra Malaysia (2.9993° N, 101.7079° E) from March 2018 to December 2019. Twenty trees were planted in the 25 L pot supplied with commercial planting soil. The plants were maintained according to the guidelines of passion fruit agricultural practice (Joy et al., 2010). Growth and development of fruit were observed one day after pollination (DAP) and subjected to pollination treatments as described by Shahidah et al. (2019). Only natural pollinated fruit was used for this experiment. A total of 15 fruits were harvested weekly from 7 to 63 DAP, contributing to a total of 145 experiment units.

Fruit size and microscopic observation

The size of the fruit was denoted by length and diameter. The length and diameter of fruit were taken by measuring the length at longitudinal and diameter at the equatorial region of each fruit using a Vernier calliper. Results were expressed in centimetre (cm). Fruit's mesocarp at equatorial region was sliced at 1 cm^3 using blades and fixed with ethanol, acetic acid and formaldehyde at 9:1:1 ratio for three days as modified from Mohamad and Ding (2019) method. After that, samples were vacuumed and post-fixed overnight in osmium tetroxide. Samples were washed with distilled water prior to the dehydration process in an increasing acetone gradient series, 30 min each concentration. The samples were then dried using a critical point drier (CPD 030, Germany), mounted on metal stubs and sputter-coated (JEOL JFC-1600, Japan) in gold and viewed under a high vacuum scanning electron microscope (SEM) (JOEL JSM-5610LV, Japan). For measuring the cell number, diameter (μm), and area (μm^2), image obtained by SEM were processed with the program ImageJ (National Institute of Health, Bethesda, MD) following the method applied by Li et al. (2010).

Statistical analysis

Data were analysed using analysis of variance (ANOVA), followed by Duncan's multiple range test at 5% confidence interval, while the correlation between all the analysed variables was subjected to Pearson's correlation at 5% interval.

Results and Discussion

In this experiment, mesocarp at fruit equatorial region was selected to study the cellular development as it has been used widely to study the growth pattern of fruit as applied in Japanese pear (Zhang et al., 2006), pineapple (Li et al., 2010) and banana (Tee et al., 2011). Mesocarp cell in the equatorial region can be a good indicator for fruit size since there is a strong correlation between cell layers with fruit weight and fruit size, as proven by Zhang et al. (2006) study. The results showed that cell diameter (Figure 1a), cell area (Figure 1b) and cell number (Figure 1c) changed significantly during growth and development. A non-significant increment in cell number was observed from 7-14 DAP, suggesting that significant mesocarp development might not yet occur at the early stage of growth. Only the ovary and pericarp undergo rapid cell division after the fruit set event is initiated (Zhang et al., 2006). It is proposed that the growth pattern of passion fruit cell is following the basic three stages of development; cell division, expansion and differentiation (Bünger-Kibler and Bangert, 1982). The cell division took place from 21-28 DAP. The mesocarp possesses 3-fold cell number increment at 21-49 DAP. This stage took 28 days before the cell reached a plateau state at 56 DAP. By 63 DAP the fruit has fallen from the tree, indicating senescence took place. A similar trend was reported by Li et al. (2010) by which the growth of pineapple fruitlet at mesocarp region possess a similar cellular trend of development through cellular division, expansion, and differentiation state.

In the second phase, cell expansion estimated happened as early as 7 DAP, suggesting the process occurred immediately after the fruit set. The mechanism of mesocarp development in fruit is believed to happen simultaneously with cell division. The result showed that the number of cells increased significantly during 7-28 DAP (Figure 1c), which concomitant with cell diameter and area increment (Figures 1a and 1b). The increase in the cell's size and diameter may happen due to cell enlargement. New cell wall materials and controlled loosening of the cell wall allows the cell to become stretch and increases its diameter and area (Rubio-Díaz et al., 2012). The cell enlargement mechanism is species-dependent. It may occur at an early or later stage of development. For tomato, this event occurred as soon as 2 DAP after the fruit set took place (Renaudin et al., 2017). Vice versa, in apple, it happened at the later stage of development after the cell has achieved a full cell differentiation (Peter et al., 2009).

The third stage of passion fruit growth began at 35 DAP as cell diameter (Figure 1a), cell area (Figure 1b) and cell number (Figure 1c) shows plateau growth until 63 DAP. During this phase, cells may

differentiate while fruit mature and ripen, and finally, senescence took place at 63 DAP. In olive development, mesocarp cell differentiation happened after enlarged mesocarp cell size reached a plateau state. The study suggests at this stage, gradation in cell size take place in the mesocarp exterior to its interior, forming a matured fruit (Rallo and Rapoport, 2001). During this stage, the mesocarp cell remains parenchymatic while sclerification occurred at the endocarp cell. Besides that, vascular tissue will become more branched vascular differentiate between mesocarp and endocarp. As the fruit further developing, the inner vascular bundles that are well-differentiated build massive sclerenchymatous sheaths and numerous twisted networks of fibre-like (Horbens et al., 2014). It is interesting to see the cell diameter (Figure 1a), cell area (Figure 1b) and fruit size (Figure 1d) possesses a similar trend; rapid development during 7-28 DAP before plateau pattern occurred. Pearson's correlation ($0.60 \leq r \leq 0.88$) study shows a medium to a high correlation between cell and fruit size (Table 1). Vice versa, cell number possess a weak correlation ($0.43 \leq r \leq 0.55$) with fruit and cell size. Plateau trend of cell number, cell size and fruit size (Figure 1a-d) from 28 DAP onwards causes the fruit to obtain its full size.

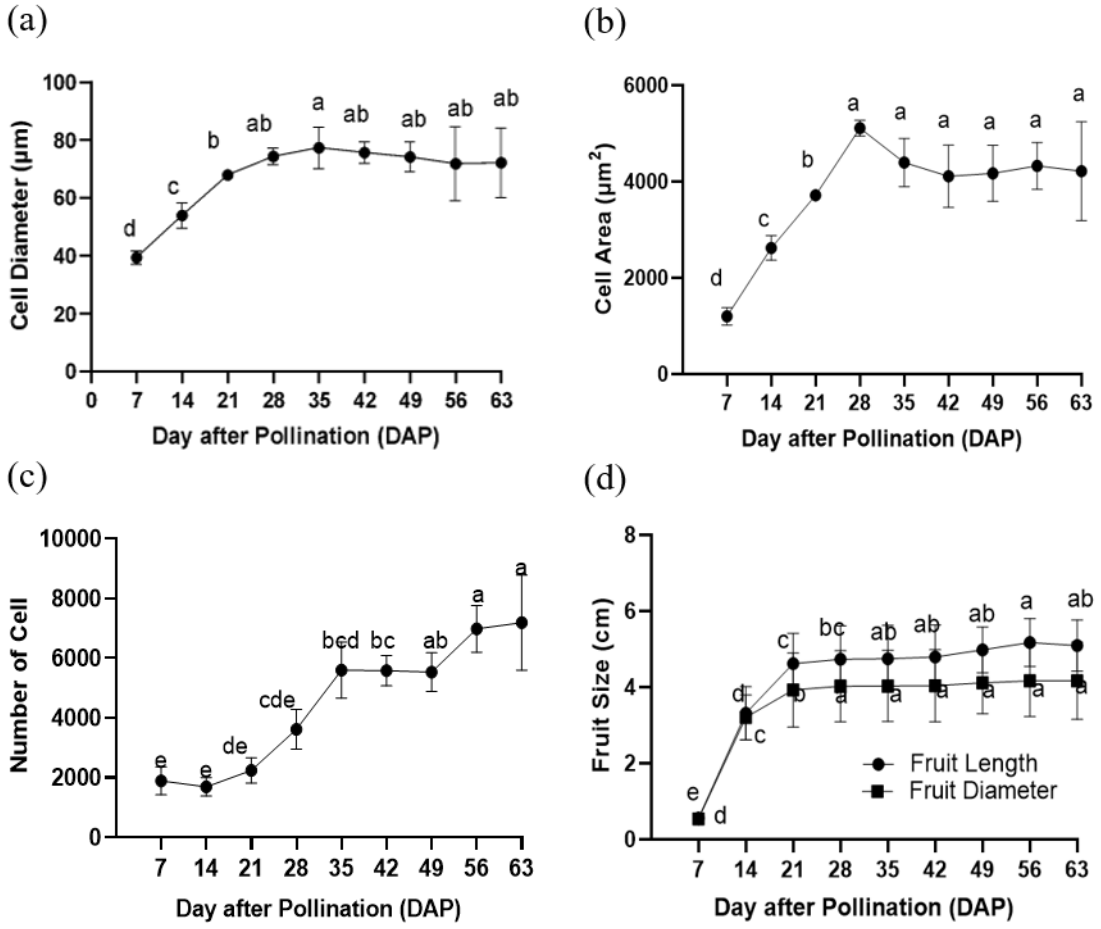

Figure 1: Changes in the (a) cell diameter, (b) cell area, and (c) cell number as processed by ImageJ, and (d) fruit size as measured by Vernier calliper.

The strong correlation between cell size and fruit size has answered our proposed hypothesis that fruit size is regulated by cell size. This result is analogous to the development of Arabidopsis (Ripoll et al. 2019), apple (Peter et al., 2009) and Karanda fruit (Mohamad and Ding, 2019) in which cell size is critical in the determination of fruit size. Thus, it can be concluded that passion fruit final size is a function of cell size rather than the cell number. Final fruit size is the consequence of complex metabolic events that occur during fruit development. Thus, factors affecting mesocarp development such as

photosynthesis, spur characteristic, and carbon partitioning can be tackled to control the final fruit size (Zhang et al., 2005).

Table 1: Correlation coefficient (r) of cell number, area, and diameter at mesocarp region, fruit length and diameter during growth and development of purple passion fruit.

	Cell diameter	Cell area	Cell number	Fruit length	Fruit diameter
Cell diameter	.	.	.	.	.
Cell area	0.88***	.	.	.	.
Cell number	0.39^{ns}	0.43**	.	.	.
Fruit length	0.76***	0.77***	0.55**	.	.
Fruit diameter	0.78***	0.83***	0.50*	0.60***	.

*^{ns} no significant difference at P=0.05; Significant at, *(P≤0.05), **(P≤0.01) and ***(P≤0.001).*

Conclusion

This study proved that the final fruit size of purple passion fruit is dependent on mesocarp cell size. The current work is a first step in examining the mesocarp cell at the cellular level. Future study will be needed to study the contributing factor from genetic and environment that can affect the final fruit size.

Acknowledgements

This research was supported by Southeast Asian Regional Centre for Graduate Study and Research in Agriculture (SEARCA), ASEAN (Reference no: 19-1404), and German Academic Exchange Service (DAAD), Germany (Reference no: 91739221).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

The Effect of Water Deficit on Growth of *Oryza sativa* L. cv. MR 219

Heary Azween, A. and Wan Natasya, W.A.*
Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (Melaka) Jasin Campus, 77300 Merlimau, Melaka, Malaysia.
**E-mail: natasyaahmed@uitm.edu.my*

Introduction

Paddy (*Oryza sativa*), commonly known as rice, is a global staple food consumed in many Asian countries. Rice can be found mainly in food products such as cereals, white flour, and noodles. However, with the increasing world population, the demand for rice production also increases, exposes the world to a pressure of water shortages (Datta et al., 2017).

Paddy cultivation requires a considerable amount of water and paddy itself is semi-aquatic rice that is extremely sensitive to water deficit (Predeepa Javahar, 2013). The water requirement and sensitivity of rice in response to water deficit are various according to their growth stages. Additionally, a timely and appropriate water level from vegetative to physiological maturity is vital because inadequate water supply in the field will interrupt the entire growth cycle of rice cultivars (Subramanian, 2012).

The water requirement for paddy is most critical during its vegetative, reduction, and spikelet formation growth stages, where the paddy needs to continuously submerge in the water. Hence, it is best to avoid imposing water stress during these periods, as the rice cultivars need adequate water until it is gradually drained before ripening and harvesting stages (Naipictuasdharwad, 2009).

Proper water management at different growth stages is crucial to ensure the optimum growth, and different paddy varieties respond differently to water deficit. This study is conducted to determine the effects of water deficit on the growth of paddy variety MR 219, and to identify the most suitable water level required for paddy at different growth stages.

Materials and Methods

The paddy variety MR 219 used in this study was obtained from Pejabat Pertanian Daerah Jasin, Melaka. Seeds are pre-soaked with fresh water for 48 hours at normal room temperature to break seed dormancy and allow pre-sprouting of seeds. On 23rd Jan 2020 seeds were sown in 16 experimental pots containing soil at 20 cm depth. Fertilizers and agronomic practices were applied as recommended by Jabatan Pertanian Malaysia.

Treatments and experimental design

The total of 16 pots served as experimental units comprised of four treatments in four replications each. Experimental design was Complete Randomized Design (CRD). Four treatments consisted of; T0 = continuously flooded at 10 cm water level until maturity, T1 = early flooding at 10 cm water level up to panicle initiation stage (55 Days After Sowing (DAS), T2 = early flooding until 30 DAS followed by saturated condition until maturity, and T3 = continuous saturated condition up to maturity as described by Juraimi et al. (2009). The soil held under saturated state during the treatment of sowing and flooding was began at 7 DAS. For T3, the water was released into the soil only when saturated (maintaining the soil to muddy state without water standing) up to a limit level of 5 mm.

The parameters measured were the number of tillers, plant height (cm), and flag leaf width (cm). The plant's height was determined from the plant base until the highest leaf's edge by using a measuring tape. Meanwhile, when tillering appeared (5 leaves stage), the number of tillers per plant was counted

and dismissed at the initial stage of panicle when the flag leaf developed. Lastly, the flag leaf width of all treatments was measured. All data were taken at 15, 30, 45, 60, 75, and 90 DAS and analyzed using One Way Anova by Statistical Package for Social Science (SPSS) software version 22.

Results and Discussion

The results obtained revealed that paddy MR 219 under saturated condition significantly affect several tillers and flag leaf width except for plant height. Figure 1 showed that the mean of plant height was tallest for paddy MR 219 in T0; control flooded treatment (66.48 cm), thus has proven as the best condition favoured for rice growth. It is well assured that there were no signs of water shortage occured throughout the growing stages. Generally, paddy that grew under T3 conditions was shorter in heigh as compared to the other treatments that received continuous floodings. The results obtained was supported by Sokoto et al. (2014) who observed that plant height reduced as soil moisture stress increased, and thereby had suggested that the decrease in plant height was due to water stress at the tillering stage.

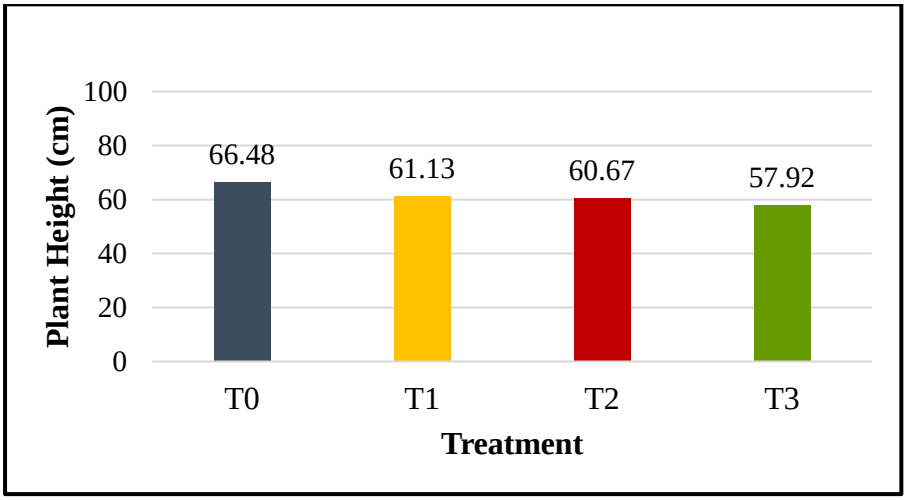

Figure 1: Mean of the number of tillers for paddy MR 219.

The number of tillers was recorded significant ($P<0.05$) when compared between treatments. The tillers' number was maintained with two tillers at 15 DAS in each experimental pot for all treatments. At 45 and 60 DAS, paddy grown under T0 which control flooded at 10 cm water level until the maturity stage recorded the highest mean number of tillers (31.29). From the result shown (Figure 2), the shorter duration of flooding in T1 (field capacity at panicle stage) (30.08) and T2 (field capacity at flowering stage) (29.96) resulted nearly similar to T0 (continuous flooding). This showed that, all flooding treatments except saturated condition significantly favoured the number of tillers (Juraimi et al., 2009). On the contrary, it was notable that plants grown under T3 (saturated condition) were comparable with under flooded conditions when the result demonstrated the least mean number of tillers at only 23.67.

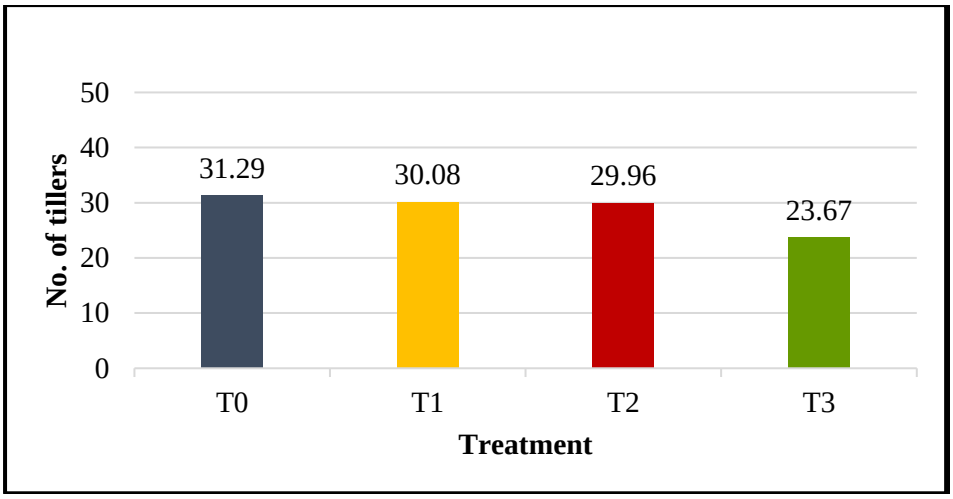

Figure 2: Mean of the number of tillers for paddy MR 219.

The results obtained for the width of flag leaf showed a significant difference ($P<0.05$) for all treatments tested. Figure 3 showed paddy grown under T0 (control flooded at 10 cm water level until maturity stage) documented the highest mean for width of flag leaf (1.15 cm). This explained that plants grown under continuous flooding significantly favoured flag leaf width as there was no shortage of water occured throughout the growing stages. At the end of the study, it was found that both treatments T1 (field capacity at panicle stage) and T2 (field capacity at the flowering stage) resulted in the almost similar mean number of flag leaf width (cm) at 1.09 cm and 1.08 cm respectively. Finally, T3 (continuous saturated condition) was found to have the least mean number of flag leaf width as compared to other flooding regimes. Prolonged water stress has leds to water loss. It forces stomatal closure to restrict gas exchange, which later interrupts the leaf water potential, stomatal activity, thus reducing gas exchange, which later interrupts the leaf water potential, stomatal activity, thus reducing cell enlargement and growth (Hasanuzzaman et al., 2019).

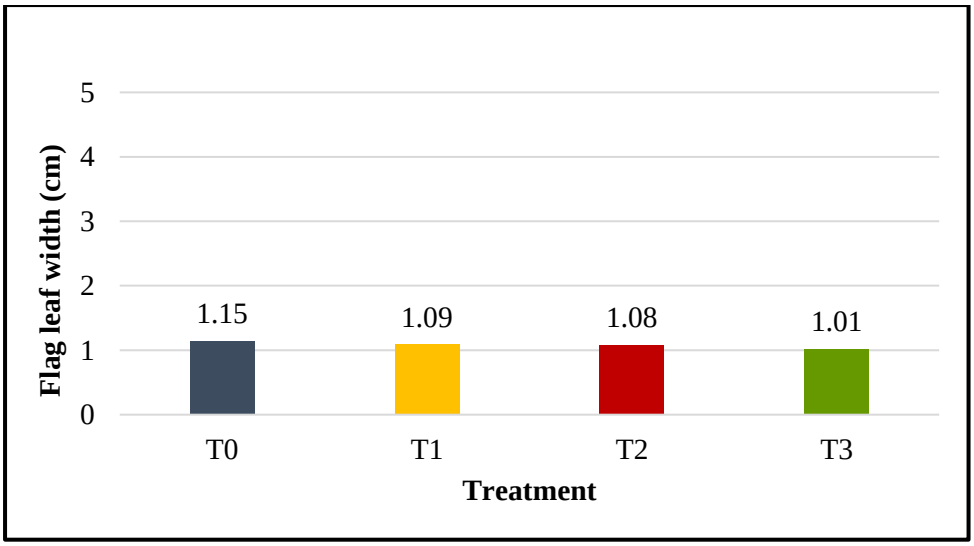

Figure 3: Mean of the number of flag leaf width for paddy MR 219.

Conclusions

The response of paddy variety of MR 219 to water deficit are varies according to its growing stages. Specifically, water deficit had significantly lowered the number of tillers and the rice plant's flag leaf width. At the beginning of the study, the flooding regime did not significantly affect the paddy's growth

at the initial stage of 15 and 30 days after transplanting. However, after 45 DAS and onwards, different flooding treatments at all stages affected all the treatments tested. The flooding treatments T0, T1, and T2 significantly increased the numbers of tiller and flag leaf width, while saturated condition (T3) had reduced the growth. In the contrary, T0 (continuous flooded) did not significantly influence the height of paddy MR 219. An unexpected result found in this study possibly attributed by the inconsistency of water depth for flooding treatments due to evaporation lost during warm weather. Therefore, imposing adequate water supply at all growth stages is crucial to meet the maximum potential of rice cultivars.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Growth Rates of Tissue Culture *Hevea brasiliensis* (Rubber) Trees

Nor Mayati, C.H.*, Izilawati, M. and Ahmad Shukri Hadafi, I.
Genetic Resources and Improvement Unit, Production Development Division, RRI Experimental Station, Malaysian Rubber Board, 47000 Sg. Buloh Selangor, Malaysia.
**E-mail*: *normayati@lgm.gov.my*

Introduction

Tissue culture approach is an alternative *in vitro* method for producing true-to-type plants and propagating root stocks of high-quality planting materials with desired genetic traits. This is to overcome the problems of incompatibility between root stock-scion within a short time frame. Tissue culture clones have been reported to be fast growing, high yielding, and resistant to a wide range of diseases with significant increase in wood volume of up to 6% (Chen et al., 2004). *In vitro* technology of *Hevea* was initiated in RRIM since 1964 but with limited success. Only tissue culture protocol for GL1 clone was established due to its highly embryogenic nature (Jaafar and Hamzah, 1998). However, GL1 clone has been superseded by many series of higher yielding clones. In 1995, some of these selected clones comprising of 2000 and 3000 series were breed and recommended, due to higher increase in latex yield and er wood volume production. These clones were also known Latex-Timber Clones (LTC) (Ong et al., 1995). For instant, RRIM 2025 clone is now favoured in forest plantings due to its desired characteristic in timber production (Nurmi-Rohayu et al., 2015).

The objectives of this study are to enhance latex yield and timber productivity. This can then lead to higher economic output such as faster growth rates, resistance to abiotic stresses such as wind damage and tapping panel dryness-TPD, leaf/root diseases, and promoting drought (low water deficit or temperature) tolerance. These capabilities for adapt to different ecological conditions in any cropping areas would surely help to generate higher profit for our local rubber growers. Thus, in this study we discussed the growth rates, wood volume, tree's height, girth diameter, and annual increment of tissue culture clones in RRIM2025 as to other selected clones i.e. RRIM600 and GL1.

Materials and Methods

Field planting and management

Tissue cultured clones, *Hevea brasiliensis* were planted in 2004 at Field 118, RRIM Kota Tinggi Experimental Station, Johor (1°48'N, 103°50'E) and comprising of 0.64 acre. Total number of trees transplanted were 316 and these clones include some of the conventional bud grafted RRIM 2025NB (control) (48 trees), tissue culture GL1MP (28 trees), replacement of RRIM 2025NBr (19 trees), tissue culture mother plant RRIM 2025MP (12 trees), twigs re-cultivated RRIM 2025V1 (101 trees), RRIM 600MP (14 trees) and twigs re-cultivated RRIM 600V1 (8 trees). The planting distance was 6.09 m x 3.65 m (Nor Mayati and Abd Razak 2018).

Rubber tapping was commenced in year 2016 and about 247 trees were evaluated. However, these were reduced to 230 (2017), 233 (2018), and 235 (2019) trees. At the end of the trial many trees were found to have dried off with few numbers of trees capable of recovering even after being treated with nanosilver solution (data not shown). As such, any mortality due to tapping panel dryness (TPD) incidence were discarded as an experimental unit. Yearly growth rates were taken from March to April based on the girth size at breast height of 150 cm above ground level, total height (straight trunk), and wood volume or clear bole volume (CBV). Other field maintenance carried out include regular manured fertilizer using NPK Blue with 720 g per tree (Table 1) and annual weeding was carried out three times per year using glyphosate (Table 2).

Table 1: Field maintenance manured with recommended fertilizers.

Year	Fertilizer type	Amount per tree (g)	Schedule
2015	SOA and MOP	300 and 300	May 2015 and Sept 2015
2016	NPK Blue	720	Nov-16
2017	NPK Blue	720	Mar-17
2018	NPK Blue	720	Mar-18
2019	NPK Blue	720	Mar-19

NKP Blue (12:12:17:2) - (720 g) = Mag J (8.6:10.8:12.6:2.1) - (900 g)

Table 2: Field maintenance weeding control with herbiside.

Yearly	Herbicide	Schedule	Responsibility
Cycle 1	Glyphosate	January	SPKT
Cycle 2	Glyphosate	Oktober	SPKT
Cycle 3	Glyphosate	December	Kontraktor SPKT

Growth performance measurement

Annual growth rate was carried out and parameters measured include: (i) girth increment and clear bole volume (CBV), calculated as follow (Ramli et al., 1995):

$CBV = \pi/12\ [(D1+D2)^2 - (D1 \times D2)] \times t$ (bole high) *yearly

i. D1 = diameter at 60 cm from ground
ii. D2 = diameter at 150 cm from ground
iii. t = (tan D1 x distance of observer from the tree) + (tan D2 x distance of observer from the tree) = planting distance

**The bole height was measured using a clinometer*

Statistical analysis

The experimental design used was Complete Randomised Design (CRD) and data were subjected to analysis of variance (ANOVA) using the SAS General Linear Model (GLM)l Analysis System. Data that was found with significantly different were then subject to Least Significant Difference (LSD) test at $P \leq 0.05$.

Results and Discussion

Tree growth rate

Clones of mother plant RRIM2025MP showed the highest girth increment as compared to all budded twigs of RRIM2025V1 and RRIM2025NB. However, the old clones of RRIM600MP, RRIM600V1, and GL1MP recorded the smallest gain although there was a slightly higher growth in RRIM600V1 (Table 3). The total height and wood volume were also found to be high but there was no significant difference between the clones of RRIM2025MP, RRIM2025V1, and RRIM2025NB. However, there was a significant difference for clones RRIM600MP and RRIM600V1 (Table 4).

Table 3: Girth and diameter trunk of the tapped clones (TC) trees.

Parameters	Girth at 150 cm (cm)				Diameter at 150 cm (m)			
	2016	2017	2018	2019	2016	2017	2018	2019
Clones								
RRIM2025MP	77.14[a]	77.35[a]	78.51[a]	77.04[a]	0.246[a]	0.246[a]	0.250[a]	0.245[a]
RRIM2025V1	71.22[a]	72.13[a]	73.04[a]	71.71[a]	0.227[a]	0.229[a]	0.233[a]	0.232[a]
RRIM2025NB	70.27[a]	70.36[a]	72.19[a]	72.32[a]	0.224[a]	0.224[a]	0.230[a]	0.230[a]
RRIM600MP	49.17[b]	50.35[b]	49.99[b]	48.84[b]	0.157[b]	0.160[b]	0.156[b]	0.156[b]
RRIM600V1	53.71[b]	54.13[b]	51.82[b]	52.44[b]	0.171[b]	0.172[b]	0.165[b]	0.167[b]
GL1MP	50.09[b]	47.22[b]	49.32[b]	49.54[b]	0.159[b]	0.160[b]	0.157[b]	0.158[b]
P value ($\alpha = 0.05$)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV	21.86	23	22.59	22.77	21.86	21.91	22.59	22.77

Table 4: Total height and clear bole volume of the tapped clones (TC) trees.

Parameters	Total height (m)				CBV (m^3)			
	2016	2017	2018	2019	2016	2017	2018	2019
Clones								
RRIM2025MP	4.057[a]	4.125[a]	4.424[a]	5.580[a]	0.268[a]	0.275[a]	0.292[a]	0.349[a]
RRIM2025V1	4.059[a]	4.178[a]	4.613[a]	5.376[a]	0.229[a]	0.240[a]	0.267[a]	0.311[a]
RRIM2025NB	4.075[a]	4.216[a]	4.677[a]	5.517[a]	0.219[a]	0.225[a]	0.261[a]	0.310[a]
RRIM600MP	3.022[c]	2.925[b]	3.372[b]	3.758[b]	0.079[b]	0.081[b]	0.088[b]	0.094[b]
RRIM600V1	3.199[bc]	3.185[b]	3.400[b]	3.767[b]	0.106[b]	0.107[b]	0.106[b]	0.117[b]
GL1MP	3.849[ab]	3.845[a]	4.146[a]	4.332[b]	0.095[b]	0.096[b]	0.100[b]	0.101[b]
P value ($\alpha = 0.05$)	0.0026	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV	26.29	22.74	20.59	25.18	55.63	52.21	51.52	57.64

Average increment of girth, diameter, total height, and clear bole volume (CBV) were compared and presented in Table 5. However, normal budded (NB), RRIM2025 were reclassified into three types based on their sources and different planting year/time. All NB in RRIM2025 is based only by it's physical appearance as the year of planting is unknown, while NBc are control trees which are clearly recorded in the existing point plan and NBr are the replacement trees that are planted whenever the original trees were dead, resulting in different age of the trees. These reclassifications are considered crucial as it showed the characteristic of the girth size growth. The highest average girth increment during four years of evaluation periods were found in older clones of GLIMP and RRIM600MP. Interestingly, RRIM2025MP and other replacement clone of RRIM2025NBr showed lower growth rates (Table 5). However, instrumental errors may occur during the course of the measurements.

Table 5: Average increment (%) of parameters evaluated for trees planted in F118, Pelepah Division, SPKT.

Parameters	Average increment (%)			
Clones	Girth at 150 cm	Diameter at 150 cm	Total height (m)	CBV (m^3)
RRIM2025MP	0.89	1.02	11.70	9.44
RRIM2025NB	1.05	1.06	12.52	15.56
RRIM2025V1	1.27	1.31	9.95	10.84
RRIM600MP	2.40	0.96	13.38	5.57
RRIM600V1	0.99	0.90	8.77	5.66
RRIM2025NBc	1.62	1.69	15.34	17.13
GL1MP	2.45	0.00	6.16	2.07
RRIM2025NBr	0.19	0.75	11.31	11.10

Conclusions

The growth of tissue culture clone in RRIM2025 were found to outperform better as compared to other bud grafted counterparts. Moreover, RRIM2025 is breed as a latex timber clone and possessed other desired characteristics such as bigger trunk size compared to clones in RRIM600 and GL1. However, care must be taken as these clones may have weaker rooting systems due to nature of its pseudo tap root, less lateral root biomass and susceptible to windfall during field planting. However, these older trees may have adapted to the soil dynamics, under ground water sources, and other environmental conditions. It is recommended that further research related to its roots morfology and other trees hydraulic capacitance are necessary.

Acknowledgement

The author gratefully thanks for the support and assistance from staffs of Crop Management Unit (SPKT) and Genetic Resources and Improvement Units for their assistance in data collections and field management. The research is funded by the Malaysian Rubber Board, Project Code: S2017/IP/2017(36)651.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Different Rootstock Age on Grafting Success and Growth Performance of *Garcinia atroviridis*

Nurhazwani, M.[1,*], Saifuddin, I.[2] and Muhammad Syakir, B.[1]

[1]Industrial Crop Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Agrobiodiversity and Environment Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: wanie@mardi.gov.my*

Introduction

Garcinia atroviridis, popular as 'Asam Gelugor' among locals is an underutilized fruit, commonly found in Peninsular Malaysia, Thailand, and India (Lim, 2012). This plant belongs to the Clusiaceae family with height can reach 20 m (Jansen, 1992), smooth grey long trunk and branches drooping. The plant leaves are dark green, shiny, and long narrow with pointed tip while its flowers are dark red and round in shape. The fruits, which are the most utilized part, are large yellowish green to yellow in colour and globular in shape (Tandon et al., 2007). *Garcinia atroviridis* is commonly used as flavouring agent in cooking. The ripe fruits are sliced, dried, and used to provide sour sensation in dishes to replace tamarind. Meanwhile, the young leafy shoots and leaves are consumed fresh or cooked as vegetable. The plant parts also have some medicinal values. It was used as pre and postpartum medication in treating stomach-ache due to pregnancy and as lotion to rub over abdomen after confinement. It also used to treat ear-ache, cough, throat irritation, dandruff, improving blood circulation, and as laxative (Lim, 2012).

However, there is lack of prioritized research on this species has been documented. Asam gelugor trees are mostly germinated from seeds and planted in home gardens and orchards. The germinated seedlings produce about 70% male trees which unable to bear fruits. To maintain the genetic characters from propagated variety, grafting is suggested as vegetative plant propagation method for most of the fruit crops. Vegetative propagation comprised of joining or combining scion and rootstock, and subsequently grows as one individual plant where the rootstock develops the root system, and the scion grows as upper fruiting part of the grafted plant. Rootstocks can be seedlings, rooted cuttings, or layered plants. Rootstocks may influence grafted plants' various physical and pomological traits such as size, growth habits of the tree, time of fruit maturity, and yield (Mukherjee and Litz, 2009). Grafting facilitates numerous advantages, including early flowering, smaller size plant with bushy canopy, and start to bear fruit earlier than seedling trees (Janick et al., 2010). Moreover, asexual propagation including grafting is an appropriate technique to maintain true-to-type of a given variety that enables to produce offspring with similar characteristics as the mother plant (Nakasone and Paull, 1998). One of the significant requirements to increase the production of asam gelugor planting materials would be rapid multiplication and production of female plant planting materials.

However, grafting's success depends on various factors such as environmental influence, grafting method, time, age, and type of rootstocks used. The suitable grafting technique must be developed to meet the increasing demand for this fruit species' planting materials. This study was carried out to determine the effect of two different rootstock ages on grafting success and to evaluate the growth of *G. atroviridis.*

Materials and Methods

Planting materials and preparation of rootstock

The *G. atroviridis* seeds were collected from Air Kuning, Perak in November 2018. Seeds were initially removed from the fruit, washed in running water and then germinated in the trays filled with planting

media. Seed was sown in about 1 cm depth sand media. Seedlings with three to four matured leaves will be transplanted into polybags size 20.3 cm x 30.5 cm containing growth media and kept under 50% shading. Plant maintenance was carried out according to the normal recommended practices. The experiment was carried out in a nursery and irrigation was applied immediately after seed sowing and repeated twice daily till the final emergence. After seed germination, irrigation was applied twice every day.

Propagation techniques

Two different age of *G. atroviridis* rootstocks (12 months old and 18 months old) were used for grafted with scions from certified female plant. Scions from certified superior female plant of *G. atroviridis* species was collected and grafted onto their respective species rootstock using top wedge grafting technique. The scion shoots of asam gelugor (10-15 cm length) at the same size with the rootstock were collected on the same day for grafting purposes. Successful grafted plants were covered with clear plastic bags and watered twice daily. General plant protection measures were carried out by applying fungicides and pesticides to control pests and diseases.

Growth measurement

Plants were sampled randomly from each treatment to determine the number of the new leaves at 30 days and 60 days which was started from the date of grafting operation. The number of new leaves per graft was manually counted based on fully expanded new leaves.

Survival percentage of the grafts was calculated after two months of grafting by using the following formula:

$$\text{Survival percentage (\%)} = \frac{\text{No. of grafted plants in the experiments}}{\text{Total no. of grafted plants}} \times 100$$

Experimental design and data analysis

This experiment was conducted in a randomized complete block design (RCBD) with two different ages of rootstock with four replications comprised of 10 plants per replication. The data obtained were analyzed 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 $P \leq 0.05$.

Results and Discussion

The highest percentage of survival of grafts was found in the plants grafted by the top wedge grafting technique using 18 months age of rootstock (90%) and the lowest was for 12 months age of rootstock (85%) after two months of grafting with no significant difference (Figure 1). The results showed that the efficacy of the top wedge grafting at the age of 12 months and above was the best age for grafting. The survival of asam gelugor scion produced by top wedge grafting might be due to the quick graft union process after grafting. Rootstock age is a factor that affects grafting success (Hartmann et al., 2002 and Kumar, 2011). The success and survival rate of grafting can be increased using proper rootstock and appropriate grafting time of the year based on the desirable growing conditions (Simon et al., 2010), and also by enhancing the skills and knowledge of gardener who involve in grafting operation (Akinnifesi et al., 2008). Furthermore, the successful rate of grafts also depends on grafting techniques used (Soleimani et al., 2010). The variety and age of rootstock as important factors for the highest percentages of graft success and survivability, and growth was reported in epicotyl grafting of mango (Jose and Velsalkumari, 1991).

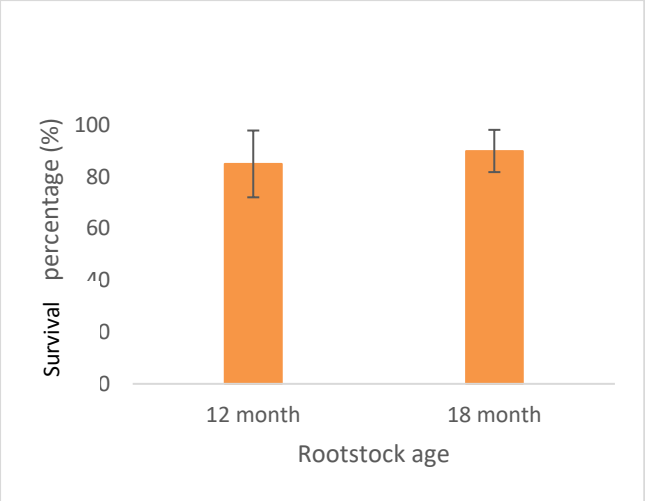

Figure 1: Survival percentage using two different age of rootstocks.

After 30 days, the number of new leaves on scion grafted with rootstock at 12 months of age and 18 months of age has no significant difference. At 60 days after grafted, the highest mean number of leaves (7.25) was produced by 18 months of age rootstock and the lowest mean number of leaves (3.00) produced by 12 months of age rootstock with significant difference (Figure 2). Results are in line with Hartmann et al. (2002) who reported that the age of the rootstock influenced the regeneration of the plant parts' due to differential activity of meristematic cells. The highest mean for the number of new leaves on the plants raised by top wedge grafting might be due to the scion inducing adequate callus formation, rapid dissociation of the barrier zone, quick healing of the grafting union, and a rapid intermingling and interlocking of the vascular tissue (Hartman et al., 1997). In general, the lowest graft union success could be attributed to the lack of intimate contact of the cambial region of both stock and scion and interference of latex exudation (Hartmann et al., 2002). It was reported that the rapid formation of callus (parenchymatous) tissues allowing the translocation of vital biochemical compounds of stock and scion might be the reason for the minimum number of days for graft union (Kilany et al., 2012). Additionally, translocation of vital biochemical compounds of stock and scion might be the reason for increasing scion height and number of leaves per graft in rootstocks. From the results, it was found that the efficacy of the top wedge grafting at 12 months of age and 18 months of age are not significant but based on growth performance indicated that top wedge grafting of 18 months of age rootstock is recommended to propagate *G. atroviridis* (Figures 3 and 4).

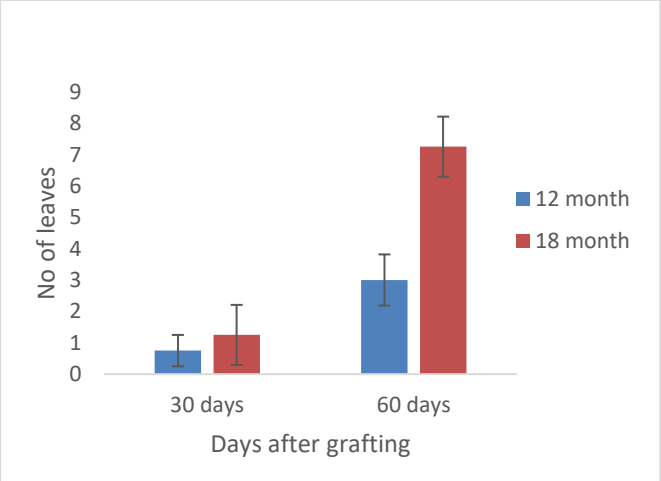

Figure 2: Number of leaves between two different age of rootstock at 30 days and 60 days after grafting.

Figure 3: *Garcinia atroviridis* at 60 days after successful grafting using two different age of rootstock.

Figure 4: Successfully grafted *Garcinia atroviridis* plants.

Conclusion

Based on the results, top wedge grafting of 18 months of age rootstock is the best approach to propagate *G. atroviridis* as it increased the success and survival rates and improved growth of the planting materials of the species.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Mangosteen: Performance of Vegetative Growth in Three Different MARDI Stations

Salehudin, M.R.[1,*], Ab Kahar, S.[2], Mohd Fairuz, N.[3], Adnan, K.[1], Mohd Ismail, M.K.[3], Zalina, E.[3], Mohd Apandi, S.[3] and Rais, H.[1]

[1]Agrobiodiversity and Environment Research Centre, MARDI Sintok, 06050 Bukit Kayu Hitam, Kedah, Malaysia.
[2]Agrobiodiversity and Environment Research Centre, MARDI Ibu Pejabat, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[3]Agrobiodiversity and Environment Research Centre, MARDI Jerangau, 21820 Ajil, Terengganu, Malaysia.
**E-mail: salehudin@mardi.gov.my*

Introduction

Mangosteen has been deemed as queen of fruits in Malaysia and has sweet and pleasant taste. It is one of the popular seasonal fruits in Malaysia. In 2015, the acreage of mangosteen plantations is 3906.3 ha with production of 22,784.1 metric ton and produced RM 59 million in Malaysia gross income. Mangosteen is usually exported to Thailand, Indonesia, and Singapore (DOA, 2015). Mesta is a variant of mangosteen which can be found commonly in Pahang and Sabah. Mesta is prominently planted in the East Coast Region (Terengganu and Pahang). Mesta is presumed to thrive in area with high rainfall and humidity (Osman and Ab. Rahman, 2006). Mesta is ovoid in shape with pointed distal end while mangosteen is ovoid with flat distal end. Mangosteen has four to eight segments of edible white aril, including one or two larger segments containing apomictic seeds (Paul and Ketsa, 2014). As for Mesta, most of its edible white arils are seedless or have undeveloped seed.

Mangosteen family has long juvenility period and slow vegetative growth. It takes around 7 to 8 years to start producing fruit. This slow growth is one of the points restricting of selecting Mesta or Mangosteen as one of focal crop in a plantation (Almeyda et al., 1976).

Materials and Methods

In this study, three locations have been selected (MARDI Sintok, MARDI Jerangau and MARDI Headquarters). Two type of planting material is used in this experiment which is 24 months old Mesta seedling and 24 months old Mesta cutting. In this study, each location is planted with Completely Randomized Block Design with three replicates and 30 plants per treatment per replicates.

Mesta plot is used for this experiment is planted in 2017. The distance for each planting point is 8 m x 8 m. Before planting, area of 20.3 cm x 20.3 cm x 20.3 cm surrounding planting point is digging ploughed by using backhoe to improve the aeration and water retention of the soil. Later 60 cm x 60 cm x 60 cm hole is dug and filled with 5 kg manure, 200 g Chrismats Island Rock Phospate (CIRP), 200 g Ground Magnesium Limestone (GML), and 200 g NPK Green (15:15:15) as basal media for planting. The fertilizer program for 3 years, with each tree is given 200 g NPK green (15:15:15) at every quarterly and 3 kg manure is applied annually. For water regime, each plot is supported with microjet propagation system and water was given every 2 days when there is no raining.

Vegetative growth of Mesta (plant height, stem girth, canopy size, and tier number of branch) is recorder quarterly). Soil sample is collected and sent to lab for further analysis. Rain and temperature data for each location is obtained from meteorological station in each location. Data recorded and obtained then later analyses by using SAS statistical software.

Results and Discussion

As shown in Table 1, the highest height increment is shown by Sintok Seedling and Sintok Cutting followed by cutting and seedling in MARDI Ibu Pejabat and MARDI Jerangau. Eventhough according to Figure 1, MARDI Sintok obtained less precipitation (1670.6 mm) than MARDI Jerangau (4255.7 mm), this may be due to the use of irrigation system and prolong shading regime of up to 6 months to combat with drought season in February and March.

Table 3: Height increment of Mesta in three locations.

Location	Planting material	Height increment (27 months)	Absolute height growth rate (cm month^{-1})	Relative height growth rate (cm cm^{-1} month^{-1})
HQ	Cutting	69.28^{bc}	3.29^{b}	0.0342^{b}
HQ	Seedling	67.48^{c}	3.23^{bc}	0.0343^{b}
Jerangau	Cutting	75.15^{b}	3.53^{b}	0.0313^{c}
Jerangau	Seedling	66.73^{c}	3.13^{c}	0.0323^{c}
Sintok	Cutting	92.81^{a}	4.40^{a}	0.0407^{a}
Sintok	Seedling	89.55^{a}	4.21^{a}	0.0371^{b}

***Each different letter is significantly different at P > 0.05 with DMRT test.*

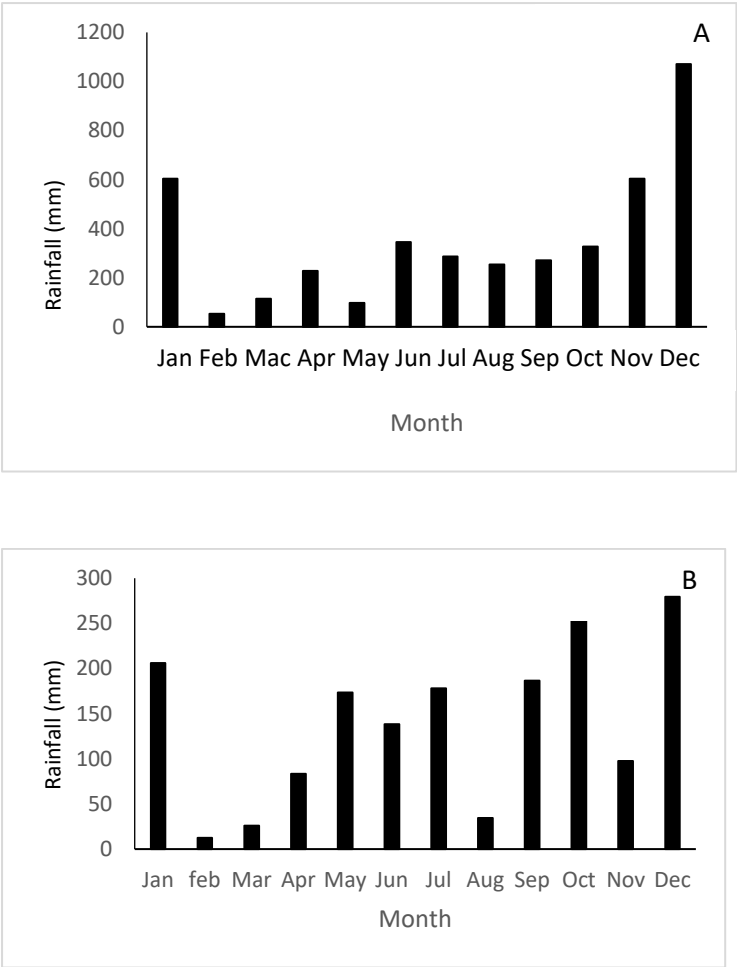

Figure 1: Rainfall data in 2019 for (A) MARDI Jerangau and (B) MARDI Sintok.

The highest amount of rain is received by MARDI Jerangau with upward trend from August to December (Figure 1). In MARDI Jerangau the dry season is in February till May. While in MARDI Sintok, the amount is scattered with the most rainfall is in September, October, and December. The dry

spell in Northern region is from February till April. Eventhough Mesta is planted in Zon 1 with less than 2000 mm precipitation, it is able to survive with proper irrigation system.

Table 4: Soil analysis of each planting location.

Location		Jerangau	Sintok	Serdang
Soil Series		Jerangau	Dampar	Munchong
Soil composition	Sand	43.48%	21.43%	46.34%
	Silt	19.57%	42.86%	29.27%
	Clay	36.96%	35.71%	24.39%
	Soil type	clay loam	silty clay loam	loam
pH		4.57	4.01	4.88
N	ppm	13.31±1.71	89.98±9.57	14.37±2.52
P	ppm	35.21±9.43	40.73±7.32	41.68±10.34
K	ppm	122.73±8.00	89.98±9.57	96.16±12.04

In this study, the N element which play critcal role in vegetative growth (Poerwanto et al., 2008) is the lowest in MARDI Jerangau compared to other location (Table 2). This may be the reason why the growth of Mesta in MARDI Jerangau is observed less vigorous compared to the other location.

From the rainfall data obtained, it is concluded that there are three elements necessary in improving survivality and recovery after transplating shock of Mesta in the field. First is the planning of planting. It is suggested to plant Mesta in Septermber or October since there is rainy season coming up due to the monsoon. Second, the importance of irrigation system and third is prolong shading. Eventhough Mesta is plant in hot and arid environment, it is able to survive and give better vegetative growth than other locations.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Influence of Organic Amendments on the Growth and Physiology of *Melastoma malabathricum*

Wan Mohamed, W.N.A., Liu, C.L., Abdullah, R. and Osman, N.*
Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
**E-mail: normaniza@um.edu.my*

Introduction

Organic amendments have shown the potential to improve growth and physiological performance in multiple species (Abdul Halim et al., 2018; Mahmud et al., 2018). These were achieved by the improvement of soil fertility due to the addition of organic matter and the support of plant development through nutrition (Maiti, 2013; Bastida et al., 2015). However, the effects of organic amendments are yet to be fully explored in Malaysian slope vegetation species. The improvement of growth and physiological responses in slope species may bring some benefits for slope protection. For instance, an increase in biomass may increase rainfall interception by the canopy (Gyssels and Poesen, 2003; Guevara-Escobar et al., 2007) and mechanical anchoring by roots (Reubens et al., 2007; Stokes et al., 2009; Saifuddin and Normaniza, 2016). Moreover, increased physiological performance such as transpiration rate may increase the drying effects by plants on slopes that, in turn, stabilise the slope (Stokes et al., 2009; Ni et al., 2018). Previous studies have reported *Melastoma malabathricum* having better physiological performances, root length, and root volume (Dorairaj et al., 2020; Rohailah, 2010; Normaniza et al., 2014; Saifuddin, 2014) compared to other species. These findings imply that *M. malabathricum* is the ideal species to be planted on slopes due to its traits that are beneficial for slope protection. Therefore, this paper aims to report the influences of three selected organic amendments, namely biochar, compost, and vermicompost on growth and some physiological performances of *M. malabathricum*.

Materials and Methods

Experimental design

The field experiment was carried out on an artificial cut slope with a gradient that ranges between 36° to 41°, at Faculty of Science, Universiti Malaya (3°07'30.9"N 101°39'11.6"E). The temperature ranged from 25 °C to 32 °C with a maximum Photosynthetically Active Radiation (PAR) of 2000 μE/m^2/s. Temperature and PAR were recorded using a portable photosynthesis system (Model LI-6400XT, LI-COR Environmental, USA). Properties of the slope soil are presented in Table 1.

Table 1: Soil properties of the slope under investigation before treatments.

Properties	Description	Properties		Description
pH	4.8	Texture class		Loam
EC, mS/cm	0.16	Particle diameter, %	0.5-1.0 mm	-
Moisture content, %	19.05		0.25-0.5 mm	5.1
Bulk density, g/cm^3	1.49		0.1-0.25 mm	17.1
Munsell colour	Hue 10 YR 7/4		0.05-0.1 mm	15.6
	(very pale brown)		< 0.05 mm	62.2

EC: Electrical conductivity.

There were five treatments including control, with three replications per treatment. Three of the treatments were organic amendments (Table 2) applied at a rate of 20 t/ha (dry weight) as practised in a previous study that compares different types of amendments (Doan et al., 2015). The treatments were:

a) CTL: Control – no additions to the soil
b) FRT: Inorganic fertilisers (15 g NPK + 15 g rock phosphate) and 15 g sphagnum moss
c) BCR: 20 t/ha rice husk biochar
d) CPT: 20 t/ha buffalo manure compost
e) VRM: 20 t/ha cow dung vermicompost

Table 2: Properties of the amendments used in this study.

Properties	Biochar	Compost	Vermicompost
pH	7.39	5.76	6.54
EC (mS/cm)	0.76	0.93	9.30
Particle diameter, %			
0.5-1.0 mm	25.5	29.8	-
0.25-0.5 mm	47.7	43.0	1.9
0.1-0.25 mm	7.5	21.7	14.8
0.05-0.1 mm	2.3	3.6	19.3
< 0.05 mm	17.0	1.9	64.0

EC: Electrical conductivity.

The study was conducted for nine months under Randomised Complete Block Design (RCBD). This duration was determined to be most suitable because following biochar treatments, (i) the least reduction in soil nitrate and ammonium were found to be between six and twelve months and (ii) with longer experiment duration, the range in soil nitrogen changes becomes greater (Nguyen et al., 2017), possibly resulting in larger variance in results.

All 150 *M. malabathricum* seedlings (10 plants per treatment plot) were transplanted following an established slope planting technique, a Microclimate Plant Propagation Technique (Normaniza et al., 2014). In this technique, a seedling of 0.8-1.0 m is transferred into polyvinyl chloride (PVC) tube (diameter 11 cm; length 29-30 cm) and allowed to acclimatise within the experimental area. This seedling is then transplanted into a 0.6 m deep hole. The organic amendments in treatments CPT, BCR, and VRM substitute inorganic fertilisers and sphagnum moss used in FRT. Therefore, CPT, BCR, and VRM are essentially modifications to the Microclimate Plant Propagation Technique's supplemental materials. As for the soil amendment application, based on the 20 t/ha amendment application rate, each plot under the amendment treatment received 9 kg of the amendment. Therefore, on average, each plant received 0.9 kg of soil amendment. 20% of the 0.9 kg amendment was mixed into the soil in the 0.6 m hole. The remaining 80% was incorporated into the top 20 cm of the soil as the incorporation of amendments into the topsoil is practised in field experiments (Tejada and González, 2009; Tejada and Benítez, 2011).

Plant growth

Plant stem diameter and height were measured in six replicates per treatment using a flexible meter ruler and a Vernier calliper, respectively, at the beginning of the experiment and a three-month interval until completion. Shoot fresh biomass was determined in three replicates by separating into stem and leaves and measured using an electronic weighing balance.

Plant physiological performance

Physiological performance measurements were taken in six replicates from the youngest fully expanded leaves. Leaf relative chlorophyll content was determined using a portable chlorophyll meter (SPAD 502, Minolta, Japan). Plant stomatal conductance, transpiration rate, and photosynthetic rate were

measured using a portable photosynthesis system (Model LI-6400XT, LI-COR Environmental, USA). Physiological measurements were undertaken between 1100 and 1300 h because during these hours, the slope species in Malaysia (i) showed maximum performances and (ii) exhibited the greatest differences in physiological performances between species (Rohailah, 2010; Saifuddin, 2014). So, the species studied capabilities could truly be captured and represented when measurements were taken around midday.

Data analysis

Statistical analysis was performed using IBM SPSS Statistic 25-26 software (IBM Corporation, Armonk, NY, USA). A T-test was carried out to analyse the significant difference between treatment means at two points in time (α=0.05). A one-way ANOVA was carried out to evaluate significance in differences among treatment means (α=0.05) followed by Duncan's New Multiple Range Test (α=0.05).

Results and Discussion

The results show that after nine months, the mean stem diameter of *M. malabathricum* in VRM was significantly greater than BCR and CTL (Figure 1a). In addition, all treatments also showed significant increment after nine months, in which the greatest exhibited in VRM (114%) and the least (71%) in CTL. Results also showed that vermicompost and compost were able to promote growth comparably with synthetic fertiliser, which could be due to the similar amount of nutrients supplied to the soil (Mahmud et al., 2018; Toselli et al., 2019). However, there was a decrease in stem diameter starting from three months. It could be attributed to declining soil moisture content (data not included) as stem diameter has a negative relationship with soil water (Meng et al., 2017). Additionally, plant height showed an increasing trend, but no significant difference was found between treatments after nine months (Figure 1b). It may indicate that plant height is not a suitable indicator for *M. malabathricum* growth under organic amendments.

Apart from that, the mean total fresh biomass of plants in the VRM treatment was significantly greater than CTL (Figure 1c). In other studies, plant biomass showed differential responses to different organic amendments (Rehman et al., 2016), with vermicompost resulting in greater values (Libutti et al., 2020). It could be due to greater N content, which likely resulted in greater above-ground biomass (Liu et al., 2006). N is directly related to plant biomass production because N is integral to many essential components, including nucleic acid, ATP, proteins, and chlorophylls (Hawkesford et al., 2012).

The mean stomatal conductance was found highest in VRM treatment, and all treatments had values greater than BCR (Table 3). Increased stomatal conductance in VRM may be due to increased stomatal aperture from turgid guard cells (Pallardy, 2007), which could have been induced by phytohormones originating from the vermicompost (Arancon et al., 2006; Acharya and Assman, 2009). These results signify that vermicompost improved the water removal and growth potential in *M. malabathricum.* The possible reason is that stomatal conductance dictates gaseous and water vapour exchange, so it determines the amount of water vapour released and CO_2 taken in by leaves. Moreover, as the stomatal opening determines plants' transpiration capabilities, a greater stomatal conductance may lead to greater transpiration rates. The highest transpiration rate was recorded in VRM, which exceeded CTL by more than 124%. Similarly, the transpiration rates in FRT and CPT were also significantly greater than CTL.

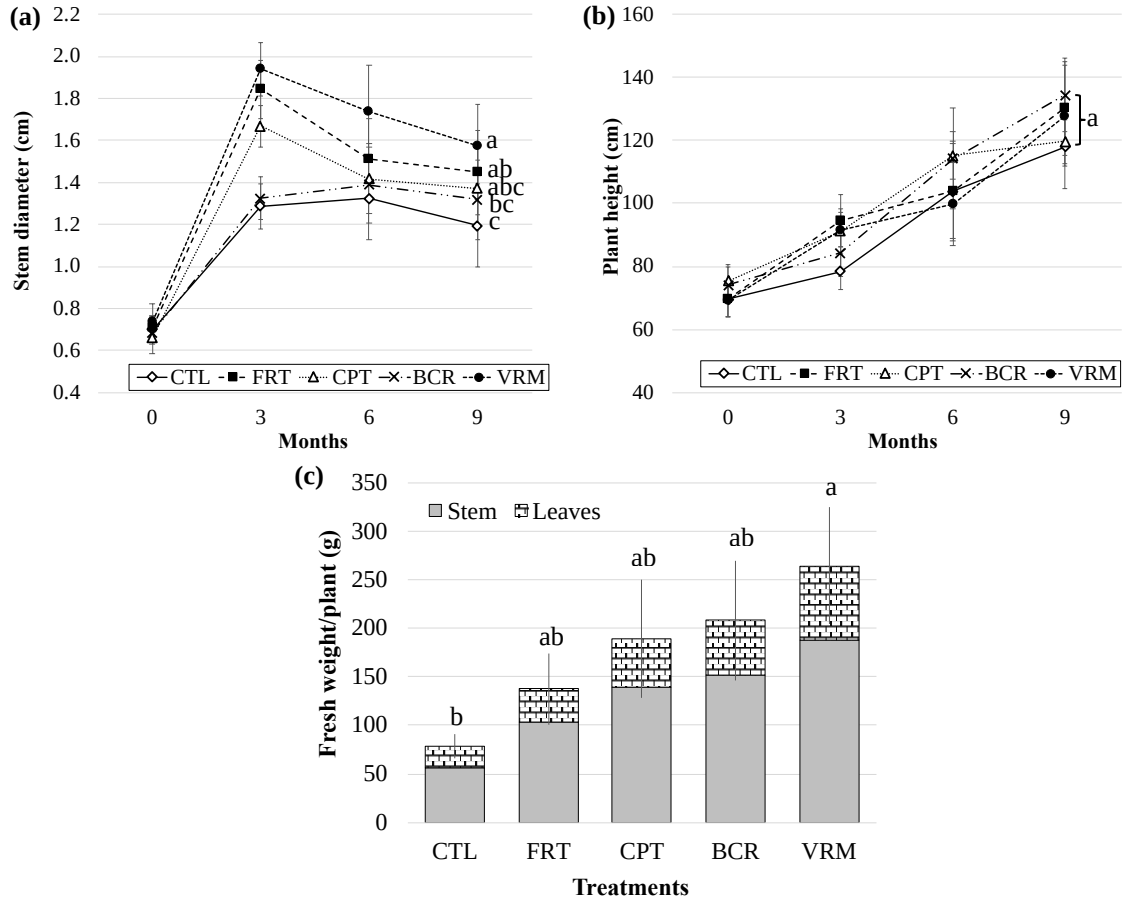

Figure 1: Growth parameters studied in *M. malabathricum* under the influence of organic amendments. (a) Stem diameter (n=6) (b) height (n=6) and (c) fresh weight (n=3) of *M. malabathricum* grown on slopes with soil amendments. Bars indicate standard deviation. Vertical lines indicate standard error. Letters indicate a significant difference between treatment means after nine months ($P<0.05$) by Duncan's MRT.

The relative chlorophyll content was significantly lower in CTL than other treatments except for BCR (Table 3). It showed that the species was able to synthesise additional chlorophyll pigments in VRM, CPT, and FRT treatments, signifying potential in improving plant photosynthetic capabilities due to relatively greater chlorophyll concentrations in leaves (Li et al., 2018; Zhou et al., 2019). The greater relative chlorophyll content could be due to greater available N in these treatments, which is an essential part of chlorophyll molecules (Liu et al., 2006). VRM treatment exhibited the greatest value, which was 68% greater than CTL, and similar observations were reported in experiments comparing several types of amendments involving other species (Kumar Srivastava et al., 2011; Tejada and Benítez, 2011; Libutti et al., 2020).

Similar to relative chlorophyll content, the mean photosynthetic rate observed in VRM and CPT was higher than in other treatments (Table 3). It is possible that in these two treatments, the combination of greater available nutrients in the soil and greater stomatal conductance that increased concentrations of CO_2 within the leaves were responsible for higher photosynthetic rates. In contrast, the photosynthetic rate was found lowest in BCR. This was attributed to lower nutrient uptake in biochar treated plants than compost treated plants (Rehman et al., 2016). Lower nutrient uptake could be due to lower soil nutrient. High C:N ratios, as is often the case in biochars, result in reduced N mineralisation and even net immobilisation to occur in the short-term following biochar incorporation (Novak et al., 2010).

Table 3: Physiological responses in leaves of *M. malabathricum* after nine months.

Treatments	Stomatal conductance ($mol/m^2/s$)	Transpiration rate ($mmol/mm^2/s$)	Photosynthetic rate ($\mu mol/m^2/s$)	Relative chlorophyll content
CTL	$0.028^{d} \pm 0.0010$	$1.01^{d} \pm 0.018$	$5.21^{b} \pm 0.155$	$37.8^{d} \pm 4.85$
FRT	$0.035^{c} \pm 0.0003$	$1.23^{c} \pm 0.006$	$5.33^{b} \pm 0.103$	$48.3^{bc} \pm 8.04$
BCR	$0.021^{e} \pm 0.0008$	$0.84^{e} \pm 0.016$	$3.08^{c} \pm 0.101$	$41.0^{cd} \pm 3.77$
CPT	$0.053^{b} \pm 0.0009$	$1.82^{b} \pm 0.020$	$6.98^{a} \pm 0.173$	$50.7^{b} \pm 4.60$
VRM	$0.067^{a} \pm 0.0013$	$2.26^{a} \pm 0.020$	$6.87^{a} \pm 0.161$	$63.4^{a} \pm 8.13$

Means in the same column followed by different letters ± standard deviations indicate significant difference at $P \leq 0.05$ by DMRT.

Conclusion

Incorporation of organic soil amendments on slopes, particularly vermicompost and compost rather than biochar, were beneficial for plant growth and physiological performances. Vermicompost and compost increased the stem diameter of *M. malabathricum* so that the effects were comparable to those in fertiliser treatment. In particular, vermicompost increased the transpiration rate to almost double that of fertiliser treatment, signifying its potential to remove water from slopes. The relative chlorophyll content in vermicompost treated plants was 68% greater than control, and this translated into a greater photosynthetic rate, which was 32% greater than control. The implication is that vermicompost incorporation resulted in increased growth capability, which was evident in the greater above-ground fresh biomass. In conclusion, vermicompost may enhance the potential of *M. malabathricum* in slope protection due to greater growth and physiological performance.

Acknowledgement

The authors acknowledge that this study was made possible by the Fundamental Research Grant Scheme from the Ministry of Higher Education: FRGS/1/2018/WAB05/UM/01/1 (FP060-2018A) and the Faculty Research Grant from the Universiti Malaya (GPF005B-2018).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Production of Volatile Sulfur Compounds and Upregulation of Methionine-γ-Lyase in 'Musang King' and 'D24' During Durian Ripening

Azizah, M.[1,*], Tan, X.Y.[1], Ding, P.[1], Mohd Sabri, P.D.[2], Daim, L.D.J.[3] and Lau, B.Y.C.[4]

[1]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Food Science, Faculty of Science and Food Technology, 43400 UPM Serdang, Selangor, Malaysia.
[3]Agronomic Selection, Sime Darby Plantation Technology Centre Sdn. Bhd., UPM-MTDC Technology Centre III, Universiti Putra Malaysia, Lebuh Silikon, 43400 UPM Serdang, Selangor, Malaysia.
[4]Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.
**E-mail: azizahm@upm.edu.my*

Introduction

The durian is the member of Bombaceae family and native to Borneo and Sumatera, and largely grown in Thailand, Malaysia, and Indonesia. After Thailand, Malaysia is the second leading country for durian production. It is the main fruit crop cultivated in Malaysia which makes up 41% of land cultivated total land specified for cultivation (DOA, 2019). Durian has been classified as premium fruit group in the Malaysia National Agro-food Policy (2011-2020), which has great potential and is projected to be in high demand especially in the China market. 'Musang King' and 'D24' are considered among top commercial durian clones that have very good demands. The high appreciation for durian might be due to the creamy texture, sulfurous aroma, and sweet taste. Despite being rich in flavour, durian fruit emits very strong aroma due to the production of volatile organic compounds (VOCs). Sulfur- and ester-containing compounds were found to occur at very high levels in the pulp of durian (Tan et al., 2020). Teh et al. (2017) found that several methionine-γ-lyase genes that are related to the production of volatile sulfur compounds (VSCs) were upregulated in durian fruit, particularly during ripening. Thus, it was suggested that methionine-γ-lyase could be involved in the formation of VSCs which contribute to the characteristic durian aroma. A better knowledge on the composition of VSCs in both clones will therefore be important in understanding the consumer preference towards different durian fruit clones.

Materials and Methods

Plant material

Fully ripened 'Musang King' and 'D24' durian fruits that fell off the trees naturally were obtained from local orchards located in Raub, Pahang, during peak season between June to July, 2020. A total of 12 uniformly sized (1.5-1.8 kg), damage-free, and fully ripened durians for each treatment were collected and transported to the Postharvest Laboratory, Universiti Putra Malaysia. The durians were cut along the suture on the back of the lobules and good condition durian arils were selected for analyses. Fresh durian pulps (~100 g) were pooled after the seeds were removed, wrapped in aluminium foil, and immediately dipped in liquid nitrogen for a few minutes and stored at -80 °C prior to protein extraction.

Determination of volatile organic compounds (VOCs)

The VOCs in durian were extracted using SPME fibre and the samples were further analysed using GC-MS/MS system. The identification of the VOCs in durian sample was accomplished by matching the mass spectra with the National Institute of Standards and Technology (NIST). The quantification of the VOCs was done by comparing the peak areas of the detected VOCs to the peak area of internal standard thiophene and results were expressed as (peak area/internal standard area) x 1000.

Proteomic analysis

The protein in durian was extracted using acetone-phenol method (Tan et al., 2021). The pellet was then digested using trypsin. Separation was done using an EASY-nano liquid chromatography (EASY-nLC) 1200 System (Thermo Scientific, MA, USA) integrated with an online Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer system (Thermo Scientific, MA, USA). Mass spectra of the peptides were acquired using Xcalibur (Ver. 4.1.31.9) and deconvoluted with Proteome Discoverer (Ver. 2.3) to create the peptide mass list. SEQUEST HT search engine, incorporated in the Proteome Discoverer, was used to match the generated mass list against *Durio zibethinus* FASTA sequences downloaded from NCBI. Spectra that matched to the sequences were further validated with Percolator algorithm (version 2.04) and identified proteins were annotated and analysed by UniProt, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses.

Experimental design and analysis

The experiment was conducted using a complete randomized block design, with four replications. The results were analysed using ANOVA of SAS version 9.3 (SAS Institute, Cary, NC) and differences between the means were compared using Least Significant Difference (LSD) at *P* value = 0.05.

Results and Discussion

In the present study, the production of VSCs in 'Musang King' and 'D24' were monitored during ripening. Analysis in both durian fruits showed that the component of the VOCs was different (Figure 1). 'Musang King' showed very high amount of volatile ester compounds (VECs) which represent about more than 80% of the total VOCs. In comparison to 'D24', it contained almost equal composition between VSCs and VESs (50 %: 49 %). Previous study reported about 52% of VECs were detected in 'D2' clone (Voon et al., 2007). The VSCs contribute to the roasted onion-like smell while ester aroma gives the fruity and flower notes (Belgis et al., 2017).

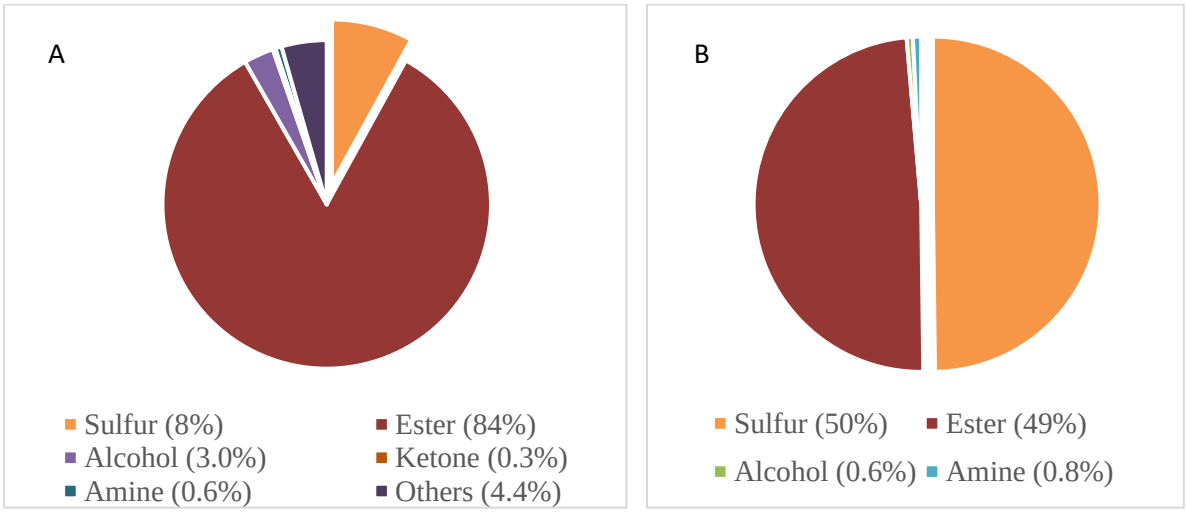

Figure 1: Pie chart showing the different amounts of VOCs detected in (A) 'Musang King' and (B) 'D24' durian fruits during ripening.

Table 1 summarises the relative composition of VSCs in both 'Musang King' and 'D24' durian fruits. It was found that the number of VSCs in 'D24' was 57% higher than those detected in 'Musang King'. This might be due to the absence of several metabolites in 'Musang King' durian such as diethyl disulfide, 2-propanethiol, 2, 2-bis (methyl sulfanyl) ethyl benzene, 2-(propyl sulfanyl) phenol and 2-ethyl sulfanyl ethanol. Diethyl trisulfide was the highest VSCs in 'D24'. It was described as sweet and

alliaceous (Naef and Velluz, 1996). However, Voon et al. (2007) demonstrated that diethyl disulfide was the major VSC in 'D24' followed by diethyl trisulfide. The total VSCs in 'D24' was found to be four folds higher compared to 'Musang King'.

Table 1: The composition of VSCs in 'Musang King' and 'D24' durian fruits during ripening.

Sulfur-containing compound	'Musang King'	'D24'
Diethyl disulfide	nd	73.44
Ethyl disulfide	36.29[b]	43.65[a]
2-propanethiol	nd	6.24
2,2-bis (methyl sulfanyl) ethyl benzene	nd	11.59
Carboisopropoxy methoxy sulfide	1.59[b]	8.44[a]
Diethyl trisulfide	103.6[b]	454.52[a]
2-(Propyl sulfanyl) phenol	nd	11.59
2-Ethylsulfanylethanol	nd	4.86
2-Hydroxyethyl isobutyl sulfide	5.65[a]	5.55[a]
3,5-dimethyl-1,2,4-Trithiolane (isomer 1)	5.65[b]	13.65[a]
3,5-dimethyl-1,2,4-Trithiolane (isomer 2)	5.47[b]	6.83[a]
Total	158.25	640.36

Means within rows followed by the same letter are not significantly different at P=0.05 by using LSD. nd=not determined

Characterisation of the proteins associated with the production of VSCs were characterized by proteomic analysis. The results indicated that the methionine-γ-lyase was present in both 'Musang King' and 'D24'. In agreement with the previous data, the relative abundance of methionine-γ-lyase was also four folds higher in 'D24' compared to 'Musang King'. Teh et al. (2017) found that several methionine-γ-lyase genes related to the production of VSCs were upregulated in durian fruit, particularly during ripening. Thus, it was suggested that methionine-γ-lyase was responsible particularly in the regulation of VSCs which contribute to the characteristic durian aroma.

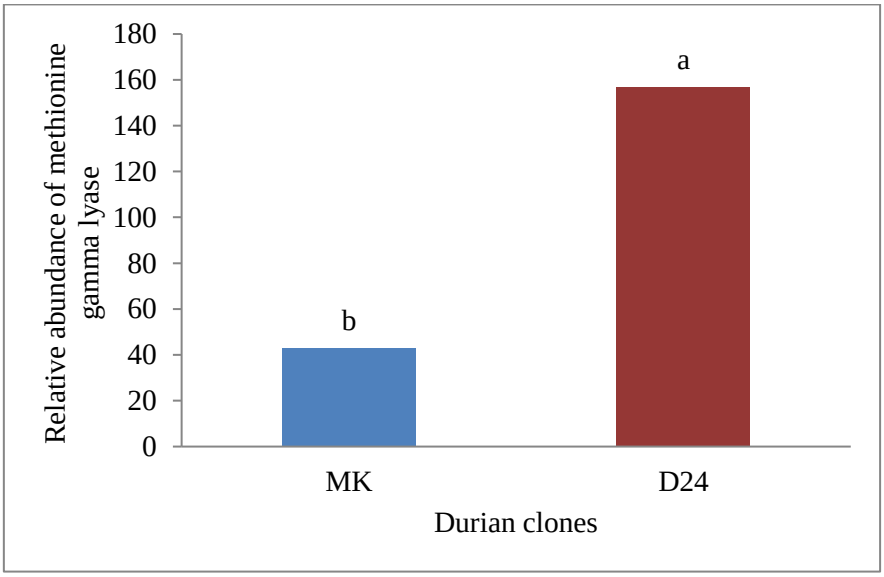

Figure 2: The relative abundance of methione-γ-lyase in 'Musang King' and 'D24' durian fruits.

Conclusions

Both durian clones, 'Musang King' and 'D24' demonstrated that different composition of VSCs contributed to the strong aroma in durian. Higher composition of VSCs were found in 'D24' with diethyl trisulfide as the major VSC. Analysis of methionine-γ-lyase showed that this protein was significantly upregulated four folds higher in 'D24' compared to 'Musang King' in accordance with the production of total sulfur compounds in the respective durian clones. These results provide the information on VSCs in durian and the protein that controls the regulation of VSC production and therefore relevant to the knowledge on the consumer preference of durian clones.

Acknowledgements

The present work was financially supported by Putra Grant - Putra Graduate Initiative (IPS) and Graduate Research Fellowship (GRF) awarded by Universiti Putra Malaysia. The authors would also like to acknowledge Sime Darby Plantation Technology Centre Sdn. Bhd. and the Proteomic and Metabolomics Laboratory (PROMET-MPOB) for permission to use their proteomic facilities and for providing valuable insights for this research.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Yield Performance of Ulam Tenggek Burung in Pilot Scale

Umikalsum, M.B.[1,*], Farahzety, A.M.[2] and Roslee, P.[3]

[1]Industrial Crop Research Center, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Horticulture Research Center, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[3]Agrobidiversity and Environment Research Center, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: umi@mardi.gov.my*

Introduction

Tenggek burung or its scientific name Melicope ptelefolia is a shrub or small tree from the family Rutaceae. This plant lives wild in the open and is found in the tropics of Mascavene Island, South Asia and East Asia. There are around 230 species reported of which 13 or 14 of them are found in Borneo (Hartley, 1994) and 10 other species are found in upland and lowland areas in Malaysia (Corner, 1952, as cited in Karim et al., 2011). This plant can grow well in bushes and peat areas as well as soils with high sulfate content. The small tree is lush and has many branches and the leaves are trifoliate with oblong leaves and acuminate at the ends. The stems are green when young and turn brown when they harden. The dark green leaves are slightly shiny while the young leaves are light green and soft. The flowers are clustered and white in colour (Figure 1). Mature flowers produce nectar that the birds can feed on. This plant is resistant to heat and rarely die in natural areas. The small tree is able to reach up to 4 to 5 meters in height. The young shoots of tenggek burung are commonly used as traditional salad or 'ulam' and have good potentials to be commercialised.

Tenggek burung is naturally propagated by seed dispersals by birds that eat the fruit. However, these plants can also be propagated by stem cuttings and tissue culture methods (Farahzety et al., 2014). Reproduction by micro stem cuttings should be performed at the juvenile stage. Plants at the juvenile stage can be obtained from seed nurseries or seedlings of tissue culture. For seed propagation, it is necessary to collect the mature fruits that are quite dark green in colour (Figure 2). Young green fruits are difficult to germinate, while black fruits have very low germination rate. Sowing can be done in a container or germination box containing perlite medium of 5 cm thick. The seeds will start germinating between the eighth and twelfths week after sowing. On average, the seed germination rate is between 40 to 50% on perlite medium (Farahzety et al., 2014). Meanwhile, the germination rate on the peat soil is 61.5% (Din Merican, 2018). Tissue culture can also be done where the explants from the lateral shoots are germinated in agar medium (Zuraida et al., 2014). The shoots are then cultured in sterile conditions. This *in vitro* shoot multiplication resulted in the production of more uniform seedling (Rahman et al., 2015).

Materials and Methods

Preparation of cuttings

The study was conducted on the plot of the Malaysian Agricultural Research and Development Institute (MARDI), Serdang. A healthy tenggek burung plant was selected as the source. Shoot cuttings with a length of between 3 to 4 cm were collected in the morning from plants that are lush, fresh, and not too young (approximately one year old). Leaves at the bottom part of the cuttings were removed. The cuttings were then soaked for 6 hours in a rooting hormone solution as described in Farahzety et al. (2014) protocol.


Figure 1: Tenggek burung flowers.


Figure 2: Tenggek burung fruits.

The nutritional content of tenggek burung for every 100 g is 83 g of water, 4.4 g of protein, 9.9 g of carbohydrates, 0.3 g of fat, 1.5 g of fiber, 51 mg of phosphorus, 41 mg of calcium, 330 mg of potassium and 9.3 mg of vitamin C. It also has high antioxidant (Abas et al., 2006) and anti-inflammatory activities (Shaari et al., 2011; Fazleen Izzany et al., 2018). Based on traditional practices, tenggek burung shoots are believed to have various benefits such as for treating high blood pressure, improving blood circulation, and refreshing the body. This study focused on evaluating the performance of tenggek burung production in pilot scale.

A pot or polystyrene box was used as planting container for the cuttings. The container was filled with coarse lumps such as small gravel or 'hydroball' and topped up with planting medium such as perlite or 'peat-moss' before planting the cuttings into the planting medium. The container was then covered with a plastic sheet. The cuttings were left for 5 to 6 weeks for the roots to be formed properly before they were transferred to the polybags.

The polybags were prepared by filling them with a mixture of soil, peat and cocopeat at the ratio of 2:2:1. Organic fertilisers such as compost or vermicompost were mixed with the mixture medium at the ratio of 1:5. After the cuttings had been transferred to the polybags, they were placed under shade with irrigation system. Two weeks after transfer, NPK Green fertiliser (15:15:15) was applied at the rate of 5 g per tree. When the plants reached the height of 50 to 60 cm, they were then planted in the field.

Cultivation and management

The mineral land was prepared in an area of 0.1 hectare by ploughing and rotor tilling to a depth of 20 to 30 cm. A week before transplanting, organic fertiliser at the rate of 5 to 10 tons per hectare was applied to the plot. Pre-emergence weedicide was sprayed on the field to control the weeds. A week after fertilisation, the seedlings of tenggek burung were ready to be transplanted. A total of 300 seedlings of tenggek burung were transplanted to the field at a distance of 1.5 m between plants in the form of an equilateral triangle as shown in Figure 3.

Irrigation was done twice a day especially for the small and young plants. Mulch such as weeds, hay and straw were used to maintain soil moisture and minimise weed growth. Weeding was done regularly and manually using a hoe. A systematic pruning was done to obtain balanced branches and canopy. The branches were pruned periodically to produce new shoots. Organic fertiliser was applied every 3 months to maintain the growth. Meanwhile, NPK Green fertiliser (15:15:15) was also used at the rate of 1 ton per hectare per month to maintain production.

Harvesting

Once the tenggek burung plant had grown lush branches, the young shoots were harvested by plucking manually. Data on the weight of the shoots were taken weekly and analysed using Microsoft Excel software.

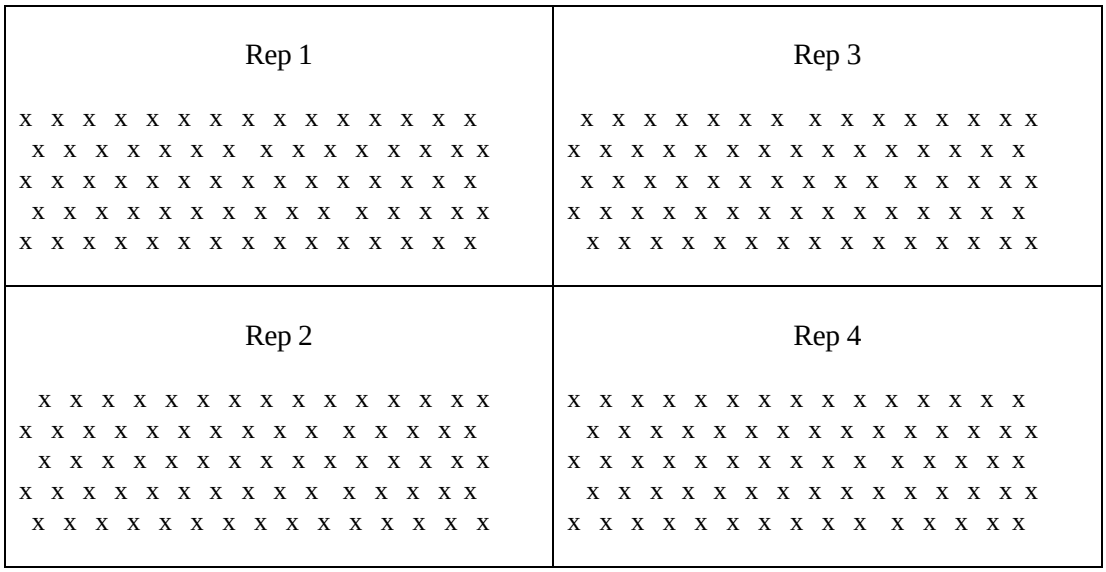

Figure 3: Plot layout of tenggek burung seedlings.

Results and Discussion

Seedlings preparation

Cuttings were collected in the morning to ensure maximum rigidity. The cuttings were selected from shoots that were lush, fresh, and disease-free but not too young approximately one year old. All leaves on the cuttings were maintained except for large cuttings. The concentration of IBA (auxin) rooting hormone used to accelerate rooting was in accordance with the dosage and not exceeding the optimal concentration as it could inhibit rooting and shoot production. Propagation of tenggek burung was best done using a closed capillary system which consisted of two components, namely water reservoir and planting medium. The bottom of the reservoir was filled with coarse lumps such as gravel or 'hydroball' with the planting medium such as perlite or 'peat-moss' placed on top (Figure 4) which allowed water to rise to the top through capillary action. This condition provided high air humidity around the cuttings and an excellent environment for cuttings to remain fresh and form roots. The cuttings of the tenggek burung began rooting within 3 weeks. However, the cuttings were left for 5 to 6 weeks in the propagation system and so the roots were formed properly before they were transferred to the polybags. The cuttings that had rooted in the propagation system were then ready to be transferred into the polybags containing a mixture of soil, peat and cocopeat at the ratio of 2:2:1 (Figure 5). The seedlings were ready to be transplanted to the field when the plant reached a height of 50 to 60 cm (Figure 6). Pruning was done to encourage branching and increase the production of shoots. Figure 7 showed the tenggek burung plants in the field after a year.

Figure 4: Tenggek burung cuttings in media container.

Figure 5: Tenggek burung transferred to polybags.

Figure 6: Tenggek burung plants transferred to the field plot.

Figure 7: Tenggek burung at the mineral soil field plot.

Yield performance of tenggek burung

The tenggek burung shoots were harvested after 4 weeks of pruning. Harvesting was done weekly. Yield for the first 2 weeks reached up to 142 g per plant (Figures 8 and 9). However, the yield began to decrease in the following week. The average yield harvested in the first month was 117 g/plant. While the average yield harvest in the second month (fifth week to eighth week) was 55 g/plant. A 50% yield decrease compared to the first month.

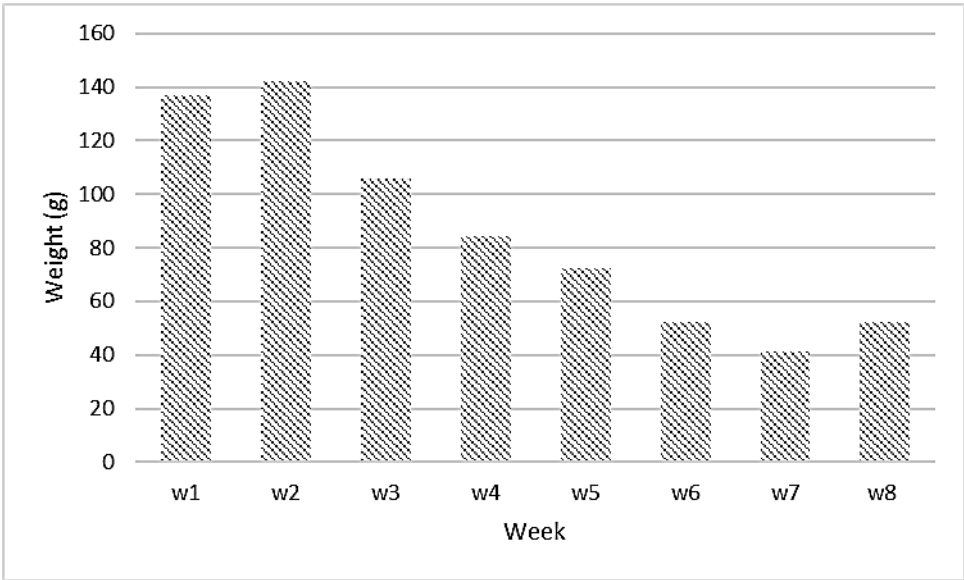

Figure 8: Yield of tenggek burung shoots for each plant.

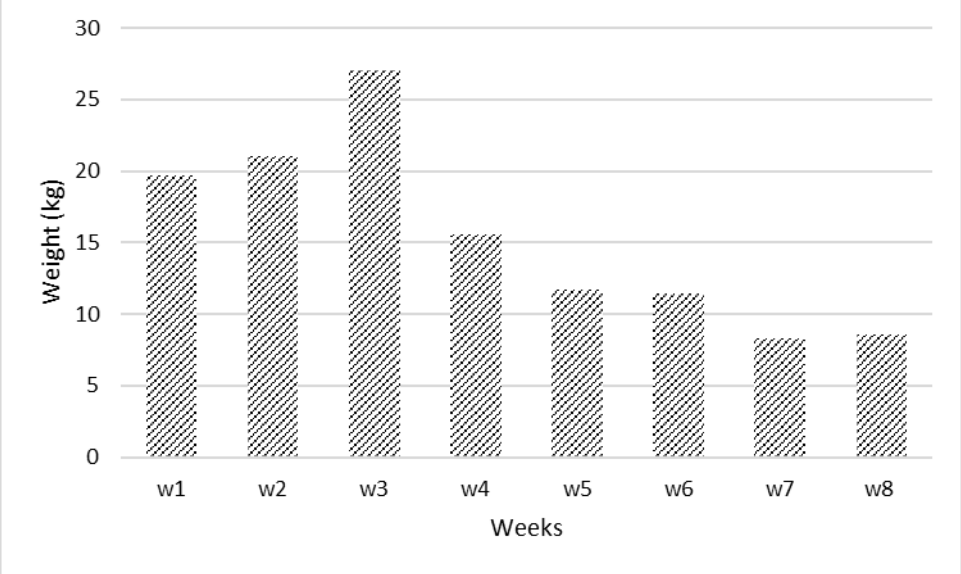

Figure 9: Total yield of tenggek burung shoots harvested every week.

The plants began to produce young shoots immediately after pruning. The first harvest was in the fourth week after pruning. The yield was increased in the first 3 weeks and then decreased in the following weeks. The average yield in the first month was 20 kg/week, while it only produced 10 kg/week in the second month.

Conclusions

This study showed that the production of tenggek burung plant increased only up to 3 weeks after pruning. The yield declined in the following week. In order to get continuous results, crop maintenance and, management such as fertiliser application, weeding and pruning need to be done on a regular basis. Further research needs to be performed to obtain good and uniform yield every week for consistent supply of sufficient materials to the market.

Acknowledgement

This project was funded under the Development Project in the Eleventh Malaysia Plan (RMK11), Ministry of Agriculture and Food Industry (MAFI) (Project Grant P-RS403).

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Chapter 2: Ecophysiology, Stress Biology, and Pest Management

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Identification and Characterization of *Fusarium* spp. Causing Slow Decline Disease of Black Pepper (*Piper nigrum*) in Belaga and Betong Districts, Sarawak

Shahidan, F.N. and Kwan, Y.M.*
Department of Crop Science, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia.
**E-mail: yeemink@upm.edu.my*

Introduction

Black pepper (*Piper nigrum* L.) is an ancient spice and medicinal plant native to the Western Ghats of Kerala, India. It is one of the most heavily consumed spices in the present day, accounting for almost one third of the global spice market. Black pepper is extensively used in food and beverage and is often considered as a functional food owing to its potential health benefits and therapeutic potential against diseases. In addition to its fundamental nutrition value, piperine (the bioactive compound of black pepper) is used widely in various insecticides, cosmetic, and pharmaceutical products due to its strong pungent aroma, antioxidant, and antimicrobial properties (Abdulazeez et al., 2016). More recently, black pepper essential oil has been developed as a natural food preservative in food packaging because of its antimicrobial properties (Myszka et al., 2017).

Black pepper is mainly exported in black and white peppercorns (95%), with the remaining 5% in the form of powder, oil, and oleoresin. Black pepper global market valued at USD3.7 billion in 2017 and is estimated to reach USD5.7 billion by 2024 (Persistence Market Research, 2017). Thus, it is known as the 'King of Spices' due to its high economic value. Malaysia is currently the eighth largest world black pepper producer, with an export volume that amounted to 19,200 tonnes, equal to RM145.6 million export revenue in 2019 (DOA, 2020). Sarawak remains the largest black pepper producer in Malaysia contribute to nearly 98% of its production. Besides, black pepper is an important cash crop contributes to the income and livelihood of approximately 67,000 farmers in rural areas of Sarawak (DOA, 2020).

Fungal diseases are important factors that affect black pepper production under the warm and humid climate in Sarawak. It has become one of the major constraints and resulted in the reduction of black pepper yield. Slow decline or yellowing disease caused by the combined infestation by nematode (*Radopholus* spp. and *Meloidogyne* spp.) and *Fusarium* spp. has resulted in an annual reduction in black pepper production as high as 30% (Ramana and Eapen, 2000). In this disease complex, the nematodes enter the plant roots as larvae and provide ingress for fungal infection. The infected plants are usually stunted, it starts with leaf yellowing, wilt and drops off progressively upward from older leaves to young leaves, leaving only the bare stem. Brownish-black lesions appear on the shoots, vascular bundle, and infected plants (Wong, 2010). Infected plants will reduce in productivity and, consequently, death within one to a few years.

Several *Fusarium* species were reported to cause slow decline disease in black pepper. There is the necessity to study the identity of the causal fungus of slow decline disease in Sarawak. Accurate identification of the fungal species would enable the employment of effective disease control and prevention strategies. Morphological characterization based on colony appearance, pigmentation, shape, and size of macro- and microconidia, and chlamydospore presence are among the most common methods to identify *Fusarium* species. Nevertheless, the differentiation of *Fusarium* species based on morphological characteristics is imprecise due to the close similarity between species. The polymerase chain reaction (PCR) technique remains the most accurate tool for discriminating fungal species and intraspecific differentiation among the isolated species.

This study aims to i) isolate *Fusarium* spp. from symptomatic black pepper plants and ii) characterize *Fusarium* isolates using morphological characteristics and phylogenetic analysis through the sequence analysis of the translation elongation factor 1-alpha (TEF-1α) gene.

Materials and Methods

Plant sampling and fungal isolation

Diseased plant samples were collected from Belaga and Betong districts in Sarawak. Samples were collected from symptomatic black pepper plants showing yellowing and wilting symptoms. Leaf, stem, and root samples were collected and kept in polyethylene plastic bags. Samples were rinsed under running water to remove soil particles. Samples were cut into 5 to 10 mm long segments and disinfected with 10% sodium hypochlorite. Samples were rinsed with distilled water, and dried before plated on potato dextrose agar (PDA) amended with rifampicin at 100 mg/L. Petri dishes were incubated at room temperature for three days. The hyphal tip of actively growing mycelia was transferred into a new PDA medium and incubated for seven days. Single spore isolation was carried out from 7-day-old cultures using serial dilution method to obtain pure fungi culture.

Morphological identification

Morphological identification was carried out to identify *Fusarium* isolates tentatively and sort the isolates into respective groups before molecular identification. Tentative identification of *Fusarium* isolates was based on the presence and shape of macroconidia, microconidia and chlamydospores on carnation leaf agar (CLA). In contrast, colony colour and pigmentation were observed on PDA. Morphological identification was based on The Fusarium Laboratory Manual (Leslie and Summerell, 2006).

Molecular identification

Molecular identification was conducted using the partial translation elongation factor 1-alpha (TEF-1α) sequences. Genomic DNA was extracted using the Fungi Genomic DNA Extraction Kit (BioTeke Corporation, China) according to the manufacturer's instruction. The primers used to amplify the partial TEF-1α gene were EF1-728F (5'-CATCGAGAAGTTCGAGAAGG-3') and EF2 (5'-GGAGTACCAGTATCA-3'). PCR amplification was carried out in a 50 µL reaction mixture containing 1X power Taq MasterMix (BioTeke Corporation, China), 0.4 µM of both forward and reverse primers and 1 µL of DNA template. PCR was performed in the MiniOpticon Real-Time PCR System (BioRad, USA). PCR cycle started with an initial denaturation at 95 °C for 2 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. Agarose gel (1%) electrophoresis was carried out with 1X Tris-acetate-EDTA (TAE) buffer at 95 V and 400 mA for 45 min. The gel was added with 10% Gelview (BioTeke Corporation, China) to visualize the PCR products. Size of the amplified products was estimated using 100 bp DNA ladder (BioTeke Corporation, China). PCR products were purified using Gel Extraction and PCR Purification Combo Kit (BioTeke Corporation, China) following manufacturer's instructions. Purified PCR products were sent for nucleotide sequence analysis.

Phylogenetic analysis

TEF-1α sequences were aligned using Clustal W and compared with other sequences in the NCBI GenBank, *Fusarium* MLST and *Fusarium*-ID databases. Database comparisons allow accurate species identification based on the closest match of basic local alignment search tool (BLAST) analysis. Phylogenetic analysis was conducted using Molecular Evolutionary Genetic Analysis (MEGA X) version 10.1 (Tamura et al., 2011). Phylogenetic tree was generated using Neighbor Joining (NJ) method with 1000 bootstrap value.

Results and Discussion

A total of 22 *Fusarium* isolates were recovered from black pepper showing slow decline symptoms of which seven isolates were isolated from Belaga while 15 isolates from Betong. It was observed that the colour of aerial mycelium of all isolates was white. Nevertheless, pigmentation on PDA was decisive for separation in groups. Isolates were grouped into three groups, following the varying pigmentation shades of pale yellow to pink to violet colour. *F. solani* isolates typically produced pale yellow to orange-brown pigmentation whereas *F. oxysporum* isolates exhibited pink to pale violet colour pigmentation. *F. proliferatum* produced purple-violet pigmentation with age. Based on morphological characteristics, three *Fusarium* species, namely *F. solani* (18 isolates), *F. oxysporum* (3 isolates) and *F. proliferatum* (1 isolate) were identified. Based on literature, *F. solani* and *F. oxysporum* were the most common species associated with slow decline symptoms in black pepper (Duarte et al., 2001; Wong, 2010). No previous reports had documented *F. proliferatum* as a cause of disease in black pepper. However, it was a known plant pathogen reported to cause fruit rot in chili pepper (Salvalaggio and Ridao, 2013). Nevertheless, *F. proliferatum* frequency percentage at 4.6% is too low to be considered as the causal agent of slow decline disease.

In the macro- and microconidia, variation of shape, length and width were observed among the three species. Macroconidia containing three reaching up to six septa. The formation of chlamydospores was detected except in *F. proliferatum*. The morphological characteristics of the three *Fusarium* species were presented in Table 1. Physiological and morphological characteristics are useful to distinguish *Fusarium* species. The shape and size of macroconidia show the greatest diversity and provide the highest species resolution (Leslie and Summerell, 2006). Morphological stability in changing environments is of fundamental importance in the taxonomic classification of *Fusarium* species. Nevertheless, morphological ambiguity caused by environmental variation (temperature, pH, light, and humidity) can be misleading. Thus, taxonomic identification needs to be reassessed with molecular data.

TEF-1α was successfully amplified from all 22 *Fusarium* isolates. A single band of 500 bp was obtained from PCR amplification. Based on the closest match of BLAST analysis, morphologically identified isolates produced the same species identity (with sequence similarity values of 98-100%) when tested against three databases. Hence, cultural and morphological characteristics were relevant in *Fusarium* species identification. From the Neighbour-joining tree constructed using EF1-alpha sequences, isolates of the same species were clustered in the same clades (Figure 1). Intraspecific variations were observed in *F. solani* isolates with the division of two major sub-clades.

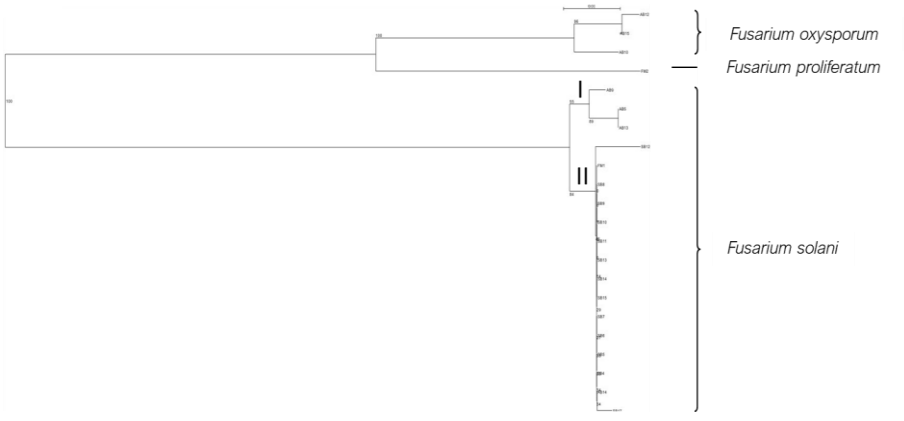

Figure 1: Phylogenetic analysis of 22 *Fusarium* isolates from black pepper based on TEF-1α sequences. Phylogenetic tree was generated from a Neighbor-Joining method using MEGA X version 10.1. Values above the branching nodes represent the percentage bootstrap value calculated from 1000 replicates. Bootstrap values >50% are indicated.

TEF-1α is useful in comparative sequence-based fungal identification due to the ability to discriminate between closely related species and individuals within species. From phylogenetic analysis, *F. solani* isolates were clustered in two sub-clades which indicated intraspecific variations. *F. solani* is regarded as a species complex and is subdivided into at least 60 distinct phylogenetic species (Sisic et al., 2018). This species is a genetically heterogenous morphospecies with diverse evolutionary lineages (Chehri et al., 2015). Genetic variation within species may have resulted from the adaptation of fungi to various environmental conditions. Intraspecific variation within a species can result in growth and variations in nutritional requirement (carbon utilization and nutrient utilization) (Johnson et al., 2012). The latter may have important consequences on the pathogenicity and virulence of the fungi. Thus, variation in plant-fungi and ecological interactions could have major implications on slow decline disease management.

Table 1: Morphological characteristics of *Fusarium* species associated with slow decline disease in black pepper.

Fusarium species	Pigmentation	Macroconidia	Microconidia	Chlamydospores
F. solani	▪ Varied from white to pale yellow, white to orange-brown	▪ Abundant in sporodochia ▪ Straight to slightly curved ▪ Curved or pointed at apical and foot-shaped basal cell ▪ Three-to-six septate	▪ Abundant in aerial mycelia ▪ Oval or ellipsoid ▪ Usually zero or occasionally one-to-two septa	▪ Singly or in pairs
F. oxysporum	▪ Varied from white to pale violet, white to light pink	▪ Abundant in sporodochia ▪ Falcate to almost straight ▪ Pointed at both ends ▪ Three-to-four septate	▪ Abundant in aerial mycelia ▪ Oval or ellipsoid ▪ Usually zero septa	▪ Singly or in pairs
F. proliferatum	▪ White to purple-violet	▪ Lack of sporodochia ▪ Slender to almost straight ▪ Pointed apical cell and poorly developed basal cell ▪ Three-to-five septate	▪ Abundant in aerial mycelia ▪ Club shaped ▪ Usually zero septa	▪ Absent

Conclusions

The present study contributed to the knowledge of *Fusarium* species associated with black pepper slow decline disease. This study has identified *F. solani* as the most predominant species in causing slow decline disease. Molecular phylogeny analysis has revealed intraspecific variation in *F. solani.* This study findings are important in the formulation of effective disease management strategies such as resistance breeding and biological control against *Fusarium* species in black pepper.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effects of Plant Growth Promoting Rhizobacteria Consortia on Total Chlorophyll Content and Osmolytes Concentration on Rice (*Oryza sativa* L.) under Drought Stress Condition

Emmyrafedziawati, A.K.R.*, Abdul Munir, A.M. and Farah Diba, A.B.
Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.
**E-mail: p92202@siswa.ukm.edu.my*

Introduction

Abiotic stress can affect an organism's physiological status directly or indirectly by altering its metabolism, growth, and development. Drought is one of the main factors which affects crop production, mainly to crops that depend on irrigation systems such as rice. The process of photosynthesis and cell growth is a significant process influenced by stress (Lotfi et al., 2015). Plants grown under drought conditions have a lower stomatal conductance to conserve water. Severe drought can cause changes in chlorophyll content by affecting chlorophyll components and by damaging the photosynthetic apparatus

Several adjustments and mitigation strategies are needed to cope with drought stress conditions by using the rhizosphere. Using these highly beneficial bacteria is an alternative strategy that can improve crop performance while increasing crop growth through different mechanisms (Dimkpa et al., 2009). Several different mechanisms are adapted to deal with drought stress. Among them is the accumulation of compatible osmolytes such as proline (Cattivelli et al., 2008) and total soluble sugar (Izanloo et al., 2008). Proline acts as a compatible solute that adjusts the osmotic potential in the cytoplasm. And proline is also used as a stress-related metabolic marker (Caballero et al., 2005). Increased proline levels indicate plant growth promoting rhizobacteria (PGPR) effectiveness in helping crops tolerant to drought stress (Grover et al., 2014).

The combination of several PGPR bacteria or with mycorrhizal fungi may have a more significant and synergistic effect in reducing drought than the use of individual genera (Knoth et al., 2014; Timm et al., 2016). The consortia can develop a strong interaction during root colonization and improve nodulation and the N fixation process (Lucas Garcia et al., 2004). Kohler et al. (2008) mentioned that numerous bacterial and fungal combinations had been applied to relieve the harmful effects of abiotic stress on plants. Applying associated microbes to crop plants under drought conditions offers new insights into novel protocols to boost plant defense response to drought.

Materials and Methods

Plant material, PGPR and treatments

Rice seed of MR 219 was obtained from the Malaysian Agricultural Research and Development Institute (MARDI) Genebank. The PGPR consortia were from *Bacillu*s and *Achromobacter* genera isolated from rice root and soil. This study was conducted in a Completely Randomized Design (CRD) with three replications (Table 1).

Table 1: List of treatments.

No.	Treatments
T1	non-inoculated seed (well-watered)
T2	non-inoculated seed (under drought condition)
T3	inoculated seed (under drought condition)

Drought stress was imposed by water retention for seven days. Rice leaves are taken at the vegetative stage to analyze the chlorophyll, proline, and total dissolved sugar contents.

Seed bioprimed

The rice seeds were sterilized with 3.5% household bleach and a drop of Tween 80. Then rinsed with sterile distilled water three times before placing the seeds on the sterilized filter paper in a petri dish. The seeds were soaked in different bacterial suspensions sufficient population density of 10^7 CFU/mL and shaken overnight at 150 rpm for biopriming. Seeds soaked in sterilized distilled water were considered as control (non-inoculated). Rice seeds were put on sterile filter paper in 100 mm petri dishes. They were irrigated with sterilized distilled water during seven days of incubation at 28±2 ºC.

Chlorophyll determination

A sample of 0.05 g leaf material was incubated in 10 mL DMSO at 60 °C for 1 h. At 645 and 663 nm, the absorbance of chlorophyll a, b, and total chlorophyll recorded and calculated as per Arnon (1949).

Proline determination

Free proline content in rice leaves was determined according to Bates et al. (1973). Leaf samples (0.2 g) from each treatment were homogenized in filtered 3% aqueous sulphosalicylic acid. An aliquot of 1 mL filtrate was then mixed with 1 mL acid-ninhydrin reagent and glacial acetic acid and the resulting mixture was heated at 100 °C for 1 h in a water bath; the reaction was stopped using an ice bath. The mixture was extracted with toluene, and the upper layer of the mixture containing toluene was measured at 520 nm. Proline concentration was calculated and expressed as μmol proline g^{-1} FW by using the calibration curve.

Total soluble sugar

The total soluble sugar was determined based on the phenol sulfuric acid method (Dubois et al., 1956). 0.1 g dry weight of leaves was homogenized with deionized water, then treated with 1% phenol and 98% sulfuric acid. The mixture was allowed to stand for 1 h and absorbance at 485 nm was determined by using a Synergy H1 microplate reader (BioTek®). Content of soluble sugar was expressed as mg/g.

Statistical analysis

One way analysis of variance (ANOVA) was performed and comparison among means was carried out using the Tukey test at $P<0.05$ with the help of GraphPad Prism 8 software.

Results and Discussion

After going through several screenings such as resistance to high temperatures, the mixed consortia were selected based on; resistance to osmotic stress conditions, and characteristics as a plant growth promoter (data not included). They were mixed in Luria Bertani (LB) broth to form a mixed consortium. This mixed consortium was used as an inoculant on rice seeds and then inoculated again on the soil at 15 days after sowing (DAS). The soils were autoclaved before treatment to ensure that the existing soil microorganisms do not influence the analysis results. Table 2 shows the total colony count of microbes in the soil samples from the rice pot. The soil sample was taken on the 55^{th} day after sowing (DAS). Soils inoculated by PGPR contained many colonies of bacteria than the non-inoculated soils.

Table 2: The total colony count in rice soil samples taken on the 55th day after sowing.

Treatments	Total Colony Count (CFU/G)
Non-inoculated (well-watered)	2.1×10^3
Non-inoculated (drought)	1.4×10^3
Inoculated (drought)	2.1×10^5

In the drought condition, rice inoculated with PGPR mixed consortia (T3) had significantly more total chlorophyll content than control (T2) ($P<0.05$). The results (Figure 1) observed the lowest content of chlorophyll pigments in non-inoculated rice under drought conditions (T2). Ommen et al. (1999) reported that leaf chlorophyll content decreased with the onset of drought stress. The application of PGPR mixed consortia positively affected drought stress rice and this demonstrates the efficiency of PGPR inoculation in maintaining chlorophyll content in drought conditions.

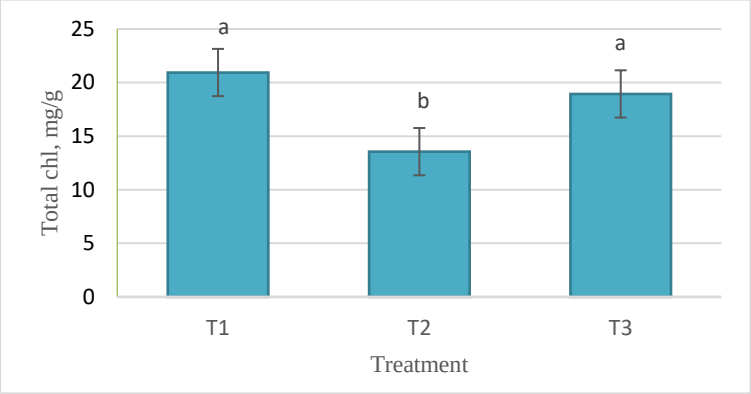

Figure 1: Total chlorophyll content of inoculated and non-inoculated rice. Means with different letters indicate significant differences according to Tukey test ($P<0.05$).

Figure 2 shows that the inoculated rice under drought conditions had the highest proline concentration than other treatments. Inoculated rice showed a 48.3% increased than the non-inoculated rice under drought stress. It was suggested that the increase in leaf proline contributes to the observed drought tolerance by protecting the plants from dehydration stress. Furthermore, proline accumulation helps to maintain the osmotic adjustment in plant cells.

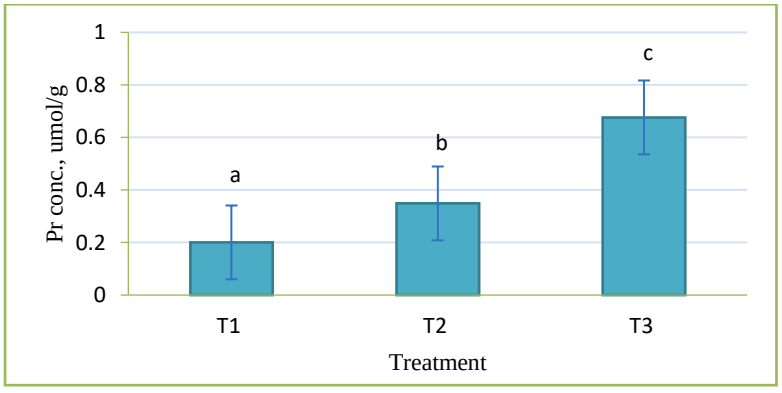

Figure 2: Proline concentration of inoculated and non-inoculated rice taken on the 55th day after sowing. Means with different letters indicate significant differences according to Tukey test ($P<0.05$).

The average amount of total soluble sugar in inoculated and non-inoculated rice leaves under drought stress was 90 mg/g and 78 mg/g, respectively. Soluble sugars may also function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor pressure. Drought is the most important factor that inhibits photosynthesis. Glucose induces stomatal closure and increases plant adaptability under drought stress (Osakabe et al., 2013). Soluble sugars maintain leaf water content and

osmotic adaptation of plants facing drought stress conditions. Generally, under abiotic stress conditions, sugar acts as an osmoprotective and also stabilizes the cell membrane.

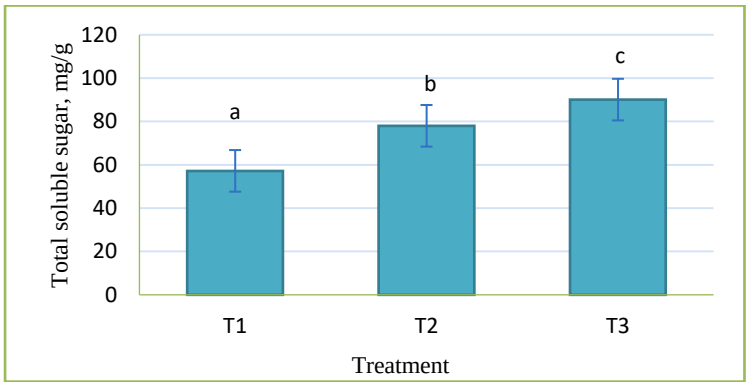

Figure 3: Total soluble sugar concentration of inoculated and non-inoculated rice taken on the 55th day after sowing. Means with different letters indicate significant differences according to Tukey test ($P<0.05$).

Conclusions

Rice inoculation with PGPR consortia turned out to have a significant effect during drought stress conditions. The total chlorophyll content in inoculated rice under drought stress is almost equal to the content of chlorophyll in non-inoculated rice under well-watered conditions. Similarly, the proline content and the amount of total soluble sugar in inoculated rice leaves in drought conditions show significant results compared to both rice that is non-inoculated either in drought or well-watered. Technically, the combination of several PGPR strains can help rice tolerance during drought at the vegetative stage as shown in this study. Other analyses, such as antioxidant defense activities are still under study.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Preliminary Findings on Spatial and Temporal Pattern of Total Nitrogen in Black Pepper Farm after Rainfall Event

Izzah, A.H. and Wan Asrina, W.Y.*
Department of Crop Science, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia.
**E-mail: asrina@upm.edu.my*

Introduction

In black pepper production, sufficient nitrogen (N) uptake is crucial to ensure a healthy formation of fleshy catkin. The common practices among farmers are applying NPK green (15:15:15) and later NPK blue (12:12:17) regardless of land topography and other agro-climatic conditions. Nitrogen deficiency is very common on undulating topography with slope >20^{o} during rainy season due to poor terracing construction (Meena et al., 2017). Eventually, the nutrient will be transported and polluted the nearest stream or river. Although, good agricultural practices have been taught to respected farmers, only a few have adopted the practices while others remain practicing the conventional methods. Because of this, a lot of black pepper farms have experienced N deficient, and later defoliation. In severe condition, crop may become stunted and subjected to death, especially on wet season (Ann, 2012). Thus, the objective of this study was to determine the rainfall effect (October 2018) on N mobility on black pepper farm located in Bintulu, Sarawak.

Materials and Methods

The research study was conducted in black pepper farm (0.06 ha) located in Samarakan, Bintulu, Sarawak (3^{o}0'42.19" N; 113^{o} 1' 34.23" E). The farm was formerly a secondary forest located at hilly terrain (slope >20^{o}) which have been slashed and burned for pepper cultivation. It was observed that agricultural terracing has not been constructed, and the soil surface was bald from any vegetation for most of the time. About 132 vines were grown in the area following standard planting practices suggested by Malaysian Pepper Board (MPB) with 110 was *Indian* cultivar, and 22 was *Rembai* cultivar on non-living pole using *Commersonia bartramia*. The soil was identified as Bekenu soil series which developed over mixed sedimentary rocks. This area is classified as humid tropical region with average rainfall 313 mm and annual temperature 26.6 ^{o}C.

About 120 surface soil samples (0 to 20 cm) were taken by blocking the farm into six main blocks with following randomised complete block design (RCBD). The collected soil samples were air-dried, pulverized, and then was analysed for total N using Kjeldahl method (Hach et al., 1985) and quantify using AutoAnalyzer3.

All results were computed using descriptive statistics for minimum, maximum, coefficient of variation (CV), skewness, and kurtosis using JMP® Version 10. Normality test was conducted to access the distribution of data using Shapiro-Wilk, and an outlier was temporarily removed to obtain a normal distribution. The outliers were introduced again to honour the data and projected their model using isotropic semivariogram to measure goodness of fits using GS+ Geostatistics for the Environmental Sciences Version 10. The model reliability was checked according to Liu et al. (2013). Ordinary Kriging was used to produce the maps on spatial and temporal pattern of total nitrogen distribution.

Results and Discussion

Descriptive statistics on soil total nitrogen

The results showed a range of N of 0.35 to 2.26 g kg^{-1} on sampling day 1 to day 5 with a CV ranged from 10.19% to 33.52% (Table 1). However, among all sampling day, only day 3 has exhibited data abnormality, thus it was temporarily removed to obtain normal distribution (Fu et al., 2010; Izzah and Wan Asrina, 2018). Skewness values close to zero has indicated normal distribution were obtained, while higher than zero indicate high values in measured samples. Apart from normal data distribution, CV was categorised into three classes according to Zhang et al. (2007) which are low (<10%), moderate (10% to 90%), and high (>90%).

Table 1: Descriptive statistics of total nitrogen from sampling day 1 to 5.

Sampling Day	Minimum	Maximum	CV	Skewness	Kurtosis
1	1.1463	1.9464	13.8446	0.8725	0.6047
2	0.5568	2.2600	24.1115	0.5509	2.9460
3	0.9909	1.4818	10.1868	0.6891*	1.0211*
4	0.8748	1.6675	15.1496	1.3374	2.5920
5	0.3465	1.1305	33.5247	1.5094	2.7849

**skewness and kurtosis values after removing outliers (normality test using Shapiro-Wilk).*

According to our results, N distribution was mainly affected by a moderate variable which indicated both factors namely intrinsic (e.g., parent material/soil properties) and extrinsic (e.g., crop management practices) were involved. The main contributor to lessen CV value may indicate that the area was surrounded by sandy loam (Bekenu soil series which developed over mixed sedimentary rocks) which has fastened the N movement (Zhang et al., 2010). Moreover, sampling day 5 has exhibits highest CV which related closely to other factors as well, such as application of fertilizer either method of application (e.g., broadcasting, strip, and others) and timing of application (e.g., seasons, time, and others). About 100 g/vine of fertilizer was added to the pole, eight hours before rainfall event, and this has closely attributed to the CV value (33.5247%). Normally, a higher CV indicated high heterogeneity and closely attributed to the recent application of N fertilizer.

Geostatistical analysis

Semivariogram model has shown total N was best modelled using spherical (modified quadratic functions) and exponential (similar to spherical but have a gradual sill) models which indicated different arising of spatial correlation (Table 2). The range may be considered shorten with value between 4.36 to 7.98. Rosemary et al. (2017) and Takoutsing et al. (2017) have emphasised that a short range/distance may illustrate extrinsic factor affecting the surrounding which mainly focuses on anthropogenic activities imply by farmers e.g., land uses. Despite the shorten range, all the sampling day exhibits a comparable sill value, thus representing moderate spatial heterogeneity compared to higher sill which has a greater spatial heterogeneity.

The spatial dependence of geostatistical analysis which calculated with nugget to sill ratios may be interpreted as strong (<25%), moderate (25% to 75%), and weak (>75%) (Cambardella et al., 1994). Consequently, all sampling day have indicated a strong spatial dependence which mainly caused by intrinsic factors such as topography, vegetation, parent material, and others from this farm (Lin et al., 2016). It was observed that spatial dependence had indicated an increasing ratio value at all five sampling events. Even though the range value increases, the value considers lower 4.36-7.98 and provides evidence of degree of influence by extrinsic factors (e.g., management practices by farmer).

Table 2: Geostatistical analysis of total nitrogen according to sampling day.

Sampling Day	Nugget	Sill	Range	Ratio	Model
	Co	Co + C	A	Co/Co + C	
1	1.00×10^{-4}	4.34×10^{-2}	7.98	0.23	Spherical
2	5.00×10^{-4}	9.30×10^{-2}	5.29	0.54	Spherical
3	1.00×10^{-4}	4.76×10^{-2}	4.36	0.21	Spherical
4	1.17×10^{-3}	3.09×10^{-2}	6.73	3.78	Spherical
5	1.30×10^{-3}	3.45×10^{-2}	6.93	3.77	Exponential

Spatial pattern

Ordinary Kriging was used to illustrated spatial and temporal pattern of total N in black pepper farms after rainfall. It was observed that the distribution of N mainly affected by intensity of rainfall and their duration and volume (Figure 1).

Among all sampling day, the N movement was accelerated with the help of rainfall intensity (13.80 mm hr^{-1}) on sampling day 2 (0.5623 g kg^{-1} to 2.2449 g kg^{-1}). Meaning, higher rain intensity has contributed to greater N movement and sparse around the farm. According to Wang et al. (2019), soil nutrients may actively move as rainfall intensity even though others factor such as vegetation cover and soil moisture also play important roles in N movement (Mazur, 2018). Ineffective fertilizer application, i.e. applying NPK blue without considering daily weather has affected N movement on sampling day 5 with high rainfall volume (44.70 mm). In this condition, higher rainfall volume has promoted N movement due to textural class (sandy loam; parent material) and eventually showed lessened N concentration in the farm area. Subsequently, chlorosis and defoliation problems which mainly subjected to N deficiency were easily observed during this time, thus, reducing berry production.

Conclusions

Rainfall intensity and volume have shown a high tendency in affecting N movement in a poorly managed farm. Farm with bare soil surface and at the same time using non-living poles as a plant's support has become an important extrinsic factor that controlled the movement of the nutrients. Therefore, it is good practice for farmer to grow legume crop prior to reduce N movement and provide N fixation in soil-plant system. Few ways to maximise nutrients uptake are following local weather forecast, and foliar spray to reduce N hunger.

Acknowledgements

The research was partly supported by the project number GP-IPB/2018/9557605 from Universiti Putra Malaysia.

Figure 1: Spatial and temporal pattern of total nitrogen (g kg^{-1}) after rainfall events in October 2018.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Nitrogen, Phosphorus and Potassium Availability on Adan Rice Planted in Lowland Area

Izzah, A.H., Adibah, M.A. and Wan Asrina, W.Y.*
Department of Crop Science, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus, 97008 Bintulu, Sarawak, Malaysia.
**E-mail: asrina@upm.edu.my*

Introduction

Adan rice is a Sarawak's local variety and cultivated for home consumption with characteristics of small grain size, pleasant taste, and fine texture. The rice can be cultivated in upland and lowland areas in Sarawak using rainfed irrigation system yearly from October to April. However, cultivating the rice on lowland area can be subjected to insufficient water supply and experience flood and drought conditions. Besides problem in water supply, insufficient nutrient supply will potentially limit productivity and cause multiple problems that eventually lead to low yield production (Mahmod et al., 2014). To sustain rice production and improve soil quality, some farmers practised crops rotational with baby corn during off-season. Though, it improves early crop establishment, but the nutrient substantially become depleted and need replenishment throughout cultivation period. Nutrient such as nitrogen is crucial to promote rapid growth and significant yield responses in all lowland rice soils (Dobermann and Fairhurst, 2000). Meanwhile, phosphorus and potassium provide substantial effect on energy storage and important roles in crop metabolism, development, and final yield (Marschner, 2012). Therefore, the objective of this was to determine effect of nitrogen, phosphorus, and potassium (NPK) availability on Adan rice grown under lowland conditions.

Materials and Methods

The study has been conducted on a hectare of rice field in Kuala Tatau, Bintulu, Sarawak during rainy season (from October 2017 to April 2018). The seedlings were raised in the nursery for a month and transplanted (~1 m apart) manually to the field. Urea (46% of N) and NPK Red (17:3:25:2 MgO + TE) were applied simultaneously on 90 days after planting (DAP) at the rate 125 kg/ha and the crop was harvested 150 to 160 DAP.

Research plot was blocked into four plots (10 m x 10 m with 15 m distance between plots) according to rice field size. About 48 surface (16 samples/phase) soil samples (0 to 20 cm depth) were collected on three rice phases (vegetative, reproductive, and ripening), air dried, and analysed for soil N, P, and K concentrations using wet digestion, Bray and Kurtz's, and double acid methods (Bray and Kurtz, 1945; Hach et al., 1985; Mylavarapu et al., 2002). Results collected were analysed and subjected to factorial design (3 X 4) at P=0.05 Tukey's Studentized Range (HSD) test. The main reasons for using factorial design are comparing the main factor of growth phases with different plot.

Results and Discussion

Results showed significant differences in nutrient availability at different plant growth phases (Table 1). Apparently, K availability was greater in vegetative phase compared to reproductive and ripening phases. Meanwhile, greater N availability was shown in reproductive phase. Field observation on the nutrient's distribution have as well indicated that there were significant differences in NPK availability between main plot and greater concentration of K in Plot 3.

Table 1: Effect of growth stage and plot on N, P, and K concentration in soil of rice field.

Characteristics	N	P	K
	mg/kg		
Growth phase			
Vegetative	2012.60[b]	4.05[a]	68.19[a]
Reproductive	2369.70[a]	4.04[a]	39.41[b]
Ripening	1801.60[b]	3.56[a]	37.71[b]
Plot			
1	1997.30[a]	3.80[a]	44.83[b]
2	2013.50[a]	3.53[a]	47.95[ab]
3	2049.90[a]	4.46[a]	54.53[a]
4	2184.60[a]	3.75[a]	46.43[b]

Values with different alphabet within column indicate significant different at P=0.05 using Tukey's Studentized Range (HSD) test.

The main contribution towards greater K (68.19 mg/kg) in vegetative phase (42 DAP) may attributed to farmer practices where the residual from burned corn stalks was left before the rice cultivation take place. Ando et al. (2014) and Mbah and Nneji (2011) had emphasized that K availability increases due to high temperature during degradation of SOM (burning corn stalks), including supply of ash content. Meanwhile, elevated N (2369.70 mg/kg) availability recorded in reproduction phase (90 DAP) closely related to amendment of urea (46%) and NPK Red (17:3:25:2 MgO + TE) to the crop. Meanwhile, Plot 3 shown greater P (4.46 mg/kg) and K concentration (54.53 mg/kg) compared to other plots. The main effect on variation of this nutrients may attributed to growing behaviour of the crops. It was observed that, Plot 3 has the best condition for the rice to grow compared to Plot 1, 4, and 2. Even though NPK showed greater concentration in some phases and plots, problems on nutrient deficiencies were visible particularly during vegetative growth phase as multiple chlorosis was observable.

Conclusions

Sufficient NPK availability to Adan rice helps in promoting its growth and yield production. However, late fertilizer application will lead to unsatisfactory rice growth by increasing nutrient starvation during tillering stage and affect panicle initiation and flowering. Therefore, farmers are encouraged to follow fertilizer management scheme as given by DOA Sarawak to enhance Adan rice production.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Malaysia Plant Red List: Rapid Assessment of Peat Swamp Forest in Pekan Forest Reserve Southeast Pahang

Mohd Afzanizam, M.[1,*], Elizabeth, P.[2,] and Azian, M.[1]

[1]Climate Change and Forestry Program, Forestry and Environment Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
[2]Ministry of Energy and Natural Resources, Reducing Emissions from Deforestation and Forest Degradation (REDD Plus) Unit, Wisma Sumber Asli, No. 25 Persiaran Perdana, Presint 4, 62574 Putrajaya, Malaysia.
**E-mail: mohdafzanizam@frim.gov.my*

Introduction

The principles, categories and criteria used in the taxon assessment for this study using two main documents namely the International Union for Conservation of Nature (IUCN) Red List Categories and Malaysia Plant Red List. There are several criteria version for IUCN Red List upgrades based on year of assessment 1998, 2017, 2018, 2019, and 2020 while for Malaysia Plant Red List version including 2001, 2010, 2013, and 2018. There are nine categories and descending severity of extinction risk. Pahang has the greatest number of threatened taxa for flora (59 taxa; 35.9%) as compared to other states in the peninsular (Chua et al., 2010). This is followed by Johor and Perak with 54 (32.9%) and 53 (32.3%) taxa, respectively. The main threat to all threatened species is land use change. The study justifies the suitability of peat swamp forest as research site and contains the peninsula's only extinct taxon (Chua et al., 2010). Hence the objectives of the study are; 1) to assess red list plant in mixed peat swamp forest using IUCN and Malaysia Red List Plant criteria, and 2) to quantify biomass and carbon stocking of red list plant. Peat swamp ecosystem support a high proportion of specialized, endemic taxa despite the inhospitable conditions (waterlogged during monsoon, anaerobic soil environment and nutrient-deprived). Plants growing in waterlogged sites (during monsoon season from November to January every year) will exhibit more stilt roots, pneumatophores, and knee roots, and have thicker roots with more pore spaces, than the same species in drier sites (Pahang Forestry Department, 2005).

Materials and Methods

The study site is located at Compartment 75, Pekan Forest Reserve, Pahang, Malaysia (Figure 1). Twenty-five ecological plots were established for species enumeration and identification. Each ecological plot sized 20 m x 20 m with cumulative 1-ha of study area. The Compartment 75 is a 200-ha area gazetted under production forest.

Forest stand metrics

All living and dead trees were enumerated in the plot. The merchantable height (mht) and diameter at breast height (dbh) were recorded. Apart from that, metrics of abundance including basal area and stand density were also calculated. Stocking density (number of trees per unit hectare of sampling area), tree basal area, and tree volume were calculated as follow:

Basal area, $ba = [\pi (dbh^2)/40000]$ (m^2)
Tree volume, $vol = ba \times mht \times 0.65$ (m^3)

Where;
mht is merchantable bole height (m).

As there is no specific volume table for peat swamp forest, 0.65 value is a generalised form factor that applies to all trees (JPSM, 1997). Only trees with dbh of more than 10 cm were analysed. Threatened category were cross-checked from two main databases namely Malaysia Biodiversity Information

System (MyBIS) and Malaysian Convention on International Trade in Endangered Species of Wild Fauna and Flora (MyCITES).

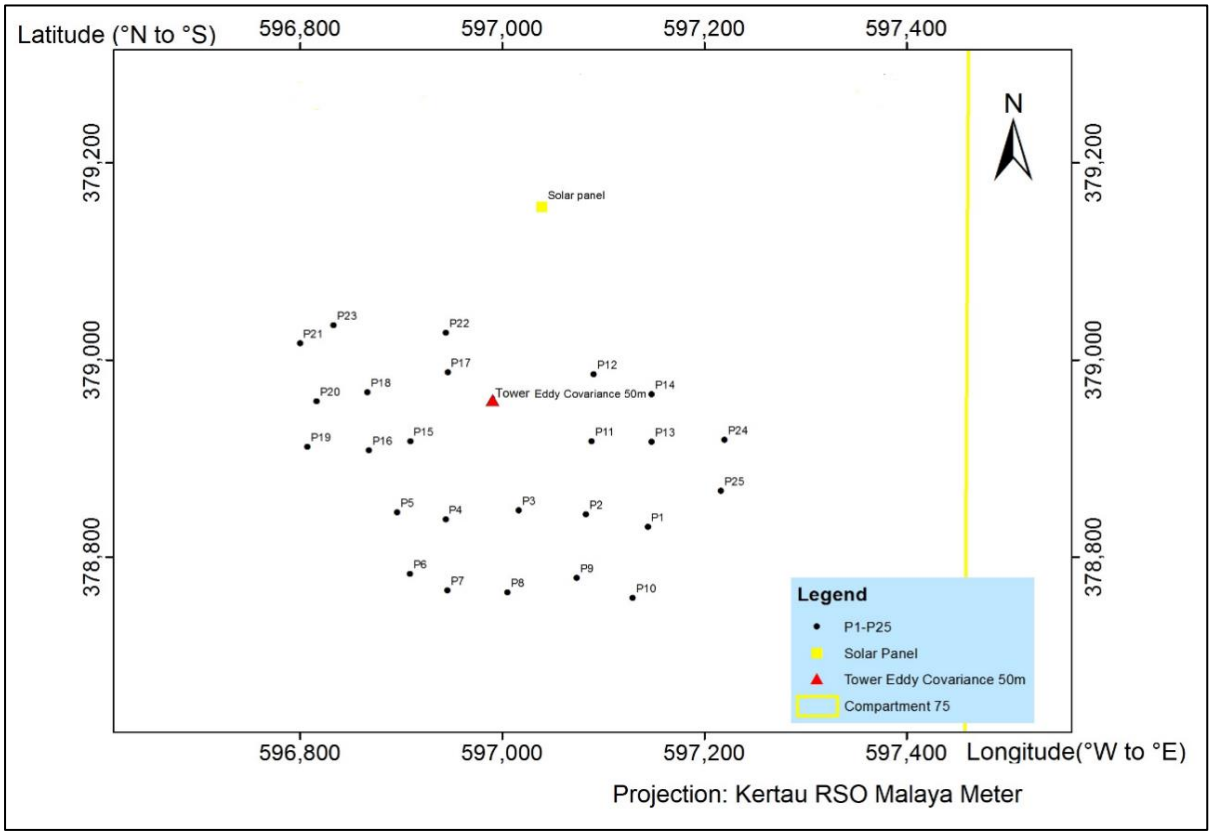

Figure 1: Location map of Compartment 75 in Pekan Forest Reserve, indicating 25 ecological plots (P1-P25).

Tree biomass and carbon stock

Allometric function used for biomass calculation using three variables/predictors, namely dbh, merchantable height (h) and wood density (wd) (Manuri et al., 2014). The chosen allometric function is best suited for this study considering similar forest type; mixed peat swamp forest and its locality in the geographic region.

Biomass (aboveground), AGB (kg/ha) = 0.0494 x $dbh^{1.7961}$ x $h^{1.2292}$x $wd^{0.9170}$, dbh in cm, h in meter and wd as oven dry mass/fresh volume in (g/cm^3) ---------- (Manuri et al., 2014),

Biomass (belowground), BGB = 20.1% of AGB ---------- (IPCC, 2006)

Total Plant Biomass = AGB + BGB

Carbon stock was calculated by multiplying the sum of biomass with C fraction. The C fraction of dry matter in biomass used for this study is 0.47 (IPCC, 2006)

Results and Discussion

Compartment 75 is generally classified as mixed peat swamp according to phase of vegetation (Anderson, 1961, 1963 and 1964) and referred to as phasic community 1. The tropical lowland peat

swamp forests in Sarawak, Malaysia and adjacent Brunei show lateral or horizontal changes in vegetation types from its periphery (seaward) to the centre (landward) of the dome-shaped peat.

Table 1: List of taxa categorised as threatened in Pekan Forest Reserve Pahang (Compartment 75).

Threatened category	Species	Remarks
Critically endangered (CR)	*Gonystylus bancanus* (Ramin melawis)	IUCN Red List (version3.1, 2018) IUCN Red List (version 2.3, 1998)
Vulnerable (VU)	*Tetramerista glabra* (Punah)	IUCN Red List (version 3.1, 2020),
	Shorea platycarpa (Meranti paya)	Peninsular Malaysia Plant Red List (2010)
	Ctenolophon parvifolius (Mertas)	IUCN Red List (version 3.1, 2019)
	Sandoricum beccarianum (Sentul)	Peninsular Malaysia Plant Red List (2013)
	Santiria rubiginosa (Kedondong kerantai) *(*var. *latipetiolata)*	IUCN Red List (version 2.3, 1998)
	Horsfieldia crassifolia (Penarahan)	Malaysia Plant Red List (2010)
	*Dacryodes macrocarpa (*Kedondong matahari)	IUCN Red List (version 2.3, 1998)
Near threatened (NT)	*Aglaia rubiginosa* (Bekak)	IUCN Red List (version 2.3, 1998), Peninsular Malaysia Plant Red List (2013)
	Planchonella maingayi (Nyatoh nangka merah*)*	IUCN Red List (version 3.1, 2019)
	Shorea leprosula (Meranti tembaga)	IUCN Red List (version 3.1, 2017)
	Myristica lowiana (Penarah arang gambut)	IUCN Red List (version 2.3, 1998), Malaysia Plant Red List (2018)

Out of three threatened categories, only Critically Endangered (CR) and Vulnerable (VU) were critically discussed in this paper as Near Threatened (NT) is not included (Table 1). Two indicator species categorized as CR were *Gonystylus bancanus* and *Shorea platycarpa*. In terms of botanical richness, peat swamp generally has less taxa hence reduced individuals per hectare of study area than inland forest. For instance, in Pekan FR, there are 465 individual trees per hectare with 62 tree species, 48 genera and 33 families while in Pasoh FR, 1474 individual trees per hectare with 235 species and 113 genera and 122 families.

Based on species composition (Table 2), *Gonystylus bancanus* (Ramin melawis) and *Shorea platycarpa* (Meranti paya) has higher stocking density and total volume as compared to other trees. Stocking densities refer to frequency of occurrence a particular species at given time. Field observation on *Shorea platycarpa* (Meranti paya) for instance are considered big, mature tree with diameter -at -breast height (dbh) ranged between 15.8 to 102 cm and height between 10.9 to 28.7 m. Diameter –at- breast height (dbh) for *Gonystylus bancanus* (Ramin melawis) ranged between 21.1 to 73 cm and height 18 to 36.4 m. In general, large dbh and height will contribute to large value for basal area and tree volume. *Shorea platycarpa* (Meranti paya) were initially categorized as Near Threatened (NT) based on Peninsular Malaysia Plant Red List in 2010 has move one tier up to become Endangered (EN) based Malaysia Plant Red List (2010, version 3.1) and Sarawak Plant Red List (2014, version 3.1). Forest fragmentation and wood harvesting for timber are among the probable reason that cause decreasing trend of *Shorea platycarpa*.

In terms of endemicity, many of the tree families or species in peat swamp forests are restricted to this habitat (Ng and Ibrahim, 2001). Hence, this study to highlight floristic richness and rarity of red listed dipterocarps will further enhance knowledge on flora conservation in peat swamp forest. In Peninsular Malaysia, 260 species of plants were recorded from the Pekan Peat Swamp Forest alone (Latiff, 2005).

Table 2: Redlisted flora composition at Pekan Forest Reserve Pahang (Compartment 75).

No.	Trees species	Family	Stocking density (stems ha^{-1})	Total basal area (m^2 ha^{-1})	Total volume (m^3 ha^{-1})
1.	*Gonystylus bancanus* (Ramin melawis)	Thymelaeaceae	17	4.68	83.15
2.	*Shorea platycarpa* (Meranti paya)	Dipterocarpaceae	14	3.54	55.41
3.	*Tetramerista glabra* (Punah)	Tetrameristaceae	15	3.14	33.51
4.	*Ctenolophon parvifolius* (Mertas)	Ctenolophonaceae	1	0.008	0.043
5.	*Sandoricum beccarianum* (Sentul)	Meliaceae	1	0.23	2.76
6.	*Santiria rubiginosa* (Kedondong kerantai)	Burseraceae	16	0.76	7.73
7.	*Horsfieldia crassifolia* (Penarahan)	Myristicaceae	4	0.07	0.71
8.	*Aglaia rubiginosa* (Bekak)	Meliaceae	10	0.87	11.58
9.	*Planchonella maingayi* (Nyatoh nangka merah)	Sapotaceae	10	0.38	3.87
10.	*Shorea leprosula* (Meranti tembaga)	Dipterocarpaceae	1	0.35	5.36
11.	*Myristica lowiana* (Penarah arang gambut)	Myristicaceae	5	0.54	7.05
12.	*Dacryodes macrocarpa* (Kedondong matahari)	Burseraceae	1	0.08	1.20
		Total	95	14.64	212.37

Carbon stocking in peat swamp forest

Of 465 trees, 95 (approximately 20% of total population) were categorised as ‘threatened’ species (Table 2). The contribution of forest to the carbon pool depends on its successional stage. Forest biomass generally increases with stand age and approaches a constant level at maturity. Biomass increment in our 1-ha forest stand can be estimated by repeated tree census, so the changes in this case biomass/carbon stock due to growth and mortality of trees can be accounted in a given area. Tree census was conducted every two-year (2016 and 2018) and tree biomass for redlist plant was presented in Table 3. Carbon stocking contributed by redlist plant were 59.46 t C/ha in 2016 and 60.06 t C/ha in 2018, respectively. This gives an increment of 0.6 t C/ha in two years of study. Assuming no mortality of redlist plant (mostly tree fall due to lightning struck), increasing living biomass will contribute to higher carbon stocking and eventually higher primary productivity (NPP). The component of biomass change was analyzed based on tree growth, recruitment, and tree deaths. From our study in Compartment 75, biomass gain by tree growth (34.35 t/ha/yr) is consistently higher than biomass loss by tree death (0.03 t/ha/yr) and only a minor fraction accounted for recruitment (0.49 t/ha/yr).

Table 3: Tree biomass for redlist plant in Compartment 75, Pekan FR in 2016.

No.	Species	AGB (t/ha)	BGB (t/ha)	Total biomass (t/ha)	Carbon stock (t C/ha)
1	*Aglaia rubiginosa*	7.78	1.56	9.35	4.39
2	*Ctenolophon parvifolius*	0.02	0.00	0.03	0.01
3	*Dacryodes macrocarpa var. patentinervia/kostermansii*	0.51	0.10	0.61	0.29
4	*Gonystylus bancanus*	38.81	7.80	46.61	21.91
5	*Horsfieldia crassifolia*	0.35	0.07	0.42	0.20
6	*Myristica lowiana*	2.53	0.51	3.04	1.43
7	*Planchonella maingayi*	2.11	0.42	2.54	1.19
8	*Sandoricum beccarianum*	0.93	0.19	1.12	0.52
9	*Santiria rubiginosa*	4.42	0.89	5.31	2.50
10	*Shorea leprosula*	1.77	0.36	2.12	1.00
11	*Shorea platycarpa*	29.10	5.85	34.94	16.42
12	*Tetramerista glabra*	17.00	3.42	20.41	9.59
			Total biomass	126.50	59.46

Conclusion

Vulnerability and extinction risk of endemic species in peat swamp forest are increasing due to many factors. Hence conservation effort is mandatory as a critical step towards preservation and conservation of biological resources in tropical peat swamp.

Acknowledgements

Authors would like to thank the Malaysia Palm Oil Board (MPOB) for providing research grant to undertake "The Study of Carbon Stock Assessment in Permanent Peat Swamp Forest in Pekan Pahang" collaboration project under the 11th Malaysia Plan Research Budget (2016-2020). Appreciation also goes to the staff of the Forestry Department of Pahang, Kuantan District Forestry Office and field assistance from FRIM staff for data collection and analysis.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Development of Rice for Tolerance to Multiple Abiotic Stresses Through Marker Assisted Breeding

Noraziyah, A.A.S.*, Mohd Ikmal, A. and Waitul Fifika, A.
Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), 43600 Bangi, Selangor, Malaysia.
**E-mail: nora_aziz@ukm.edu.my*

Introduction

In Malaysia, the current self-sufficiency level (SSL) on rice (*Oryza sativa* L.) is around 70% despite the government's various types of subsidies. The remaining 30% has been imported from other rice-producing countries such as Vietnam, Thailand, and Pakistan. High dependency on import can cause a major threat to the country's social, economic, and political stability. However, to increase rice SSL is not an easy task due to several issues such as limited land suitable for rice, high production cost, and increasing severity of abiotic and biotic stresses due to climate change. Climate change-induced abiotic stress (such as drought, submergence, and salinity) adversely affecting rice growth and productivity. Malaysia's rice cultivars though high-yielding, are highly susceptible to abiotic stresses. Thus, developing high-yielding rice that can withstand abiotic stresses through a breeding program is a sustainable and viable option for improving productivity, reducing farmers' risks, and bringing marginal land into use. Selection of parental lines and breeding method is crucial to ensure the effectiveness of a breeding program. Conventional breeding seems to be an effective way to develop new rice cultivars; however, it is tedious and highly dependent on the breeder's subjective evaluation and empirical selection. The pressure is higher when it involves multiple stresses. As compared to conventional breeding, marker-assisted breeding (MAB) is more effective to produce new plant cultivars that tolerant to abiotic stresses in a shorter time.

Using MAB, beneficial alleles from traditional varieties, landraces, and wild relatives can be introgressed to develop climate-ready rice cultivars. Molecular markers mainly microsatellites (SSRs) and single-nucleotide polymorphism (SNPs) have been applied to locate genes and quantitative trait loci (QTLs) linked to the various traits of interest on rice chromosomes. Information about genes and QTLs linked to abiotic stress-tolerance is important, where this information can be used by plant breeders to develop promising lines. From previous studies, several major and consistent effects QTLs such as $qDTY_{1.1}$, $qDTY_{2.2}$, $qDTY_{3.1}$, $qDTY_{6.1}$, and $qDTY_{12.1}$ for drought-tolerance, *Sub1* for submergence tolerance, and *Saltol* and *SKC1* for salinity tolerance were identified (Septinengsih et al., 2009, Thomson et al., 2010; Kumar et al., 2018). Some of these identified QTLs have been successfully introgressed into Malaysian rice through MAB to improve tolerance to either single or multiple abiotic stresses (Noraziyah et al., 2016; Mohd Ikmal, 2020). Several breeding lines with good performance under non-stress (NS) have been selected through these breeding programs. In this study, these selected breeding lines were screened under multiple abiotic stresses to understand the effects of the introgressed QTL/s in providing abiotic stress/es tolerance.

Materials and Methods

Plant materials, phenotyping, and experimental design

Eleven rice genotypes consist of nine breeding lines and two mega-varieties, MR219 and IR64-*Sub1* were evaluated under reproductive stage drought stress (RS), vegetative stage submergence stress (VSS), and non-stress (NS). Two lines, MR219PL-335 and MR219PL-91, mega-varieties and salinity tolerant check, Nona Bokra were also screened under vegetative stage salinity stress (VSL). The information on the plant materials used in this study is shown in Table 1. The QTL's presence was confirmed using the following SSR markers: $qDTY_{2.2}$ – RM236, RM297, and RM12460; $qDTY_{3.1}$ –

RM416 and RM520; *qDTY*$_{12.1}$ – RM28099, RM28130, RM511, RM1261, and RM28166; *Sub1* – ART5, AEX, and SC3. The screenings were conducted in Kompleks Rumah Tumbuhan, UKM, Selangor from year 2018 to 2020. All experiments were carried out in Randomized Complete Block Design (RCBD) with three replications.

Table 1: Information on the breeding lines and check varieties.

Genotype	QTL	Background/Type	Reference
MR219PL-335	*qDTY*$_{3.1}$ + *qDTY*$_{12.1}$	MR219/*qDTY*$_{2.2}$/*qDTY*$_{3.1}$/*qDTY*$_{12.1}$	Noraziyah et al., 2016a
MR219PL-91	*qDTY*$_{3.1}$	MR219/*qDTY*$_{2.2}$/*qDTY*$_{3.1}$/*qDTY*$_{12.1}$	Noraziyah et al., 2016a
91-19	*Sub1*	UKMPL-91*/IR64-*Sub1*	Mohd Ikmal, 2020
91-37	*Sub1*	UKMPL-91*/IR64-*Sub1*	Mohd Ikmal, 2020
91-49	*Sub1*	UKMPL-91*/IR64-*Sub1*	Mohd Ikmal, 2020
335-112	*Sub1* + *qDTY*$_{3.1}$	UKMPL-5*/IR64-*Sub1*	Mohd Ikmal, 2020
335-155	*Sub1*	UKMPL-5*/IR64-*Sub1*	Mohd Ikmal, 2020
335-206	*Sub1* + *qDTY*$_{12.1}$	UKMPL-5*/IR64-*Sub1*	Mohd Ikmal, 2020
335-239	*Sub1*	UKMPL-5*/IR64-*Sub1*	Mohd Ikmal, 2020
MR219	No QTL	MR151/MR137 and susceptible to abiotic stresses	Zainudin et al., 2012
IR64-*Sub1*	*Sub1*	IR64/IR40931(*Sub1*) and tolerant to submergence	Septinengsih et al., 2009
Nona Bokra	*SKC1*	Landrace and tolerant to salinity	Ren et al., 2005

Drought, submergence, and salinity screenings

RS was imposed 30 days after transplanting. The soil surface was allowed to completely dry and become cracked to create RS. Irrigation by flash flooding for 24 h was only given when the check varieties showed severe leaf rolling and the water table level dropped below 100 cm. For the NS, 5 cm standing water was allowed throughout the experiment period. Days to flowering (DTF), plant height in cm (PH), number of panicles per plant (NP), chlorophyll content (CC), percentage of spikelet sterility (SFP), and grain yield in ton/ha (GY) were recorded according to Standard Evaluation System (IRRI, 2013). VSS was set up according to Ikmal et al. (2019a). Ten pre-germinated seeds per genotype per replication were sown in the plastic tray and placed in a 1 m height tank. On the 21st day after germination, the seedlings were completely submerged with rainwater in the tank for 14 days. Survival rate (SRVSS) was taken after 10 days of de-submergence according to Ranawake et al. (2014). The elongation percentage (EP) and the percentage of non-structural carbohydrate change (NSCC) were obtained and calculated according to Yoshida et al. (1976). Percentage of changes in total chlorophyll content (TCC) was obtained and calculated according to Mackinney (1941) and Arnon (1949). VSL was set up according to Gregorio et al. (1997). Ten pre-germinated seeds per genotype per replication were arranged in the styrofoam seedling float. VSL was imposed 3 weeks after germination. NaCl was dissolved in a plastic container for salinisation to get water electrical conductivity (EC) of 8 dS/m. The rice seedlings were stressed for 14 days. Survival rate (SRVSL) and visual salt injury (VSI) was recorded on the final day of stress imposition according to Gregorio et al. (1997). All data were analysed using PBTools version 1.4.

Results and Discussion

The results revealed that the *qDTYs* influenced GY under RS through the improvement of the morpho-physiological traits. Even though *qDTYs* were for the GY's improvement under RS, the effects of these *qDTYs* were also detectable under NS condition. As shown in Table 2, all the breeding lines recorded higher GY compared to MR219 under both RS and NS conditions. Breeding lines with *qDTYs* also had better morpho-physiological traits (flowered earlier and produced higher NP and CC) contributing to higher GY compared to MR219 under both NS and RS (Table 2). In this study, it is evident that the presence of *Sub1* plays a vital role in determining survival under submergence (Table 3). Breeding lines with *Sub1* had a higher SR, which is contributed by lower EP, lesser TCC and maintaining an adequate

amount of energy reserve shown by lower NSCC (Table 3). The EP will be inhibited through the mediation of *Sub1* leading to the increment of SR due to limitation of carbohydrate usages as shown in IR64-*Sub1* (Sarkar et al., 2009; Singh et al., 2014). If any of the contributing traits is profoundly affected, it is compensated by another trait to ensure survival under VSS. Under VSL, both breeding lines, MR219PL-335 and MR219PL-91 recorded higher SRVSL than the two mega-varieties, MR219 and IR64-*Sub1* (Table 3). Also, the performance of MR219PL-91 was also comparable to Nona Bokra, the salinity tolerance check although MR219PL-91 did not carry any major salinity tolerant QTLs such as *Saltol* and *SKC1* (Table 3). This indicates that the *qDTY* that regulate drought tolerance also improve salinity tolerance in this rice population.

Table 2: Mean of DTF, PH, NP, CC, GY, and CC of breeding lines and check varieties.

Genotype	DTF NS	DTF RS	PH NS (cm)	PH RS (cm)	NP NS	NP RS	GY NS (ton/ha)	GY RS (ton/ha)	CC NS	CC RS
MR219PL-335	77.00	84.00	107.50	84.00	21.50	13.50	9626.00	2605.91	40.60	26.10
MR219PL-91	76.00	85.00	101.00	78.00	19.50	16.50	10297.60	3278.13	32.75	19.80
91-19	77.50	86.50	121.00	90.00	16.50	14.00	9178.90	4020.56	49.60	22.30
91-37	74.50	86.50	105.00	72.00	22.00	11.50	12011.00	4306.11	42.70	27.70
91-49	78.00	87.50	107.50	92.00	23.00	11.50	10037.80	2599.61	44.20	32.30
335-112	80.00	89.00	103.00	97.00	18.00	8.50	8488.00	2590.58	44.40	27.50
335-155	74.50	86.50	103.50	93.00	15.50	11.50	7062.00	3373.63	42.50	35.20
335-206	76.50	91.50	108.00	84.00	23.00	14.00	13893.00	3830.40	41.40	21.30
335-239	75.50	99.50	105.00	86.00	23.00	13.50	11084.00	4796.35	43.50	28.70
MR219	75.50	107.50	118.00	88.00	17.50	6.00	5960.00	951.32	34.90	31.20
IR64-*Sub1*	77.00	85.50	107.00	82.00	15.50	12.00	6939.00	342.66	37.55	20.30

Table 3: Mean of SFP, SR, EP, NSCC, TCC, and VSI of breeding lines and check varieties.

Genotype	SFP NS (%)	SFP RS (%)	SR VSS (%)	EP VSS (%)	NSCC VSS (%)	TCC VSS (%)	SR VSL (%)	VSI VSL
MR219PL-335	75.89	74.26	46.70	47.23	44.13	87.17	52.33	3
MR219PL-91	84.60	90.14	33.33	35.88	42.23	86.48	66.67	1
91-19	73.43	81.14	100.00	18.58	30.48	45.10	-	-
91-37	92.06	66.33	100.00	28.40	40.66	84.57	-	-
91-49	90.36	80.37	100.00	-	-	-	-	-
335-112	67.93	79.07	80.00	13.15	34.40	73.18	-	-
335-155	65.00	76.14	100.00	6.73	22.20	90.72	-	-
335-206	77.37	73.43	100.00	-	-	-	-	-
335-239	68.48	86.41	100.00	-	-	-	-	-
MR219	91.55	89.71	10.00	31.61	38.61	79.07	25.00	7
IR64-*Sub1*	92.01	82.17	60.00	9.87	27.33	60.40	0.00	9
Nona Bokra	-	-	-	-	-	-	66.67	1

The effect of *Sub1*, *qDTYs*, and combination of the QTLs was confirmed in this study. These QTLs acted either synergistically or singly to cause effects on the morpho-physiological traits under RS, VS, SL, and NS conditions. However, the combination of two QTLs (*Sub1*+$qDTY_{3.1}$, *Sub1*, and $qDTY_{12.1}$ and $qDTY_{3.1}$ + $qDTY_{12.1}$) did not significantly impact compared to single QTL. This indicates that the many QTLs introgression or pyramiding into specific varieties would not always give positive results. Similar results were observed by Dixit et al. (2017), Kumar et al. (2018), and Ikmal et al. (2019b). Interestingly, breeding lines such as 91-19, 91-37, and 335-239 which carry *Sub1* QTL only, recorded the highest GY under RS and overcome the performance of other breeding lines with *qDTY/s* (Table 3). This indicates that submergence QTL, *Sub1* may also plays an important role in tolerance to RS and suggested similar mechanisms for abiotic stresses in plants (Ciarmiello et al., 2011).

Conclusions

Although the introgression of these QTLs through MAB has been suggested as a fast-track and effective approach for abiotic stress improvement of mega rice varieties, the target QTL/s must have a large and consistent effect on GY for MAB to be worthwhile. Breeding lines used in this study will serve as precious genetic materials for more detailed studies. Future research elucidating the effects of *Sub1* and *qDTYs* such as studying genes underlying the QTLs and their interaction, plant hormones production, biochemical processes, and pathways related to tolerance and QTL by environment interaction are suggested. Furthermore, screening of these breeding lines under other abiotic stresses such as salinity, acidity, heavy metals toxicity, and low input ecosystems is necessary to identify rice varieties with broad-spectrum tolerant.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Flower Strips on the Beneficial Insect Abundance in Kuini (*Mangifera odorata*) Orchard

Nurin Izzati, M.Z.[1,*], Aimi Athirah, A.[2], Saiful Zaimi, J.[1], Siti Noor Aishikin, A.H.[1], Rosliza, J.[1], Mohd Shahidan, S.[1], Zul Helmey, M.S.[3] and Mohd Shukri, M.A.[3]

[1]Agrobiodiversity and Environment Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia.
[2]Socio-economic, Market Intelligence and Agribusiness Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia.
[3]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, Malaysia.
**E-mail: nurin.izzati@mardi.gov.my*

Introduction

Mangifera is a common genus found in Malaysia, and it belongs to the Anacardiaceae family, which contains about 69 edible fruit species (Kostermans and Bompard, 1993). One of the species in this family is *M. odorata* or locally called kuini, and is categorised as rare fruits (Pozi et al., 2014). It originated from Guam, the Philippines, Thailand, and Vietnam, and was widely distributed in Malaysia, Indonesia, and Singapore. The fruit is an oval shape with green exocarp to yellowish green in colour. The flesh is yellow-orange, rich in fibre with a strong sweet taste and strong aroma (Orwa et al., 2009). In addition to its delicious taste, *M. odorata* also emits a strong aroma to attract various insects (Wong and Ong, 1993). This fruit has the potential to be commercialised because it contains high nutrients and can be eaten fresh or processed (Brooke and Lau, 2013).

The use of pesticides is to control the invasion of pests in kuini fields and has been practised for a long time. This chemical spraying can affect beneficial insect populations such as predators, parasitoids, and detritivores (Acheampong and Stark, 2004; Nik Mohd Noor et al., 2012). Besides, monoculture systems of agriculture are also among the sources of this beneficial species that cannot survive independently (Alteiri, 1999; Finch and Collier, 2000; Jones and Gillett, 2005). Agriculture with integrated pest management (IPM) methods was introduced to balance ecology and sustainability as well as to reduce the use of pesticides in crop protection. One of the IPM approaches is to reduce the use of pesticides by using cultural methods through the introduction of ecological engineering or previously known as habitat manipulation. It works naturally to promote the activities of biological to control pest by using their natural enemies and it environmentally friendly (Badrulhadza et al., 2018). Through this practice, biological activities can be enhanced by providing alternative food sources to natural enemies or predators when there are no victims (pests) or host as the main food source. The presence of flower strips in or near the main crop area will indirectly increase biodiversity and make the ecosystems more stable (Jones and Gillett, 2005). This method is also one of the ways to avoid the adverse effects of monoculture cultivation while at the same time providing more quality food and shelter (refugia) to insect species or other organisms (Figure 1) that act as natural enemies to the main crop pest (Kremen and Miles, 2012).

Considering this potential, a year-long study was conducted in selected seasons (i.e., flowering, fruiting and off-season) to investigate the effect of flower strips planted near kuini plot in abundance and distribution of beneficial insect at MARDI Station Sintok, Kedah. Through this study, information gathered will be useful as baseline data for implementation for pest management through the practice of ecological engineering or habitat manipulation.

(a)  (b) 

Figure 1: Beneficial insects attracted to the flowers of (a) *Turnera* sp. and (b) kuini in the orchard.

Materials and Methods

Development of planting methods for flowers strip

The planting of flowers was carried out in early 2018 in strips of *Turnera* sp. This flower is selected because of its durable characteristics and flowering throughout the year. These features are important to ensure sufficient food supply such as flower nectar and flowers for survival and to maintain the reproduction of beneficial insects on the field. Besides, the aroma of flowers is also an attractive factor for some insects as an indication of a suitable location for protection and oviposition (Finch and Collier, 2000; Proffit et al., 2011). *Turnera* sp. was planted in a long line, about 0.5 m near the kuini plot (Figure 2). The control plot was left without flower strips within 60 m from the experimental plot.

Figure 2: Flower strips of *Tunera* sp. planted near kuini orchard.

Sampling method

Insect sampling was performed using a yellow sticky trap (YST). Yellow plastic with size (15 x 15 cm) was sprayed with insect glue (Neopiece) and pounded on the wooden pole. It was then placed in between the *Turnera* sp. borders and the area between kuini trees with three replicates per tree. Another type of YST was hung on the tree and was obtained from KOPPERT Company. Fifteen kuini trees were randomly selected to represent the experimental plot (CT), and another fifteen were in the control plot (CC) (Figure 3). Three (3) YST were placed on each tree, with one at the top, middle, and bottom of the tree. After left exposed for 24 hours, the traps were covered with transparent plastic sheets for transportation, and the insects were identified up to the species level in the laboratory.

Figure 3: Installation of yellow adhesive traps (YST) on kuini trees, and the area in between kuinitrees and *Turnera* sp.

Data analysis

Collected data were analysed by using Minitab 19.0 software on Microsoft Excel statistical program. Each data was analyzed using a one-way analysis of variance (ANOVA) with Tukey's test at a significance level of $P<0.05$.

Results and Discussion

The total number of insects recorded for all three samplings was 2,084 individuals in the experimental plot (CT) and 2,504 individuals (CC). The highest individual of insects was recorded during the flowering season, where 1,462 individuals in CC and 993 individuals in CT (Figure 4). From the overall individuals recorded, the top five (5) families of the beneficial insects were Formicidae (25.19%), Encyrtidae (14.66%), Dolichopodidae (14.44%), Braconidae (14.33%), and Apidae (10.31%). Meanwhile, the top five (5) families from pests recorded were Thripidae (35.27%), Chrysomelidae (17.15%), Agromyzidae (14.33%), Chloropidae (12.21%), and Pyralidae (11.02%). The total number of beneficial insects was found to be higher in the CT compared to the CC with a ratio of 1 (CC): 2 (CT), by which 485 beneficial insects were recorded in CT and 247 beneficial insects were from the CC (Figure 5). Overall, the number of pests was higher in CC (1,505) than in CT (2,077).

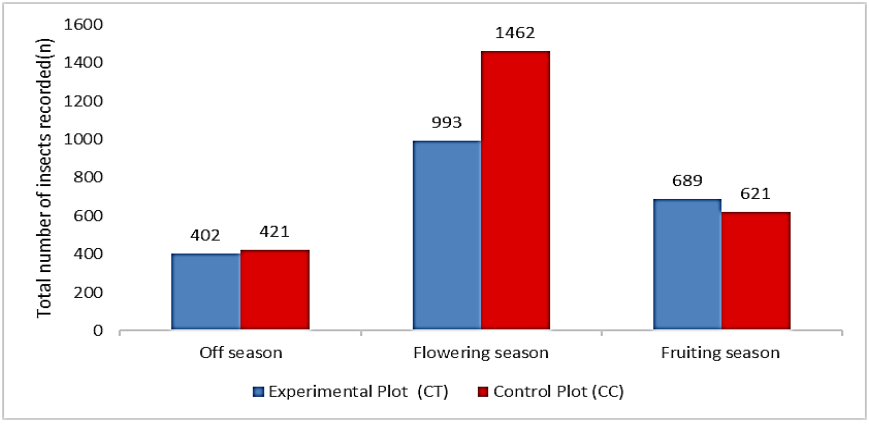

Figure 4: The total number of insects recorded during three (3) seasons (off, flowering, and fruiting seasons).

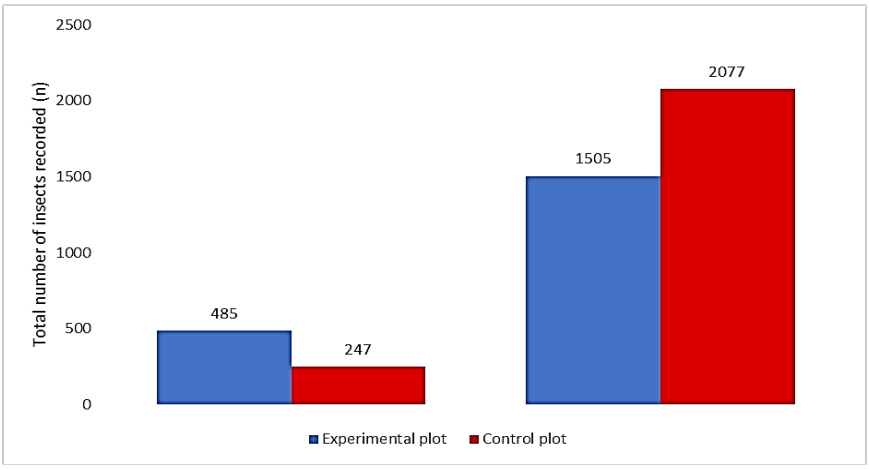

Figure 5: The total number of beneficial and pest insects recorded experimental (CT) and control (CC) plot.

Based on the ANOVA analysis, there was a significant difference ($P<0.05$) in the presence of beneficial insects for the entire season on the CT compared to the CC (Table 1). No significant differences were recorded in the average occurrence of pests during the entire season. However, there was a decrease in average on the presence of pests in the CT compared to the CC.

Table 1: Mean comparison between beneficial insects and pests in experimental (CT) and control (CC) plot.

	Beneficial insects (n)	Pests (n)
Experimental plot (CT)	17.96[a]	55.74[a]
Control plot (CC)	9.15[b]	76.9[a]

**Means that do not share a letter are significantly different.*

The use of flowers in enhancing the presence of beneficial insects has been extensively demonstrated by studies conducted by previous researchers (Steffan-Dewenter and Tscharntke, 2001; Hagen and Kraemer, 2010). The main crop area surrounded by area surrounded by wildflowers has a positive impact on the abundance and diversity of pollinator insects such as bees, syrphids and other Diptera species (Bäckman and Tiainen, 2002; Croxton et al., 2002). It is important to know the data on increasing the presence of pollinator insects in an environment, whether they can increase pollination rates, but the information is still being studied. However, from these early results, it was found that the importance of flowers in enhancing the presence of natural enemies can be seen mainly from parasitoid

species. Similar results have been reported by Wratten et al., 2003; Carreck and Williams, 2004 and Ricketts, 2004.

In Malaysia, the cultivation of flower strips to increase parasitoids' presence began around the 1990s, but it just concentrated only in oil palm areas. Beneficial cultivation of flower strips such as *Cassia cobanensis*, *Turnera subulata* and *Antigonan leptopus* have created a natural environment for the propagation of natural enemies to the major pests of palm oil (*Metisa plana*, *Pteroma pendula* and *Mahasena corbetti*). Studies show that these plants produce nectar and pollen for the survival of natural enemies of pests such as parasitoids. Nectar is required by parasitoid to enhance the life cycle and the lack of nectar resources can cause death and deterioration of the organism population (Basri et al., 1999). In Indonesia, planting *Turnera* sp. in the oil palm plantation is part of the Standard Operating Procedure (SOP) in managing oil palm plantations. Besides that, the high presence of families Formicidae (n = 232) may be due to the presence of extrafloral nectar (extrafloral nectaries - EFN) secreted in the vegetative part of *Turnera* sp. in return for food to ants that will enhance the survival of the plant and preventing damage from herbivorous pest attacks (Cuautle et al., 2005; Cruz et al., 2017).

Conclusions

This study concludes that the cultivation of flower strips in the edge area near the kuini plot increases the presence of beneficial insects (predators, parasitoids and pollinators), which in turn, it may help to reduce pests and improve kuini fruit production. However, further study is needed to evaluate the effectiveness of flower strips during the fruiting season.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effects of Different Environmental Condition on Stem Cuttings of *Strobilanthes crispus*

Syafiqah Nabilah, S.B.*, Norhayati, S, Farah Fazwa, M.A. and Mastitah, M.T.
Herb and Tree Improvement Branch, Forestry Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.
**E-mail: syafiqah@frim.gov.my*

Introduction

Strobilanthes crispus is an herbal plant originated from the countries of Indonesia to Madagascar (Nurraihana et al., 2013). According to Ghasemzadeh et al. (2015) this plant is locally known as Pecah beling or Bayam karang which belongs to the Acanthacea family. It is a woody shrub that can spread and reaches to 1-1.5 m in height. The stem of the *S. crispus* is purple in colour when young and become brown and woody when matured. The diameter of the stem is in the range from 0.2-0.7 mm including the bark. The leaf shape is elliptical with rough surface covered with short hairs. The flowers are dense, short, yellow colour, funnel-shaped, and has panicle spikes. The seeds of *S. crispus* often ovate and lenticular if flattened (Sunarto, 1977).

There are plenty phytochemical groups and constituents that have been obtained in *S. crispus*. According to Ismail et al. (2000), the dried leaves of *S. crispus* contains high amount of water-soluble minerals which contributes to high antioxidant activities also it consists of alkaloids, tannins, caffeine, polyphenols, vitamins (C, B1, and B2), and high mineral content including potassium (51%), calcium (24%), sodium (13%), iron (1%), and phosphorus (1%). Asmah et al. (2006) successfully isolated bioactive components such as stigmasterol and β-sitosterol. Besides that, Liza et al. (2010) have identified eight flavonoid compounds from the leaves of *S. crispus* by using HPLC. The identified compound included (+)-catechin, (−)-epicatechin, rutin, myricetin, luteolin, apigenin, naringenin, and kaempferol. These compounds exhibit high biological activity and display antioxidant, anti-inflammatory, and antiallergic properties.

This plant is highly demanded for raw material used in developing various natural products such as supplements and functional foods. Propagation of the planting materials in commercial scale is needed to ensure continuous supply of the raw materials. To date, there are lacks literature reporting on the macropropagation technique for *S. crispus.* Macropropagation technique through stem cutting is convenient and inexpensive method to obtain the raw materials in mass quantity. However, there are several factors that influence the successful of cuttings such environment, light intensity, humidity, and others. Therefore, the objective of this research is to study the effects of growth medium and growth condition on the performance of *S. crispus* stem cuttings.

Materials and Methods

Preparation of stem cuttings

Stem cuttings of *S. crispus* were collected from the source bush at Forest Research Institute Malaysia (FRIM). Stem part with three nodal segments were used as the cutting materials (Figure 1). The leaves were cut to about two third of their original size in order to encourage root and shoot growth. The base of each cuttings was applied with commercial rooting hormone Seradix (0.1% Indole Butyric Acid) using the basal quick-dip method. A total of 30 stem cuttings were used for each experiment.

Preparation of growth media and growth condition

The stem cuttings treated with Seradix were immediately planted onto two set of growing media containing mixture of; M1- 2 top soil: 1 sand, M2- 1 soil: 1 sand. The experiment was conducted in square based plastic pot (Length: 53 cm x Width: 40 cm x Height: 8 cm). The plants were kept in two growth conditions; C1- Open greenhouse, C2- Greenhouse with enclosed growing chamber. The greenhouses were installed with automatic misting system that operated for 1 min thrice a day (8.00 am, 12.00 pm and 4.00 pm) to water the plants.

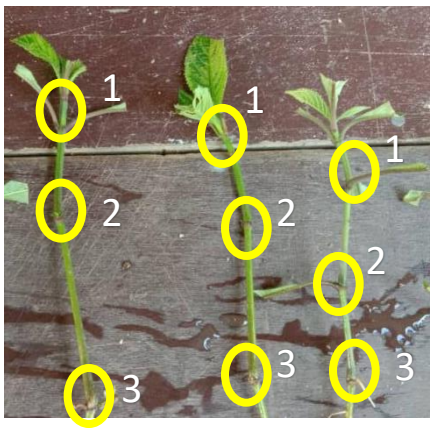


Figure 1: Stem cuttings of *Strobilanthes crispus* with the nodal segment.

Assessment of cuttings

The study was conducted for a total of 4 weeks and the measurement of the stem cuttings conducted every week, included survival and rooting percentage, number and length of roots per cutting. Any plants with their leaves and/or stems that turned brown were considered dead and their measurements were stopped. Analysis of variance (ANOVA) was conducted to compare each of parameters measured between the different media and environmental treatments. Statistical analysis was conducted using SPSS version 22.

Results and Discussion

Survival and rooting percentage

The stem cuttings grown in 2 top soil: 1 sand at open greenhouse (M1:C1) had the highest survival percentage at all weeks (100%) (Figure 2a). Stem cuttings treated in 1 top soil: 1 sand in enclosed growing chamber (M2:C2) had the lowest percentage of survival at week 2 (10%) and gradually dead after week 2. None of the cutting rooted at week 1 (Figure 2b). The stem cuttings treated with M1:C1 had the highest rooting percentage (100%) followed by M2:C1 (36%) and M1:C2 (33%) after 4 weeks of cutting.

Effects of growth media and growth conditions on root development

There was no significant difference between growth media used and the growth condition for the root number produced. Stem cuttings treated in M2:C1 produced highest root number per plant in the range of 16.04 to 30.36 followed by M1:C1 with the range of 14.53 to 22.67. There were significant differences observed on the root length of *Strobilanthes crispus* in M1:C1 treatment (14.45 cm±1.65) compared to others after 4 weeks of cutting.

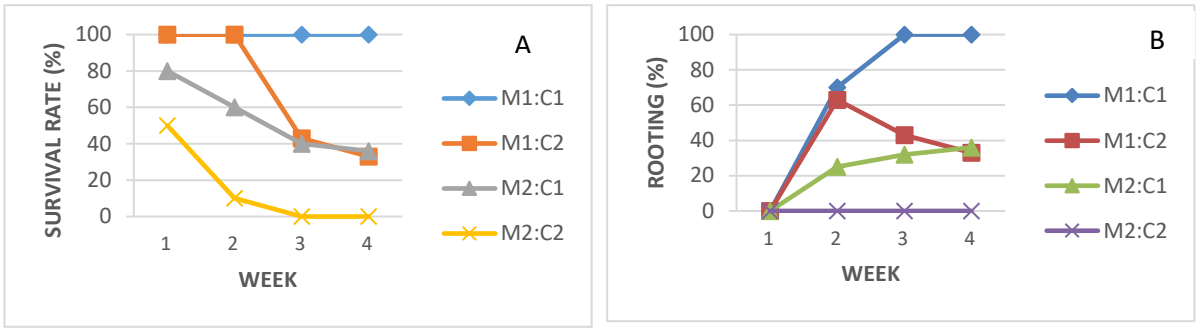
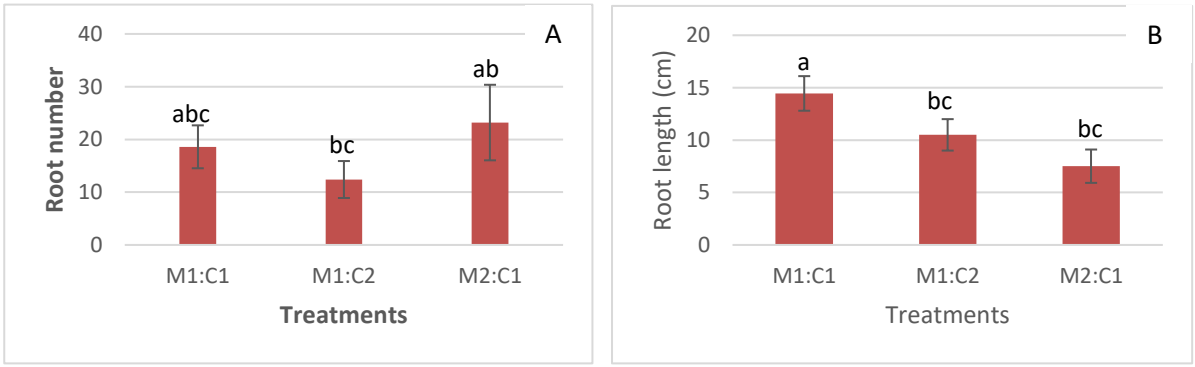


Figure 2: (A) Survival rate and (B) Rooting percentage of *Strobilanthes crispus* stem cuttings treated with different growing media and growing condition over 4 weeks.



.Figure 3: (A) Root number and (B) Root length of *Strobilanthes crispus* stem cuttings treated with different growing media and growing condition at week 4. Values are Means±Standard Error (SE). Means with different letters showed significant difference at P<0.05 according to Duncan's Multiple Range Test

The successful rooting of stem cuttings could be influenced by many factors including the rooting medium, the environmental conditions as well as the physiological status of the stock plant itself (Arteca, 1996). This study revealed that stem cutting grown in growth media M1:2 top soil:1 sand and placed at C1: open greenhouse gave 100% survival rate and 100% rooting percentage. The combination of sand and soil as the growth media in this study is to achieve the high porosity and water retention while providing adequate aeration. The ratio of 2 top soil: 1 sand is better compared to 1 top soil: 1 sand. Both materials are low cost and easily available for the farmers to conduct the propagation of *S. crispus.* Stem cuttings of *S. crispus* preferred open greenhouse environment over the enclosed propagation chamber. In the enclosed propagation chamber, the system is limited to low irradiance which limit the photosynthesis through the depletion of carbon dioxide during daytime hours (Rosenberg et al., 1992). In addition, the high relative humidity that sustains the water potential within the enclosed propagation chamber may not be suitable for *S. crispus* as the stem will rot faster in such condition. The present study reported no significant effects of the media and environment treatment on the root number, but the root length of the cuttings was higher at M1:C1 treatment. The current work also suggested that more studies on different environmental conditions need to be conducted as this factor may improve the growth rate, number and length of root and other parameters.

Conclusions

From the present findings, growing *S. crispus* stem cutting in 2 top soil: 1 sand at open greenhouse system resulted in high survival, rooting percentage with longer root length. The result may be important as pioneer study on macropropagation of this medicinal plant.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Lemon Myrtle Essential Oil as a Potential Botanical Pesticide Against Bacterial Leaf Blight Disease *(Xanthomonas oryzae* pv. *oryzae)* in Paddy

Hazalina, Z.[1,2,*], Norhayu, A.[2], Dzolkhifli, O.[2], Siti Izera, I.[2] and Noor Azlina, M.[1]

[1]Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute, MARDI Head Quarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
**E-mail: hazalina@mardi.gov.my*

Introduction

Bacterial leaf blight (BLB) of rice, a deadly disease that is among the most destructive afflictions of cultivated rice (*Oryza sativa* and *Oryza glaberrima*). In severe epidemics, this annual crop loss may be as high as 75 %, and millions of hectares of rice are infected. The disease was first observed in 1884-1985 in Kyushu, Japan, and the causal agent, the Gram-negative bacterium *Xanthomonas oryzae* pv. *oryzae*, also referred as Xoo was identified in 1911 (Yasmin et al., 2017). It is a vascular disease resulting in tannish-gray to white lesions along the leaf veins. In 2016, some 4,440 ha of paddy fields in Sabak Bernam, Selangor, Malaysia have been infected with the BLB disease, which dries up the leaves and kills the plants (Chukwu et al., 2019). It was reported to be more serious than the previous one, which occurred during the second harvest season in 2013. The losses caused by this disease worldwide could jeopardize global food security and therefore, it is important to discover the solution to this problem. The current practice was by using chemical copper-based pesticide for controlling the diseases. However, there are concerns that frequent applications of copper-based pesticide may led to the emergence of copper-resistant strains in agriculture, hence raising doubt on the long-term sustainability of agricultural production. Moreover, copper-based pesticide also has adverse effects on the environment and biodiversity, such as contamination of soil and groundwater, with a significant impact on soil biota. There is an obvious need to search for an alternative botanical antimicrobial agent which are more environmentally safe approaches so as to overcome the loss of grain yields in rice.

Since ancient time, plant and their active compounds or secondary metabolites, have been demonstrated to possess potent as bactericidal, fungicidal, insecticidal, and nematocidal activity and these inspire and influence the modern agrochemical research. Essential oils (EOs) have become immensely popular among consumers worldwide. They are volatile, natural, and complex compounds found in great quantities in oil sacs or glands which are mainly present at different depths in the fruit peel, flavedo parts, and cuticles. EOs are aromatic oily liquids extracted from different parts of plants such as leaves, barks, seeds, flowers, and peels. The antibacterial action of the EOs is due to their ability to penetrate through the bacterial membranes into the cell, causing the inhibition of their vital functions (Bajpai et al., 2012). Therefore, this study was to show the effects of seven essential oils against *Xanthomonas oryzae* pv *oryzae* growth and initiated by performing *in vitro* screening via filter paper disc diffusion assay on peptone sucrose agar (PSA). Based on its antibacterial performance, the EOs that demonstrated strong antibacterial activity, by showing the huge inhibition zone was selected for further characterized using gas chromatogram – mass spectroscopy (GC-MS) which further used to identify for their phytochemical compounds.

Materials and Methods

Essential oils and bacterial strain

Essential oils extracted from lemon myrtle (*Backhousia citriodora*), kaffir lime (*Citrus hystrix*), cinnamon (*Cinnamomum zeylanicum*), tea tree (*Melaleuca alternifolia*), lemongrass (*Cymbopogon*

citratus), citronella (*Cymbopogon nardus*), and paper bark (*Melaleuca cajuputi*) were obtained using steam distillation conducted at MARDI Kuala Linggi, Negeri Sembilan. The steam distillation was employed due to its robustness and efficiency as compared to hydro distillation process. Bacteria strains of *Xanthomonas oryzae* pv. *oryzae* (Xoo) was received from MARDI Seberang Prai, Penang, Malaysia. The strain was cultured on potato sucrose agar (PSA) at 30 °C for 48-72 h. Large quantities of the mass-produced bacterium on PSA agar were collected by washing the colony surface on the agar plate with 1mL of sterile 0.01M phosphate buffered saline (PBS) at pH 7.4. Bacterial 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 again with PBS 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 1.0 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.

Filter paper disk diffusion assay for antibacterial activity determination

Antibacterial activity of EOs were determine by performing the filter paper disc diffusion assay. The Xoo bacteria were spread on peptone sucrose agar (PSA) using sterile cotton bud. A 3 µL each essential oil was drop on 6 mm sterile filter paper disc respectively and air dried for 5 min. A filter-paper disk, impregnated with the EO to be tested, is then placed on the surface of the agar. The agar plates were incubated in 30 °C for 3 days. Streptomycin sulphate antibiotic was used as positive control. Meanwhile, mineral oil was used as a negative control. The diameter length of inhibition or halo zone were recorded in millimetre.

Minimal bactericidal concentration (MBC) determination

The minimal bactericidal concentration (MBC) values for lemon myrtle EO against Xoo was also studied through the broth dilution method according to Gormez et al. (2013) with slight modification. The bacterium inoculum was prepared in lysogeny broth cultures and adjusted to optical densities (OD) at 600 nm of 0.1 equal to 0.5 McFarland Standard turbidity. The EO was prepared by diluting 10% Dimethyl sulfoxide (DMSO) in lysogeny broth. Then 2 folds dilutions were performed to prepare a 500 µL desired concentration percentage of EOs range from 0.5% (v/v) to 0.032% (v/v) in sterile 1.5 mL Eppendorf tube. Each tube was added with previously prepared Xoo inoculum then were incubated at 30 °C for 24 hours. Then 100 µL Xoo cultured in lysogeny broth were transferred and spread on peptone sucrose agar (PSA) using sterile cotton swab. The petri dish was sealed with parafilm an incubated in the oven at 30 °C for 3 days. The growth of Xoo on PSA petri dish was observed and recorded. The PSA plate with the lowest concentration of lemon myrtle EO showing no visible growth was regarded as the MBC value.

Determination of active compound in lemon myrtle essential oil using gas chromatography mass spectrometry (GC-MS)

The lemon myrtle essential oil was obtained by steam distillation and 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 initial 60 °C for 10 min, then increase at 3 °C/ min and at 180 °C for 15 min. 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. The sample injection flow rate at 1 mL/min and the total run time was 65 min. Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in mass spectral library of the GC/MS data system.

Results and Discussion

Filter paper disc diffusion assay for antibacterial activity

There was an antibacterial activity of essential oils against Xoo growth in PSA, as shown in Figure 1 and Table 1. Essential oils from lemon myrtle, lemongrass, citronella, and cinnamon showed a wide length of inhibition zone diameter (>15 mm) as well as streptomycin sulphate. However, tea tree and kaffir lime essential oils showed least inhibition zone diameter (less then 10 mm) while no inhibition zone was observed on Xoo growth using paper bark and mineral oil.

Table 1: Antibacterial activity of EOs determine by filter disk diffusion assay.

Essential oil	Inhibition zone diameter (mm)
Negative control	No inhibition
Positive control	26±1.73
Paper bark (*Melaleuca cajuputi*)	No inhibition
Tea tree (*Melaleuca alternifolia*)	9.33±1.53
Cinnamon (*Cinnamomum zeylanicum*)	21.67±3.05
Citronella (*Cymbopogon nardus*)	15±3.61
Lemongrass (*Cymbopogon citratus*)	25.67±2.52
Kaffir lime (*Citrus hystrix*)	12.33±1.15
Lemon myrtle (*Backhousia citriodora*)	39.0±1.0

** Significantly different (P<0.05).*
** Values are the mean diameter of the inhibitory zone (mm), ± SD of three replicates.*
** The diameter is included of the paper disk (6 mm).*

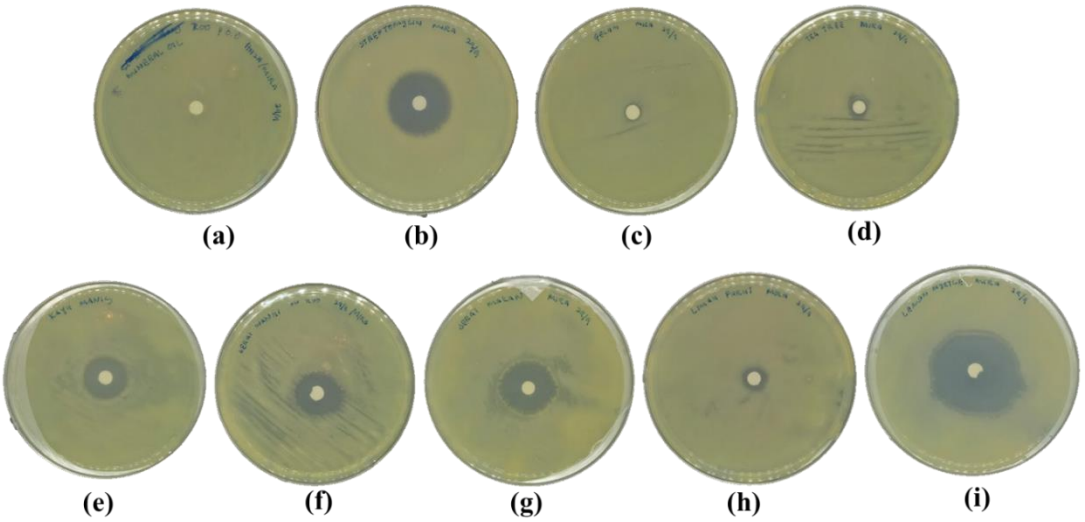

Figure 1: Diameter measurement of inhibition zones obtained from filter paper disc diffusion assay for; (a) negative control (mineral oil), (b) positive control (5 mg/mL streptomycin sulphate), (c) paper bark (*Melaleuca cajuputi*), (d) tea tree (*Melaleuca alternifolia*), (e) cinnamon (*Cinnamomum zeylanicum*), (f) citronella (*Cymbopogon nardus*), (g) lemongrass (*Cymbopogon citratus*), (h) kaffir lime (*Citrus hystrix)*, and (i) lemon myrtle (*Backhousia citriodora*).

Minimal bactericidal concentration (MBC) determination

The effect of an antimicrobial against microorganism is often measured as the minimum bactericidal concentration (MBC). In this study, the MBC value is the lowest concentration percentage of lemon

myrtle essential oil resulting in killing 99.9% of the *Xanthomonas oryzae* pv. *oryzae* bacteria tested with about 0.075% (v/v) against *Xanthomonas oryzae* pv. *oryzae* bacteria as highlighted in Table 2.

Table 2: The minimum bactericidal concentration percentage value of lemon myrtle (*Backhousia citriodora*) essential oil.

Concentration (%)	Day 1	Day 2	Day 3
0.5	-	-	-
0.4	-	-	-
0.3	-	-	-
0.25	-	-	-
0.2	-	-	-
0.15	-	-	-
0.125	-	-	-
0.1	-	-	-
0.075	-	-	-
0.063	-	+	+
0.05	-	+	+
0.038	-	+	+

+: indicates XOO bacterial growth on PSA.

Determination of active compound in lemon myrtle essential oil using gas chromatography mass spectrometry (GC-MS)

This essential oil comprises of complex mixtures of numerous molecules, and their biological effects are suspected to be synergism of all molecules or reflect only those of the main molecules present at the highest levels according to gas chromatographical analysis (Bakkali et al., 2008). The GC-MS analysis carried out identified the lemon myrtle essential oil chemical composition (Table 3) and the total lemon myrtle essential oil ion chromatogram is illustrated in Figure 2. Citral as a mixture of two aldehydes, geranial (citral a) and neral (citral b) were identified to be the major compounds in lemon myrtle comprising of 43.63% and 40.04% respectively. Both geranial and neral have the same molecular formula but different stereochemistry structure and these are shown in Figure 3. Besides citral compound that covers 83.67% in lemon myrtle, the minor compounds were eugenol, isocitral-E, geraniol, mentha-2,8-dien-1-ol, citronellal, and linalool. Other trace compounds identified are less than 1.0% of the essential oils were α-pinene, camphene, β-pinene, cineole, myrcene, γ-terpinene, terpinen-4-ol, piperitone, α-copaene, E-caryophyllene, and γ-cadinene.

The possible effectiveness of lemon myrtle essential oil could be due to the presence of citral as an active component as it displays spasmolytic, anti-microbial, anti-inflammatory, analgesic, and chemo-preventive properties (Aprotosoaie et al., 2018). Citral ($C_{10}H_{16}O$) is also called 3,7-dimethyl-2,6-octadienal which is a pale-yellow liquid like, with a strong lemon odour that also occurs in some other plants. This essential oil is however, insoluble in water but soluble in ethanol (ethyl alcohol), diethyl ether, and other mineral oils. In addition, with the present of eugenol it can also played a role as an antiseptic, anti-inflammatory, antifungal, antibacterial and possessed anaesthetic properties.

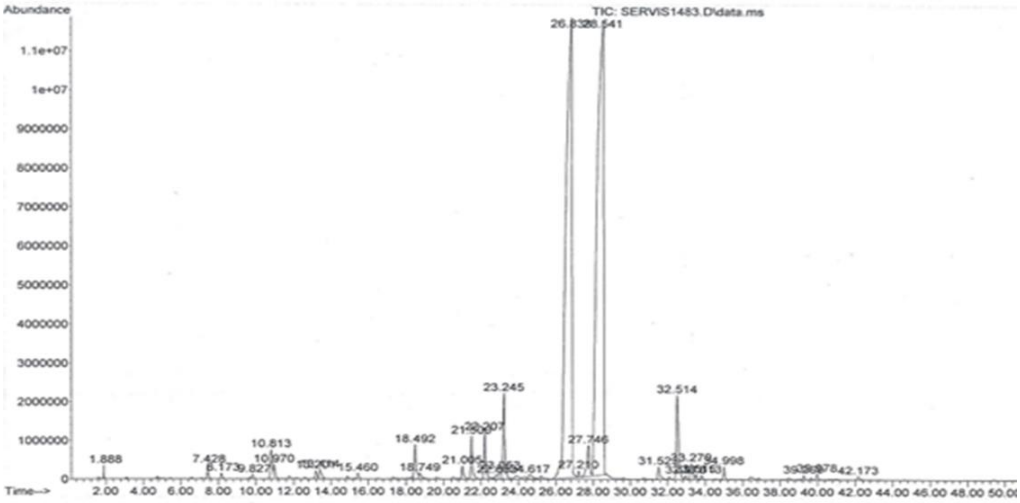

Figure 2: Gas Chromatography-mass spectrometry (GC-MS) chromatograph of lemon myrtle essential oil.

Table 3: Percentage of chemical compound in lemon myrtle essential oil identified using GC-MS.

No	Compounds	Retention time (minutes)	Percentage (%)
1	α-pinene	7.43	0.29
2	camphene	8.17	0.14
3	β-pinene	9.82	0.12
4	cineole-dehydro-1,8	10.81	0.84
5	myrcene	10.97	0.39
6	ortho-cymene	13.20	0.22
7	γ-terpinene	15.46	0.15
8	linalool	18.49	1.08
9	citronellal	21.50	1.16
10	mentha-2,8-dien-1-ol	22.21	1.30
11	terpinen-4-ol	22.88	0.17
12	isocitral-E	23.25	2.39
13	neral	26.84	40.0
14	piperitone	27.21	0.12
15	geraniol	27.75	1.34
16	geranial	28.54	43.6
17	eugenol	32.51	3.09
18	α-copaene	32.97	0.11
19	geranyl acetate	33.51	0.13
20	β-elemene	33.81	0.10
22	E-caryophyllene	34.99	0.30
23	γ-cadinene	39.27	0.14
24	eugenol acetate	39.98	0.17
25	caryophyllene oxide	42.17	0.15

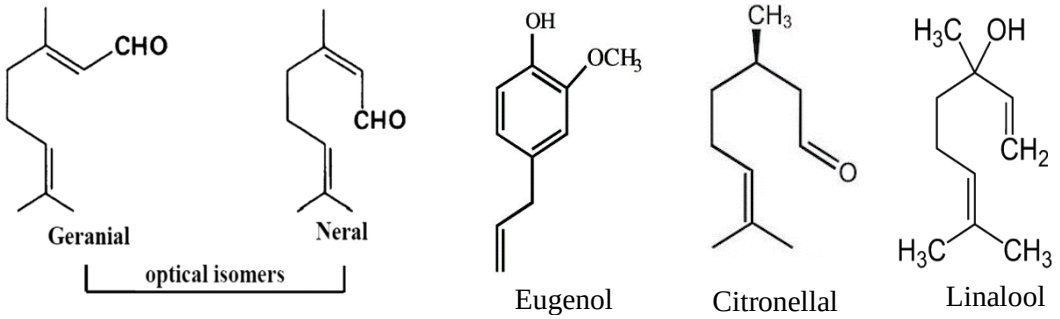

Figure 3: Stereochemistry structure of citral, eugenol, citronellal, and linalool.

Additionally, citronellal and linalool compounds are monoterpenoids. Citronellal is an aldehyde, with distinctive lemon aroma and has a role as a metabolite and antifungal agent. Citronellal displayed a good activity against abnormal cell growth or cancer because of its antioxidant properties including its potential to maintain the immunity, cell regeneration, protection from free radicals, anti-aging, and heal wounds (Sinha et al., 2011; Russo et al., 2015). Meanwhile, linalool which is a colourless liquid, relatively soluble in water and can be an alcoholic with a floral scent when present at high concentration. It is found in many plants such as *Lavandula* sp., coriander, basil, cinnamon, and rosewood (Quintans et al., 2013). Numerous studies have been reported on linalool contributions and these include antimicrobials, anti-leishmaniosis, anti-inflammatories, and antioxidants biological activities (Beier et al., 2014).

Conclusions

Using botanical or plant-based pesticides can be a good environmental-friendly approach for controlling and managing the BLB disease. The results from this *in vitro* studies revealed and concluded that the EOs of lemon myrtle (*Backhousia citriodora*) possessed vast amount of antibacterial properties against Xoo. However, this potential properties for pest management in agriculture needs to be further explored and validated.

Acknowledgement

This work was financially supported by Projek Pembangunan MARDI, project code: P-RB401-1001. The authors wish to thank Mdm. Kogeethavani Ramachandran (Paddy and Rice Research Centre, MARDI Seberang Perai) for providing *Xanthomonas oryzae* pv. *oryzae* stock cultures.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Chapter 3:
Seed Technology and Quality Planting Materials

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Responses of Seed Germination and Seedling Growth to Seed Priming in *Moringa oleifera*

Farahzety, A.M.*, Yap, W.S.P. and Singh, A.
School of Biosciences, Faculty of Science and Engineering, University of Nottingham Malaysia, 43500 Semenyih, Selangor, Malaysia.
**E-mail: hbxfa1@nottingham.edu.my*

Introduction

Moringa oleifera is the most underused multipurpose tropical crop, also known as Horseradish or Drumstick-tree or Ben oil tree. It is a fast-growing perennial soft-wooded tree, drought tolerance and well adapted to grow in adverse conditions (Palada and Chang, 2003). The tree is famous for its versatility as a source of food for humans and livestock. In Malaysia, Moringa is well-known as kelor or merunggai. Reproduction of Moringa can be done by sexual (seed) and asexual (cuttings, air-layering, and tissue culture). Propagation from cuttings is unproductive because the stem cuttings must be longer and thicker (Fuglie and Sreeja, 2001) and this process results in a high mortality rate for the mother plant (Ramachandran et al., 1980). Therefore, seeds remain dominant in the Moringa propagation method because it easy, cheap, and produce a large number of new plants (Fuglie, 2000; Palada and Chang 2003; Radovich, 2012). However, Moringa seeds have been reported to lose viability and vigour quickly (Sharma and Raina, 1982; FAO/IPGRI Genebank Standards, 1994; De Oliveira et al., 2009; Fotouo-M et al., 2015). Loss of viability is commonly associated with extended storage conditions (Tommasi et al., 2006; Basu, 2007). Many studies have indicated decreased seed viability with increased dehydration and storage (Bonner, 1990; Kioko et al., 2006). Seed priming is a technology known to improve seed performance. Seed priming is a regulated hydration technique that enables metabolic pre-germination without actual germination. It involves imbibing the seeds in various solutions for a defined time under controlled conditions, then drying them back to their original moisture content, so that radicles do not emerge before sowing. There are various techniques for priming seeds, namely hydropriming, halopriming, osmopriming, matrix priming, hormopriming, nutripriming, nanoparticle priming, et cetera and were effective in increasing crops germination and seedling establishment (Rashid et al., 2006; Yu-Jiee et al., 2009; Sedghi et al., 2010; Imran et al., 2013; Singh et al., 2014). Seed priming is one of the most efficient, practical, and short-term approaches to improve seed vigour and germination synchronisation (Kaya et al., 2006). The efficacy of different priming agents, however, varies on different crop types. Hence, the study is conducted to apply seed priming technique to maximises *M. oleifera* total germination, germination rate and initial seedling development in the nursery.

Materials and Methods

Plant materials and seed treatments

Moringa seeds at the same batches were bought from local shop name Galoremy in Kepong, Selangor. All seeds were sterilised with 1% sodium hypochlorite for 25 min, followed by washing twice with distilled water (Fotouo-M, 2015). The sterilised seeds were subjected to hydropriming (distilled water), osmopriming (KNO_3) at 1%, and nutripriming (SeedActivator) at 1% each for the duration of 4-hours, 8-hours, and 12-hours, and the unprimed seeds as control treatment. The temperature of the priming solutions was at 29 °C. Seeds were weighted prior to priming application and after treated seeds were rinsed with distilled water, dried on paper towels, and ventilated until they regained their original weight at room temperature.

Germination test

Treated seeds were germinated in petri dish with double layer Whatman No. 1 filter paper in a plant growth chamber at 12 hours photoperiod, 12 hours light (30 °C)/12 hours dark (20 °C), at 60% relative humidity (RH). The experiment was arranged in a Completely Randomised Design (CRD); each treatment comprised four replicates. Seed germination was monitored and counted daily when the radicle emerged up to 14 days. Seeds were considered germinated once the radicle emerged. Germinated seeds were discarded from the petri dish after every count. Germination percentage (GP) was measured as the number of germinated seeds divided by the number of sown seeds x 100. Mean germination time (MGT) was calculated following equation of Ellis and Roberts, (1981). $MGT = \sum(Dn)/\sum n$, where n is the number of seeds that germinate on day D, and D is the number of days counted from the beginning of the germination test. The time to reach 50% germination (T50) was calculated according to Coolbear et al. (1984) modified by Farooq et al. (2005). $T50 = ti + [(N / 2 - ni) (ti - tj)] / ni – nj$. Where N is the final number of emergence, and ni and nj are the cumulative number of seeds germinated by adjacent counts at times, ti and tj, respectively, when $ni < N / 2 < nj$. The germination index (GI) was calculated as described by the Association of Official Seed Analysis (1993). GI = Σ number of germinated seeds/number of days.

Root pouch assay

The priming treated seed was germinated in the petri dish before transferring to the pouch using a tweezer by inserting the seeds with radicles into a pre-made slits growth pouch (PhytoTC.com). The pouches were stacked vertically in a rack in a plant growth chamber (Model PRC1200SL, Hettich) for 14 days with a photoperiod 12 hours light (30 °C)/12 hours dark (20 °C), at 60% RH and arranged in a CRD with six replications. Each growth pouch contained four seeds that were evenly placed along the seed line. Root systems were shielded against light during growth by covering with black cardboard on each side of the pouch. Root morphological parameters including total length, volume, average diameter, number of tips, number of forks and number of crossing were measured at the end of the experiment. The acquired root images were analysed using WinRHIZO Pro 2007b (Regent Instruments Inc., Quebec, Canada).

Pot experiment

The sowing media used were sand: peat moss (9:1), and topsoil: coco coir dusk: peat moss (5:2:1) (volume by volume; v/v) and filled in non-woven pots (15 cm x 7 cm). The substrates were mixed thoroughly with organic fertiliser BioRichar at 4 g per pot equivalent to 0.2 tan/ha before planting. The experimental design was a Randomised Complete Block Design (RCBD) with two factors. The factors were the priming treatments and growing media. 20 seeds used per treatment per replication with a single seed sown in every pot. The seedlings growth was monitored daily for over 14 days. Throughout this period, the seedling was watered daily, which wetted the growing medium to field capacity. The morphological growth parameters measured were seedling height (cm), stem girth (mm), and the number of leaflets. The total leaf area per plant was measured in cm^2 by destructive sampling using a leaf area meter (LI-3100, Li-COR Inc., USA).

Statistical analysis

All data were analysed using analysis of variance (ANOVA) and mean was separated by Duncan Multiple Range at $P \leq 0.05$ (Version 9.4. SAS Institute, Cary, N.C.).

Results and Discussion

Seed germination

The effects of the different priming treatments initially observed on seed germination. Seed priming increased germination percentage, mean germination time, time to reach 50% germination and germination index of moringa seeds. The soaking process allowed the seeds to be partly hydrated to a point where the metabolic process of germination began. The seed priming technique was able to increase the germination rate. In this study, the highest germination percentage recorded for seed subjected hydropriming at (63.25%), followed by nutripriming (61.75%) and osmopriming (60%) with the same duration of 4 hours soaking time. Figure 1 shows the germination curves that indicate the germination rate of moringa seed subjected to different priming application and duration. A narrow distribution curve shows rapid germination rate and uniformity, while broader curve illustrates poor homogeneity in germination. The time (days after seeding) at which the curve's peaked, is the mean germination time (MGT) for the particular priming treatments. From the seed of hydropriming, nutripriming, and osmopriming curves, it shows that these primed applications with 4 hours duration had the highest germination rate and uniformity.

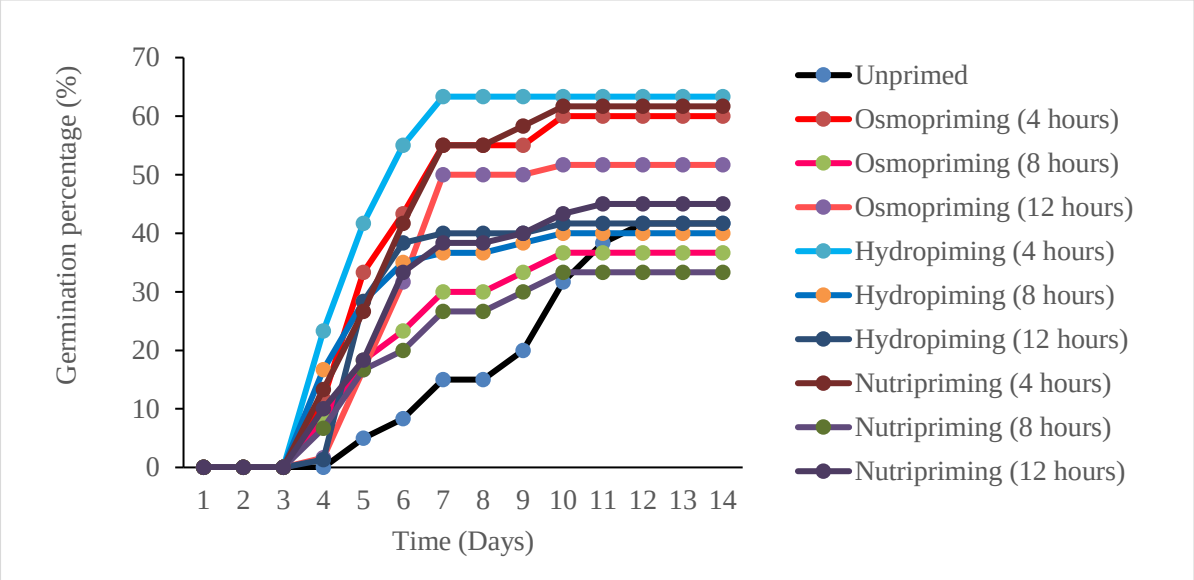

Figure 1: Cumulative germination percentage of moringa seed under different priming application and duration over 14 days.

All pre-germination treatments have significant early mean germination time (MGT), time to 50% germination (T50), and higher germination index (GI) as compared to the unprimed seeds (Table 1). Enhanced germination may be because of improved water imbibition during priming application, as moringa seeds have a thick outer coat and may take longer to start germination. Water imbibition is the first stage of germination and insufficient moisture level hinders germination. The study revealed that prolonged priming durations did not positively affect the germination percentage, mean germination days and time to 50% germination. In this study, after a certain period, seeds became mouldy and deteriorated as seeds were moistened daily. These findings support by Lee and Kim, (1999 and 2000), and Farooq et al. (2006a), that longer priming durations may negatively affect germination percentage.

Table 1: Mean germination time (MGT), time to reach 50% germination (T50), and germination index (GI) of moringa seed with different priming application and duration.

Treatments	MGT (days)	T50 (days)	GI
Unprimed	8.96^{a}	8.79^{a}	0.76^{c}
Osmopriming (4 hours)	6.02^{b}	5.40^{b}	1 66^{a}
Osmopriming (8 hours)	5.15^{b}	4.40^{b}	1.93^{a}
Osmopriming (12 hours)	5.76^{b}	4.99^{b}	1.66^{a}
Hydropiming (4 hours)	6.09^{b}	5.12^{b}	1.21^{abc}
Hydropiming (8 hours)	5.26^{b}	4.60^{b}	1.26^{abc}
Hydropiming (12 hours)	6.24^{b}	5.65^{b}	1.29^{ab}
Nutripriming (4 hours)	6.25^{b}	5.58^{b}	0.87^{bc}
Nutripriming (8 hours)	5.25^{b}	4.38^{b}	1.23^{abc}
Nutripriming (12 hours)	5.95^{b}	5.40^{b}	1.02^{bc}

Means with the same letter were not significantly different at P≤0.05.

Root morphology

Root growth is essential indicators of robust seedling establishment, as fewer and shorter roots are unable to cope with minor stress and ultimately result in crop yield declines. The effects of different priming application and duration on roots development were analysed (Table 2). The length of the roots, root volume, number of fork and number of crossing of the hydropriming of 4-hours seedlings were 41%, 60%, 91% and 107% greater in comparison to unprimed seedlings, respectively.

Table 2: Effects of different priming treatments and duration on root characteristics after 14 days grow in seed pouches.

Treatments	Length (cm)	Root Vol (cm^3)	AvgDiam (mm)	Tips No	Fork No	Crossing No
Unprimed (Control)	170.88^{bc}	0.292^{c}	0.514^{a}	260.60^{ab}	271.47^{bc}	28.53^{b}
Osmopriming (4 hours)	183.80^{abc}	0.328^{bc}	0.530^{a}	260.73^{ab}	322.00^{bc}	32.60^{ab}
Osmopriming (8 hours)	126.39^{c}	0.273^{c}	0.561^{a}	233.73^{b}	219.27^{c}	25.67^{b}
Osmopriming (12 hours)	140.62^{bc}	0.314^{bc}	0.557^{a}	228.53^{b}	255.60^{bc}	36.40^{ab}
Hydropriming (4 hours)	240.52^{a}	0.467^{a}	0.537^{a}	344.20^{ab}	517.80^{a}	59.20^{a}
Hydropriming (8 hours)	192.05^{ab}	0.453^{a}	0.530^{a}	373.87^{a}	447.07^{ab}	46.07^{ab}
Hydropriming (12 hours)	163.64^{bc}	0.329^{abc}	0.538^{a}	270.20^{ab}	370.00^{abc}	36.40^{ab}
Nutripriming (4 hours)	137.84^{bc}	0.278^{c}	0.549^{a}	240.20^{b}	297.73^{bc}	26.33^{b}
Nutripriming (8 hours)	173.65^{bc}	0.342^{bc}	0.538^{a}	277.87^{ab}	310.13^{bc}	36.53^{ab}
Nutripriming (12 hours)	195.69^{ab}	0.301^{c}	0.532^{a}	268.47^{ab}	316.87^{bc}	33.33^{ab}

Means with the same letter were not significantly different at P≤0.05.

Seedling growth

Varied findings were observed on the effects of different priming application and durations in the seedling growth of Moringa at 14 days after seeding in different growing media (Table 3). All priming treatments showed taller seedlings compared to control. Nutripriming of 4 hours gave the robust stem girth (2.34 mm) comparable with seedling treated with osmopriming at all durations and nutripriming at 12 hours. Least number of leaflets/plants were observed on unprimed and osmopriming of 8 hours seedlings compared to other priming treatments. Seedling treated with nutripriming of 8-hours and 4-hours hydropriming had the highest total leaf area 95% and 65% greater than unprimed seeds. Primed seeds showed slight improvement in all parameters tested when compared to unprimed (control). Presence of growth promoters and micronutrients in nutripriming application does improved seedling growth, but no remarkable differences observed compared with hydropriming application. Similarly,

Nouman et al. (2012) recorded that hydropriming improves germination and stand establishment of moringa. They also reported induced tolerance against adverse conditions like abiotic stress, especially during emergence and early seedling growth. In this study, growing media did not significantly affect seedling biomass.

Table 3: Vegetative growth measurement of moringa seedlings at 14 days after seeding treated with different priming treatments and growing media.

Treatments	Height (cm)	Stem girth (mm)	Number of leaflets	Total leaf area (cm^2)
Growing media (M)				
Sand: peat moss (9:1)	23.09^{a}	2.27^{a}	4.89^{a}	131.92^{a}
Top soil: coconut coir: peat moss (5:2:1)	21.76^{a}	2.20^{a}	4.81^{a}	146.09^{a}
Priming (P)				
Unprimed (Control)	17.17^{d}	2.09^{e}	4.00^{d}	102.54^{e}
Osmopriming (4 hours)	18.94^{cd}	2.29^{ab}	4.61^{bc}	106.91^{de}
Osmopriming (8 hours)	21.67^{bc}	2.27^{abc}	4.33^{cd}	112.93^{cde}
Osmopriming (12 hours)	25.17^{a}	2.29^{ab}	5.17^{ab}	107.76^{de}
Hydropriming (4 hours)	23.67^{ab}	2.19^{cd}	5.11^{ab}	168.82^{ab}
Hydropriming (8 hours)	23.06^{ab}	2.18^{cde}	5.50^{a}	158.45^{b}
Hydropriming (12 hours)	24.56^{ab}	2.24^{bc}	4.78^{bc}	141.94^{bcd}
Nutripriming (4 hours)	22.44^{ab}	2.34^{a}	5.06^{ab}	146.06^{bc}
Nutripriming (8 hours)	23.72^{ab}	2.13^{de}	5.00^{ab}	197.46^{a}
Nutripriming (12 hours)	23.83^{ab}	2.31^{ab}	4.94^{ab}	147.21^{bc}
M	n.s	n.s	n.s	n.s
P	**	**	**	**
M x P	n.s	n.s	n.s	n.s

***Significant at 1% probability level, ns: Not significant. Means with the same letter were not significantly different at P≤0.05.*

Conclusions

Seed germination and early growth of moringa seedlings are major determinant in successful growing of the crop, and thereby contributing to the crop stand density and yield. Higher yield and productivity of the moringa is found to dependant on practical methods applied. The study revealed that seed priming techniques using different solutions could significantly improve moringa plant performance by increasing seed germination rate and seedling growth. As in this study, amongst all the treatments, hydropriming for 4 hours is recommended as the most cost-effective priming treatment as it gave the optimal value of germination percentage, germination rate and seedling growth.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Evaluation of Sorghum Seed Viability using Tetrazolium Test

Faizah, S.A.R.[1,*], Muhammad Najib, O.G.[1], Halimah, H.[1], Nurhazwani, M.[1], Hanim, A.[1], Zaitialia, M.[2] Amyita, W.U.[1] and Ahmad, F.M.[1]

[1]Industrial Crops Research Centre, MARDI, 43400, Serdang, Selangor, Malaysia.
[2]Seed, Planting Materials and Livestock Production Programme, Technology Commercialization and Business Centre, MARDI, 43400, Serdang, Selangor, Malaysia.
**E-mail: fsalvana@mardi.gov.my*

Introduction

Sorghum (*Sorghum bicolor* L.) belongs to the grass family, *Poaceae*. It is mainly cultivated in drier areas and has become a crop of choice for farmers because it is resistant to drought compared to other animal feed crops (Habyarimana et al., 2004). In Brazil, this crop is almost exclusively grown as animal feed, in the form of pasture, silage, and feed composition.

Assessment of seed physiological quality is a key factor in determining whether the seed can be used in the production process. Decisions to plant crops especially during uncertain weather conditions need to be quick and thus rapid methods to determine seed quality such as the tetrazolium test was established. This test could rapidly determine seed viability, especially for storage and commercial purposes. Using the tetrazolium test is very important for seed quality assessment as it requires a short time, usually less than 24 hours to determine seed viability. Having time cut short to evaluate seed viability is a crucial part in the seed production process as this can hasten decision making at different stages of the process. As sorghum seeds may exhibit dormancy when freshly harvested, the use of tetrazolium test over standard germination test is really advantageous where evaluation time can be cut from the usual 17 days to yield results.

The test is based on the activity of dehydrogenase enzymes that catalyse respiratory reactions in the mitochondria, resulting in the formation of a stable and non-diffusible red compound, formazan. Thus, it is possible to analyse seed viability by identifying the stain areas in the embryo (ISTA, 2008). The physiological quality of the seed determines its viability and vigour, which are evaluated through laboratory analyses that express this potential in a precise, rapid, and efficient way. For this purpose, the germination test is the most used to verify the viability of seeds and its result represents the maximum potential of the seed lot obtained under favourable environmental conditions.

Therefore, this study aimed to determine an improved methodology of the tetrazolium test and its efficiency to evaluate the viability of sorghum seeds.

Materials and Methods

Seed materials

Seeds of four sorghum varieties were used in the test: hybrid brown mid rib (BMR), mega sweet (MS) and sugar glaze (SG) and inbred SPV 422. This research has been carried out at the Quality Control Laboratory, Seed, Planting Materials and Livestock Production Programme, Technology Commercialization and Business Centre, MARDI Headquarters, Serdang, Selangor.

Moisture content test

For moisture content determination, four replicates of 20 seeds were used. Following the International Seed Testing Association (ISTA) 1985 recommended method, the Low Constant Temperature oven method was used in determination of seed moisture content where the seeds were placed in the oven at

103 ºC±1 ºC for 16±1 hours. Seeds were chopped into small pieces to increase the surface area of the seed to allow a complete removal of water content before being weighed and placed in the oven. Aluminium petri dishes were used for moisture content determination. The initial weight of aluminium petri dish (W_1), the weight of the aluminium petri dish with seeds (W_2), and the weight of dried seed after removing from the oven at 103 ºC±1 ºC for 16±1 hours (W_3) were recorded. The petri dish and dry seeds after removal from the oven has to be cooled in desiccators for about 20-30 minutes. Finally, the percentage of moisture content (% MC) was calculated using the following formula:

$$\% \text{ MC} = \frac{W2 - W3}{(W2 - W1)} \times 100\%$$

W1 = Weight of aluminium petri dish, g
W2 = Weight of aluminium petri dish + seed before drying in the oven
W3 = Weight of aluminium petri dish + seed after drying in the oven

Germination test

Four replicates of 25 seeds for each variety were subjected to germination test. Each cleaned, germination plastic container was filled with sterilized sand as a germination media. Then, the sand was moistened with distilled water. A total of 25 seeds were placed on top of the sand and the plastic germination containers were covered using the lid to prevent moisture loss. The media was watered if found dried. Germination was assessed at day 8 after sowing and results were expressed as the percentage of normal seedlings. The germination percentage and germination rate were calculated and recorded. The germination percentage was calculated using the following formula:

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Tetrazolium (TZ) test

Excised embryos of sorghum were subjected to tetrazolium test. Tetrazolium solution (2,3,5-triphenyltetrazolium chloride) was prepared by diluting the tetrazolium salt with distilled water at 0.1%, 0.5% and 1.0%. The solution was kept in a volumetric flask covered with aluminium foil since this solution is light sensitive. Embryos that had been excised were placed in a beaker covered with aluminium foil. The tetrazolium solution was added until all the embryos were entirely immersed by the solution.

For this experiment, four subsamples of 30 seeds were used for each treatment.

Seed preconditioning:

(i) Direct immersion for 10 hours (h) at 20 °C in 40 mL of water contained in a 100 mL glass beaker.

Post-preconditioning seed preparation:

(i) The seeds were longitudinally sectioned in the centre of the embryonic axis and ¼ of the length of the endosperm. Each seed was submersed in solution of 2,3,5- triphenyltetrazolium chloride (TTC), at the concentrations of 0.1, 0.5 and 1.0%, in the dark, at temperatures of 30 ºC for 3, 4 and 5 h, using four subsamples of 30 seeds, inside plastic cups. After the periods of coloration, the solutions were drained and the seeds were washed in running water and evaluated for uniformity, location, and intensity of coloration of embryonic tissues, being classified into two categories: viable and non-viable.

After each period of staining, the seeds were kept on filter paper (staining on paper) or immersed in refrigerated water at 5-10 °C (immersion staining) until the time of assessment, which was performed

on the same day of staining. The viability assessment of sorghum seeds was adapted from the methodology of International Seed Testing recommendation. The staining intensity and pattern of the embryo were observed under microscope. Seeds-stained red were classified as viable, while improperly stained or unstained seeds were considered unviable, and the data obtained used to calculate the percentage of viable seeds.

Statistical analysis

The experiments were carried out in a complete randomized design (CRD) with four replications. The SAS software was used for analysis of variance (ANOVA). Treatment means were compared by Tukey's test ($P \leq 0.05$).

Results and Discussion

The initial seed moisture content values were similar for all sorghum varieties, varying from 7.91% to 9.55% (Table 1), a factor considered fundamental for standardizing viability tests and obtaining consistent results. The seed quality evaluation by the standard germination test (Table 1) separated the variety into high viability variety namely MS, BMR and SPV and the lower viability variety (SG).

Table 1: Mean germination values and seed moisture content of four variety of sorghum seeds.

Variety	Germination (%)	Moisture content (%)
Brown mid rib	97[a]	8.51[a]
Mega sweet	98[a]	7.91[a]
Sugar graze	80[b]	9.55[a]
SPV 422	96[a]	8.80[a]

Mean with the same letter are not significantly different at P≤0.05% level according to Tukey's HSD.

The immersion staining procedure recommended by ISTA (2008) for sorghum seeds is using TTC concentration of 1.0% at 30 °C. However, the results obtained in the present study revealed that using a concentration of 0.1% TTC yielded the same results as using 1.0%. Using 0.1% TTC for staining sorghum seeds is not only more economical, but also allows proper staining of seed tissues, without reducing the classification of viability.

Table 2 shows the results of the viability of sorghum seeds by the tetrazolium test conducted with seed preconditioning immersion. Immersion staining in the tetrazolium solution of 0.1% for three hours produced the same seed viability classification for the lot as that of the germination test (Table 1), variety BMR, MS and SPV were ranked as having the best quality and variety SG was considered to have the least performance.

Table 2: Seed viability of four sorghum varieties conducted with pre-conditioning by immersion with different concentrations of tetrazolium salt.

	Staining method								
	3 hours			4 hours			5 hours		
Variety	Tetrazolium chloride concentration								
	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
BMR	93[a]	90[a]	91[a]	90[a]	86[a]	88[a]	91[a]	86[a]	92[a]
MS	95[a]	91[a]	93[a]	93[a]	91[a]	90[a]	91[a]	90[a]	90[a]
SG	73[b]	73[b]	71[b]	70[b]	70[b]	70[b]	69[b]	69[b]	70[b]
SPV 422	92[a]	86[a]	91[a]	86[a]	90[a]	90[a]	88[a]	91[a]	88[a]

BMR (brown mid rib), (MS) mega sweet, SG (sugar glaze). Mean with the same letter are not significantly different at P≤0.05% level according to Tukey's HSD.

Figure 1: Scale of the seed viability and vigour for sorghum seed.

From the TZ test, a scale of seed viability and vigour is obtained to categorize the seed according to their staining pattern (Figure 1). The formation of this scale starts with viable seeds with high vigour on top while the lower viability seeds in the middle and the dead seeds located at the bottom of the scale. This scale will then be used to determine other sorghum seeds tested using the TZ test.

Viable seeds were considered as that which embryo showed uniform intense red colour, firm, with embryonic axis and cotyledon node region coloured and cotyledon with more than 50% of its surface coloured (Figure 2A). Non-viable seeds were those with totally white or incomplete staining pattern, soft tissues, characterizing dead tissue (Figure 2B).

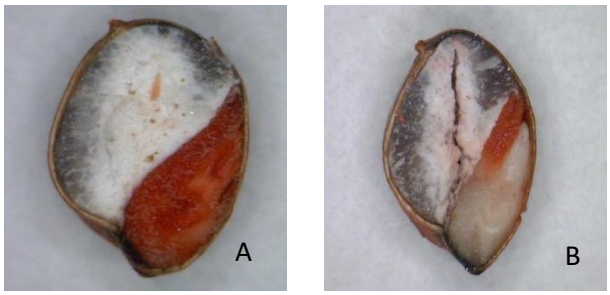

Figure 2: Sorghum seeds after staining (Viable, A and non-viable, B).

Conclusion

This study indicates that it is possible to analyse the quality of sorghum seeds by using tetrazolium test at lower concentration of TTC, 0.1% for 3 hours compared to the recommended concentration (1.0% TTC) by ISTA. Reduction of TTC concentration will reduce the amount of TTC needed for the solution which in the end will reduce the cost needed to conduct the test. Seed viability is crucial information for farmers to predict the number of seeds needed for planting.

Acknowledgements

The author would like to thank all parties that has helped conduct this research from the beginning until the completion of this study. Special thanks to Industrial Crop Research Centre for supporting this research and (project) for funding that has made this research completed. The author would also like to

thank MARDIs Technology Commercialisation and Business Centre for providing the laboratory and equipment need to conduct this study.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Biopolymer Coating to Seed Quality of Chilli (*Capsicum annuum* L.) var. Kulai

Amyita, W.U.[1,*], Uma Rani, S.[2], Norhayu, A.[3] and Azizah, M.[2]

[1]Pest and Disease Management Programme, Industrial Crop Research Centre, MARDI, 43000 Serdang, Selangor, Malaysia.
[2]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
**E-mail: amyita@mardi.gov.my*

Introduction

Chili (*Capsicum annuum* L.) belongs to the family Solanaceae is one of the important vegetables cum spice crops in the world. It is widely cultivated throughout warm temperate, tropical, and subtropical countries (Selvan et al., 2014). In most countries, chilli is grown and consumed worldwide as a fresh vegetable and processed, such as spices, condiments, sauces, and pickles because of its versatility and high nutritional value (Chauhan et al., 2018). It is also one of the important vegetable cash crops grown in Malaysia due to its high nutrients and economic value. The importance of chilli as vegetable is reflected by the increasing local demand and high import value. Despite the economic importance, there are several abiotic and biotic factors limiting chilli production. The lack of high-quality chilli seeds (i.e. low germination percentage and vigour) and the prevalence of seed-borne diseases are among the constraints that significantly reduce the quality and yield of chillies (Ananthi et al., 2014; Abdullah et al., 2017).

A high-quality seed with better germination, vigour, and seed health is the prerequisite for a good crop. Various seed treatments have been suggested to improve seed germination, the emergence, and the seedling's health. The primary way of enhancing seed performance is to apply agrochemicals on seeds by dressing or slurry technique. The disadvantages of this technique are the non-uniform coating of the seed and dust-off material during transport and handling. Maximum germination and seedling establishment of seed treated with pesticides, growth regulators, biologicals or micronutrients rely on firm attachment of these products to the seed coat during packaging, handling, and planting (Williams and Hopper, 1998; Junges et al., 2013). Coating seeds with liquid-based polymeric adhesives improve the adherence of agrochemicals on seeds. It creates an artificial seed coating layer that covers the entire seed surface without altering the shape or size of the seeds. It is usually formed by inert materials to provide adequate handling, promote microenvironments, and protect seeds against pathogens and insect damage (Ziani et al., 2010; Zeng et al., 2012).

A synthetic polymer is commonly used as a seed coating agent. However, the coating drawback is that a substantial amount of polymer remains in the soil takes a long time to degrade. Currently, more emphasis is given on the use of natural polymers or biopolymers originated from plants or biological sources for seed coating. It may act as seed stabilizing and protecting agents from biological and environmental stresses, enhance the nutrient absorption by plants resulting in plant growth promotion and, yield improvement (Chandrika et al., 2019). However, critical to the advancement of new seed coating polymers is an assurance that there is compatibility between the polymer and seed germination, and plant growth (Keawkham et al., 2014). Kaufman (1991) suggests that the ideal coating would be neutral in its effect on the speed, uniformity, and germination percentage of a seed lot. Therefore, this study was carried out to determine the effect of biopolymer coating on germination, seedling vigour, and health of chilli var. Kulai.

Materials and Methods

Seed samples

The seed of chili (*C. annuum* L.) var. Kulai (germination percentage of >80%) purchased from MARDI Station, Klang, Selangor was used in this study. The chili seeds were one year old and stored at 10±2 °C before used in the experiments. Seeds with no cracks or other visible deformations were selected.

Preparation of coating solutions and seed coating

The coating solutions were prepared by dissolving the biopolymer emulsion (polysaccharide-based formulation) in water to four concentrations (2.5, 5, 10, and 20% v/v). Seeds were coated with the biopolymer solutions at 25 mL/kg of seeds to ensure thoroughly coating coverage of seed coat. Seeds and coating solutions were placed into a conical flask and was tightly closed before vigorously shaken to ensure even distribution of coating solutions on the seed. The coated seeds were air-dried to its initial weight inside the laminar air flow hood at room temperature for 4 hours. Non-coated seeds were used as a control.

Seed quality assessment

Germination tests were conducted with four replications of 100 seeds per treatment. Seeds were placed on two layers of moistened paper towel in a closed plastic germination box (20 cm × 20 cm × 10 cm) and incubated in a growth chamber at a temperature of 25±2 °C under 12/12-hour photoperiod. Seeds with visible radicle protrusion (>0.5 cm) were considered as germinated. The number of germinated seeds was recorded daily for 14 days for germination count. Seedlings obtained from standard germination test were used for seedling evaluation. Normal or abnormal seedlings were classified according to the rules of the International Seed Testing Association (ISTA, 2015).

Ten normal seedlings were selected randomly in each treatment from all the replications on the 14th day (final count) of the germination test. The root length was measured from the tip of the primary root to base of hypocotyl, and the shoot length was measured from the tip of the primary leaf to the base of the hypocotyl using a ruler. The mean shoot and root length were calculated and expressed in centimetres (cm). Next, the dry matter of these seedlings was measured. The fresh weight of seedlings was recorded before dried in an oven at 70±2 °C for 24 hours. Then, the dried seedlings were cooled in a desiccator for 30 min and their average dry weight was recorded in milligram (mg) per seedling (ISTA, 2015).

The mean germination time (MGT) was calculated based on the equation MGT = $\sum(n \times d)/N$ where n = number of seeds germinated on each day, d = number of days from the beginning of the test, and N = the total number of seeds germinated at the termination of the experiment (Ellis and Roberts, 1981). The coefficient velocity of germination (CVG) was calculated using the following formula; CVG = $\sum ni/(ni \times ti)$ X 100 where, n is the number of seeds germinated on day i and t is the number of days from seeding corresponding to n (Scott et al., 1984). Germination rate index (GRI) was calculated using a formula by Maguire (1962); GRI = G1/T1 + G2/T2 + G3/T3 + Gn/Tn, where: G1 = number of germinated seeds on T1; T1 = day of first count; Gn = number of germinated seeds between Tn–1 and Tn; Tn = day of final count. Germination index (GI) was calculated using the formula; GI = $\sum gi/ti$, where gi is the germination at the moment i, ti is the number of days counted from the beginning of the germination till the moment i (Scott et al., 1984). The seedling vigour index (SVI) was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973); SVI-1 = (Mean root length + Mean shoot length) (cm) x Germination (%) and SVI-2 = Seedling dry weight (mg) x Germination (%).

Seed health test was carried out based on the procedure of ISTA (2015). For each treatment, 100 seeds per replication were used which twenty seeds were placed in petri dish of 90 mm diameter containing three layers of moistened filter papers. The plates were incubated at 20±2 °C under an alternating cycle of 12

hours darkness and 12 hours light of Near Ultraviolet (NUV) for 7 days. After incubation period, the seeds were examined under stereo binocular microscope for detecting the fungi grew over the germinating seeds. Percentage of fungi infection was calculated following the formula; Percentage of fungi infection (%) = (No. of infected seeds with fungi/Total no. of seed) X 100.

Experimental design and statistical analysis

The experiments were carried out in a Completely Randomized Design (CRD) with four replications. The SAS software was used for the analysis of variance (ANOVA). Treatment means were compared using the Tukey's Honestly Studentized Range (HSD) test with a confidence interval of 95%.

Results and Discussion

Seed germination and vigour

The data on germination percentage, mean germination time (MGT), coefficient velocity of germination (CVG), germination rate index (GRI), and germination index (GI) were presented in Table 1. It shows that different concentrations of biopolymer had no significant effects on the germination percentage of chilli seeds. However, germination percentage for the coated seeds was higher (83 to 92%) than the control (81%). The time taken for coated seeds to germinate (MGT) was significantly longer (3.9 to 4.7 days) than the control (2.9 days) which indicated late germination. CVG was also significantly reduced when seeds were coated with high concentration of biopolymer (>10%), which indicated slower rate of germination. Results also showed that coating significantly reduced the GRI and GI. Since there is an empirically direct proportionate relationship between GRI and GI, so the similar trend was observed in GRI as that in GI. Several studies have indicated that the seed coating can act as a mechanical barrier for water absorbance and radical emergence (Taylor et al., 1998; Amirkhani et al., 2016). Thus, the polymer coating used probably creating a barrier for imbibition of water into seeds which caused increased of germination time and lowered the germination rate of chilli.

Table 1: Germination percentage (%), mean germination time (MGT), the coefficient velocity of germination (CVG), germination rate index (GRI), and germination index (GI) of chilli following coating with different concentrations of biopolymer emulsion.

Treatments	Germination (%)	MGT (day)	CVG	GRI	GI
T0 (0% biopolymer)	81^{a}	2.93^{d}	47.06^{a}	34.99^{a}	1185^{a}
T1 (2.5% biopolymer)	92^{a}	3.86b^{c}	49.75^{a}	26.34^{bc}	1037^{bc}
T2 (5% biopolymer)	88^{a}	3.59^{c}	46.18^{a}	28.53^{b}	1085^{ab}
T3 (10% biopolymer)	90^{a}	4.11^{b}	51.14^{a}	25.44^{bc}	1045^{b}
T4 (20% biopolymer)	83^{a}	4.72^{a}	26.06^{b}	21.11^{c}	925^{c}

Note: Means with a different superscript letter in the same column are significantly different by Tukey HSD test at P<0.05.

Seedling growth and vigour

The data on seedling shoot length (SL), root length (RL), fresh weight (FW), dry weight (DW), and seedling vigour index (SVI) were presented in Table 2. RL, DW, and SVI of chilli were not significantly affected by biopolymer coating except for SL and FW. Results show that the SL of seeds coated with biopolymer was significantly shorter than the control. Additionally, the seedling FW of seeds coated with biopolymer at concentrations of >5% was significantly reduced when compared with the control. The SVI-1 value was lower after coating due to the reduced seedling shoot length as compared to the control and as for SVI-2 value, it was lower due to the lower seedling dry weight and percentage of germination. SL, RL, and SVI are important indicators to determine whether the coated seeds affect seedling growth. It showed that coating affect the seedling growth especially when seeds were coated at high concentrations of biopolymer (>10%).

Table 2: Seedlings shoot length (SL), root length (RL), fresh weight (FW), dry weight (DW), and seedling vigour index (SVI) of chilli following seed coating with different concentrations of biopolymer emulsion.

Treatments	SL (cm)	RL (cm)	FW (g)	DW (g)	SVI-1	SVI-2
T0 (0% biopolymer)	3.0[a]	4.4[a]	0.0288[a]	0.0030[a]	594[a]	246[a]
T1 (2.5% biopolymer)	2.8[ab]	4.5[a]	0.0252[ab]	0.0027[a]	601[a]	248[a]
T2 (5% biopolymer)	2.6[b]	4.4[a]	0.0239[b]	0.0026[a]	617[a]	228[a]
T3 (10% biopolymer)	2.9[ab]	4.2[a]	0.0242[b]	0.0025[a]	640[a]	227[a]
T4 (20% biopolymer)	2.6[b]	3.8[a]	0.0245[b]	0.0028[a]	587[a]	229[a]

Note: Means with different superscript letters in the same columns are significantly different by Tukey HSD test at P<0.05.

Seed health

Result shows that the fungal incidence was significantly decreased after seeds were coated with higher concentrations of biopolymer (>5%) (Table 3). According to Robani (1994), polymers give additional protection to the seeds by acting against pathogens and ensure more excellent safety during handling. The polymer coating acts as binding material to keep the seeds intact, as it covers the minor cracks and aberration on the seed coat, thus blocking the fungal invasion. It may also act as a physical barrier, which reduces leaching of inhibitors from seed covering and restricts oxygen movement. Hence, respiration of embryo is reduced, and thereby reducing the ageing in seeds (Vanangamudi et al., 2003). However, the biopolymer efficacy to inhibit fungal growth is not conclusive as the seed itself was very healthy.

Table 3: Fungi infection (%) on chilli seed following coating with different concentrations of biopolymer emulsion.

Treatments	Fungi infection (%)
T0 (0% biopolymer)	9[a]
T1 (2.5% biopolymer)	5[ab]
T2 (5% biopolymer)	4[ab]
T3 (10% biopolymer)	0[b]
T4 (20% biopolymer)	0[b]

Note: Means with different superscript letters in the same column are significantly different by Tukey HSD test at P<0.05.

Conclusion

Based on this study, the biopolymer coating did not affect the seed quality of chilli var. Kulai in terms of germination, health, seedling growth, and vigour. However, biopolymer coating affects the time taken for seeds to germinate and its rate of germination especially when seeds were coated with high concentrations of biopolymer (>10%). Thus, coating with biopolymer improved the physiological quality of seeds and it give additional protection to the seeds against infection of fungi pathogens.

Acknowledgements

The authors would like to thank MARDI for the financial support (HBBP Scholarship) provided to the first author.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Seed Priming Treatments on Seed Quality of Tomato (*Solanum lycopersicum*)

Zaitialia, M.[1,*], Nurul Atilia Shafienaz, H.[2], Amyita Witty, U.[2] and Muhamad Arizal, B.[1]
[1]Commercialization Technology and Business Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
[2]Industrial Crop Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: mzai80@mardi.gov.my*

Introduction

Solanum belongs to the Solanaceae family and is commonly eaten raw or cooked as vegetable. Tomato consists of several species and varieties that can be characterized based on their varying sizes and shapes. According to Ghoohestani et al. (2012), tomato is one of the most important fruit vegetables in the world and has the highest production volume and cultivation among vegetable products. Tomato is a rich source of nutrients such as carbohydrate, protein, vitamin, and lycopene for human diet (Siti Norhayati et al., 2019). Tomato production increased in volume to accommodate market demand and food consumption. However, production of tomatoes may encounter several obstacles due to diseases and low germination of seeds which may drastically reduce the quality and yield of tomatoes. Abdullah et al. (2017) stated that seed quality can be enhanced in several methods including seed priming.

Seed priming is a modern technique used to modify the seeds to be more effective in germination and resistance to harsh condition and be able to promote rapid plant growth as well. Nowadays, it is used on various species of seeds to enhance germination and emergence percentage. Besides, priming also improves the uniformity of germination and seedling establishment under stress condition (Ansari et al., 2013). It is also considered as feasible and cheap technique (Patel et al., 2017). There are several types of seed priming methods to increase seed effectiveness, namely hydropriming, osmopriming, solid matrix priming, hormonal priming, biopriming, halopriming, and others (Divya and Nirmala Devi, 2015). Seed priming has been proved to advance germination for many plant species in agriculture like in soybean (Helsel et al., 1986), parsley (Phill and Kilan, 2000) and pepper (Khan et al., 2009).

Potassium nitrate (KNO_3) is one type of chemical used for halopriming. It is recommended by ISTA to be used in dormancy breaking treatment for *Capsicum* spp. Senaratna et al. (2000) found that KNO_3 influenced the growth of plumule from seed. Besides, KNO_3 is widely used in promoting germination (Gashi et al., 2012). However, studies on how it affects plant growth and performances are still lacking. Besides, the performance of the seeds after being treated by seed priming depends on the priming technique, environment, crop variety, seed imbibition behaviour and imbibition time (Demir and Okcu, 2004).

Salicylic acid (SA) as a phenolic compound has the function to modulate plant responses to environmental stresses (Senaratna et al., 2000). Priming seeds using SA is called hormonal priming. According to Lutts et al. (2016), seeds will undergo the imbibition process in the presence of plant growth regulators. SA is one of the endogenous growth regulators that are derived from phenolic-nature group. It influences various processes in plants including seed germination (Cutt and Klessing, 1992). This study aims to determine the effect of priming on germination, seedling emergence and growth of tomato seed var. MAHA 18.

Materials and Methods

The experiment was conducted in Seed Quality Control Laboratory in Malaysian Agricultural Research and Development Institute (MARDI) Headquarters.

Seed treatments and solution preparations

Tomato var. MAHA 18 seeds were obtained from Commercialization Technology and Business Centre (CB) in MARDI Headquarters. They were soaked with three different priming methods *viz* water for hydropriming, KNO_3 for halopriming and SA for hormonal priming. Nine treatments studied consisted of control (no priming, T1), hydropriming (priming with distilled water for 4 days: T2, and 24 hours: T3), halopriming with KNO_3 solution (priming with 0.2%: T4, 0.7%: T5, and 1.2%: T6) for 4 days and hormonal priming with SA solution (priming with 50 mg/L: T7, 100 mg/L: T8, and 150 mg/L: T9) for 24 hours.

Firstly, 1,000 mL stock solution of 1 g/L SA was prepared. Then, it was diluted using successive dilution with distilled water to obtain the desired concentrations of 150 mg/L, 100 mg/L, and 50 mg/L. KNO_3 solutions of 0.2%, 0.7%, and 1.2% were prepared by diluting 0.2, 0.7, and 1.2 g KNO_3 powder into 100 ml distilled water, respectively. Seed weight and moisture content of seed were determined before and after seed priming. Initial weight of two samples of 50 seeds was determined and recorded. Then, the seed samples were dried for 17±1 hours at 103 °C in oven. After the seeds were dried, the final weight of the samples was measured, and moisture contents were determined using the following formula:

Moisture content (%) = [(initial weight-final weight)/initial weight] x 100.

The ratio of seed to solution used was 1:5 (w:v) (Rehman et al., 2011). After priming, seeds were rinsed three times with distilled water and left to dry at room temperature for 24 hours or until the weight turned close to their initial weight.

Germinations test

25 seeds/replicate per treatment were sown in peat moss medium under shade at ambient temperature of 33-35±3 °C during days and 20-23 °C at night. The relative humidity was 70-90%.

Evaluation of vigour

Number of germinated seeds was recorded daily for 14 days after commencement of germination. The criterion of germination for seed is the first appearance of hypocotyl above the medium.

The percentage of normal seedlings was evaluated at the 14th day after sowing (ISTA, 2015).

Mean germination time (MGT) was calculated by using following equation (Ellis and Roberts, 1981).
MGT (days) = $\sum(n \times d)/N$
where,
n = number of germinated seeds on each day
d = number of days from the beginning of the test
N = total number of seeds germinated at the termination of the experiment

Germination index (GI) was calculated as described in the Association of Official Seed Certifying Agencies (AOSCA, 1993).
GI = $\sum \frac{gi}{ti}$
where,
gi is the germination at the moment
ti is the number of days counted from the beginning of the germination till the moment i

Seedling vigour index (SVI) was calculated by multiplying seedling length to germination percentage, where seedling length is the total length of radicle and plumule (Patel et al., 2017).

On the 14th day after germination, shoot length (SL), root length (RL), seedling fresh weight (SFW) and seedling dry weight (SDW) of randomly selected 10 seedlings per replication for each treatment were measured and recorded. Prior to measurement, seedlings were washed and surface dried using tissue paper. Immediately, the length of shoots and roots were measured before they were separated and weighed to obtain SFW and dried at 70 °C for 24 hours using oven to obtain SDW.

The experiment was carried out using completely randomized design with three replicates. Data were subjected to analysis of variance (ANOVA) and significant differences among means were determined by Least Significant Difference (LSD) test at 5% probability using the SAS statistical software (SAS 9.4).

Results and Discussion

The process of germination starts with uptake of water by the dry seed and terminates with radicle penetration through the seed covering layers (Bewley et al., 2006; Weitbrecht et al., 2011). Water uptake by seed is controlled by the difference in water potential between the seed and the external medium. Water potential is defined by the sum of osmotic potential, matric potential, and pressure potential. Matric potential plays the major role in the soil because of the effects of soil particles on water potential. In addition, primed seeds have completed Phase I (hydration) and Phase II (lag phase) of germination, therefore they only require a favourable water potential gradient for water uptake to begin growth (Korkmaz, 2005).

Seed priming treatments had significant effects on all parameters of tomato seed. There was a significant difference between control and primed seeds (Table 1 and Table 2). Significantly, higher germination (90.7%) was reported with T5, followed by T3, T7 and T8 (80%). Seed priming with SA (T7 and T8) improved MGT and speed of germination (GI). Shorter emergence in terms of MGT was observed with T7 (4.68 days) followed by T1 (4.9 days) and T8 (4.98 days). A higher speed of germination (GI) was reported in T7 (25.13), followed by T8 (24.29) and T1 (23.41). Hydroprimed seed with water for 24 hours improved germination up to 3.5% compared to the control. It was in contrast to four days incubation with germination percentage decreased to 2.1%. However, speed of germination of both treatments were decreased 8.8% to 45.7% compared to the control (Table 1).

In terms of SVI, tomato seed was significantly improved by seed priming treatments, where seeds primed with water for 24 hours increased SVI significantly by 36.5% compared to control. The maximum SL was recorded when the seed was primed with T5 (3.82 cm) followed by T4 (3.77 cm) and T3 (3.76 cm). The maximum RL (3.14 cm) was recorded by T3 and followed by T9 (2.77 cm) and T6 (2.57 cm) (Table 2).

In general, tomato seeds responded differently to different priming treatments and duration. For seeds primed with 0.7% KNO_3, there was increment in germination percentage to be 17.3% compared to the control. Our findings were in agreement with Lara et al. (2014); they reported that tomato seeds primed with KNO_3 were related to the activity of the enzyme nitrate reductase in the production of nitrite/nitric oxide, which promoted faster germination. Moreover, study done by Agoncillo (2018) on hot pepper showed that seed priming using KNO_3 at concentration of 3.0% for 6 days significantly improved initial germination percentage and speed of germination.

Table 1: Effect of nine priming treatments on germination percentage, mean germination time and germination index.

Treatments	Germination percentage (%)	Mean germination time (days)	Germination index
Control (T1)	77.3 ± 10.07^{abc}	4.90 ± 0.31^{bc}	23.41 ± 3.56^{ab}
Hydropriming for 4 days (T2)	61.3 ± 14.05^{cd}	5.97 ± 0.56^{ab}	12.71 ± 1.89^{c}
Hydropriming for 24 hours (T3)	80.0 ± 6.93^{ab}	5.62 ± 0.17^{abc}	21.34 ± 1.53^{ab}
0.2% KNO_3 for 4 days (T4)	72.0 ± 10.58^{bcd}	5.47 ± 0.12^{abc}	19.74 ± 2.92^{b}
0.7% KNO_3 for 4 days (T5)	90.7 ± 8.33^{a}	6.24 ± 1.18^{a}	22.18 ± 2.28^{ab}
1.2% KNO_3 for 4 days (T6)	56.0 ± 4.00^{d}	6.36 ± 1.05^{a}	13.18 ± 2.56^{c}
50 mg/L SA for 24 hours (T7)	80.0 ± 12.00^{ab}	4.68 ± 0.29^{c}	25.13 ± 4.94^{a}
100 mg/L SA for 24 hours (T8)	80.0 ± 10.58^{ab}	4.98 ± 0.66^{bc}	24.29 ± 3.56^{ab}
150 mg/L SA for 24 hours (T9)	77.3 ± 12.22^{abc}	5.07 ± 0.33^{bc}	22.77 ± 2.50^{ab}

Means with different subscript letter are significantly different by LSD test at P 0.05.

Table 2: Effect of nine priming treatments on seedling vigour index, seedling fresh weight, seedling dry weight, shoot length and root length.

Treatments	Seedling vigour index	Seedling fresh weight (g)	Seedling dry weight (g)	Shoot length (cm)	Root length (cm)
Control (T1)	405.3 ± 45.59^{bc}	0.753 ± 0.03^{ab}	0.074 ± 0.01^{a}	3.51 ± 0.13^{b}	1.74 ± 0.04^{c}
Hydropriming for 4 days (T2)	344.7 ± 110.05^{c}	0.447 ± 0.06^{c}	0.048 ± 0.01^{b}	3.12 ± 0.20^{c}	2.42 ± 0.39^{abc}
Hydropriming for 24 hours (T3)	553.4 ± 83.17^{a}	0.682 ± 0.11^{b}	0.055 ± 0.01^{b}	3.76 ± 0.15^{ab}	3.14 ± 0.64^{a}
0.2% KNO_3 for 4 days (T4)	439.1 ± 87.46^{abc}	0.808 ± 0.08^{ab}	0.061 ± 0.01^{ab}	3.77 ± 0.06^{ab}	2.30 ± 0.46^{bc}
0.7% KNO_3 for 4 days (T5)	533.4 ± 35.75^{a}	0.796 ± 0.14^{ab}	0.057 ± 0.01^{b}	3.82 ± 0.22^{a}	2.08 ± 0.44^{bc}
1.2% KNO_3 for 4 days (T6)	348.7 ± 16.47^{c}	0.746 ± 0.16^{ab}	0.048 ± 0.01^{b}	3.69 ± 0.19^{ab}	2.57 ± 0.57^{ab}
50 mg/L SA for 24 hours (T7)	449.3 ± 63.89^{abc}	0.834 ± 0.10^{ab}	0.060 ± 0.01^{ab}	3.54 ± 0.17^{b}	2.09 ± 0.39^{bc}
100 mg/L SA for 24 hours (T8)	459.0 ± 67.37^{abc}	0.873 ± 0.06^{a}	0.057 ± 0.01^{b}	3.67 ± 0.03^{ab}	2.06 ± 0.12^{bc}
150 mg/L SA for 24 hours (T9)	494.5 ± 68.62^{ab}	0.764 ± 0.10^{ab}	0.052 ± 0.01^{b}	3.65 ± 0.17^{ab}	2.77 ± 0.42^{ab}

Means with different subscript letter are significantly different by LSD test at P<0.05.

Conclusion

The different priming treatments (water for hydropriming, KNO_3 for halopriming and SA for hormonal priming) enabled significant increases in the germinability and vigour of tomato cultivar MAHA 18 seed. For high germination percentage, all priming treatments can be used except for priming treatment with 1.2% KNO_3 and hydropriming for 4 days. These treatments caused low germination rate, speed of germination and SVI in tomato seeds. In terms of seedling vigour, it is recommended to prime tomato seeds with water for 24 hours, or KNO_3 at low concentration of 0.7% for four days.

Acknowledgements

This work was done with funding from Development Project RMK 11 for vegetables (P-RH403). Thanks are extended to colleagues from Seed Quality Control Laboratory, CB3 and Industrial Crop Centre, IC, MARDI. Special thanks are conveyed to my practical students from Universiti Malaysia Terengganu for the work well done.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Seed Quality of Papaya Lines Selected for Tolerance to Papaya Ringspot Virus Disease

Zubir, M.N.[1,*] and Hassan, M.A.[2]

[1]Horticulture Research Centre, MARDI Pontian, 82000 Pontian, Johor, Malaysia.
[2]Horticulture Research Centre, MARDI Headquarters, 43400 Serdang, Selangor, Malaysia.
**E-mail: znizam@mardi.gov.my*

Introduction

Papaya ringspot virus (PRSV) disease is the second devastating disease of papaya after papaya dieback disease (PDD). Both diseases were proclaimed as dangerous diseases under Malaysia Plant Quarantine Act 1976. PRSV disease is caused by a species member of the genus *Potyvirus* that causes damage on cucurbit crops such as squash and melons, and papaya (Desbiez and Lecoq, 2020). The infected plant has stunted growth and the fruit is malformed with bumpy shape, often showing ringspot symptoms from which the name is derived. Currently, cultivated papaya variety in Malaysia is susceptible to PRSV disease and control of this disease consists mostly of preventive actions. Conventional breeding of papaya was conducted to develop new variety of papaya that can resist or tolerate PRSV as one of the long-term strategies to improve papaya production in Malaysia. MARDI initiated a breeding programme for resistance to PRSV in 1991 using Tainung No. 5 and Cariflora as tolerant parents. The F1 hybrids with Eksotika showed very good tolerance to PRSV. The single seed descent method was used to generate F2 to F5 in which finally four selected lines namely L41 (excluded in this study), L90, L248, and L13 were chosen for their highest tolerance to PRSV. Based on ranksum method to select the best overall performance for simultaneous selection for disease tolerance and fruit attributes, L13 and L248 were deemed to have the best potential (Chan and Ong, 2003). Later in this paper, L13, L90, and L248 were designated as P13, P90, and P248, respectively.

The quality of seed is equally important to the attributes related to selection of certain variety either for high yielding or resistance/tolerance to diseases. High quality seed is important for agricultural production because poor quality seeds reduce potential yield. Traditionally, papaya is propagated through seeds although other method such as tissue culture was also practiced to produce clonal and disease free planting materials. However, propagation method through seed is still widely practiced due to its low cost and the abundance of planting materials. Papaya seed behaves similarly to orthodox seeds in which its viability is extended by reducing its moisture content and kept at lower temperature (Berbert et al., 2008). There are four parameters for seed quality attributes listed by FAO (2010). These parameters are physical qualities, physiological qualities, genetic quality, and seed health. This study specifically discussed on physiological qualities of papaya seed and focused on seed performance of selected lines of papaya that are tolerant to PRSV.

The objective of this study was to evaluate physiological qualities of papaya seeds of P13, P90, P248, and Eksotika in terms of seed germination rate and seed vigour. Seed germination rate was annotated by germination percentage (GP), germination capacity (GC), mean germination time (MGT), and time to 50% germination (T50) while seed vigour was annotated by germination index (GI).

Materials and Methods

Seed source

The selected lines of papaya hybrid that are tolerant to PRSV disease, i.e. P13, P90, and P248, were compared to susceptible papaya variety of Eksotika. All papaya trees were cultivated on tropical peat soil at Malaysian Agricultural Research and Development Institute (MARDI) in Pontian, Johor, Malaysia. The standard agronomical practices were followed as recommended by Chan et al. (1994). Self-pollination on hermaphrodite flower was conducted by protecting the flower with folded parchment

paper before flower anthesis to prevent entry of any foreign pollen from possible pollinator. The fruit was harvested at maturity index of two and seed collection was done after the fruit had undergone postharvest ripening at room temperature to achieve the stage with 75% of the fruit skin turned yellow in order to produce good germination and high vigour of papaya seeds with better storability (Yogeesha et al., 2013). Only hermaphrodite fruits were selected for seed extraction. The seeds were carefully extracted from halved fruit and the gelatinous sarcotesta was removed by gently rubbing the seeds on stainless steel wire mesh. Seed floatation test was carried out to remove floating seeds that usually had low seed viability (Hartmann et al., 2001). Small sized seeds were also removed through sieve test with 3.15 mm aperture (PRADA). The seeds were treated with fungicide (a.i. 2 g/L benomyl) and air dried at room temperature at 75±10% relative humidity until they reached moisture content of 8-12% (Yogeesha et al., 2013). Moisture content of the seed was determined with Moisture Analyzer MX-50 (A&D Company Limited Japan). The seeds were kept at 10 °C before germination test was conducted.

Germination test

Germination test was conducted with four replicates of 100 seeds each at a total of 400 seeds per treatment. Seeds were placed on paper towel saturated with distilled water and germinated at 28±2 °C under a 12-hour photoperiod (Salomao and Mundim, 2000). Seed germination was recorded daily for 15 days where only radicle protrusion of length more than 1.0 cm was taken as the criterion of successful germination. GP was calculated based on cumulative germination on Day 15. The experiment was carried out in Completely Randomized Design (CRD). In addition to GP, the results were also expressed as GC, MGT, T50, GI, seedling fresh weight (FW), and seedling dry weight (DW).

GC was calculated based on formula by Ranal and Santana (2006):

$$\mathrm{GC} = \frac{\text{total number germinated seed}}{\text{total number of non germinated seed}}$$

MGT (days) was calculated based on the equation of Ellis and Roberts (1981) modified by Moradi Dezfuli (Moradi et al., 2008).

$$\mathrm{MGT\ (days)} = \frac{\Sigma D\mathrm{n}}{\Sigma n}$$

where,
n is the number of seeds, which germinated on day D and D is the number of days counted from the beginning of germination.

T50 (days) was calculated according to the formula of Coolbear et al. (1984) as modified by Farooq et al. (2005).

$$\mathrm{T50}\ (days) = t_i + \frac{[(N/2)-n_i)(t_i-t_j)]}{(n_i-n_j)}$$

where,
N is the final number of germination and n_i, n_j are cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i<N/2< n_j$.

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

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

Seedling FW and DW was determined by the below formula.

$$\text{Seedling FW or DW (mg)} = \frac{\text{total weight of seedling germinated}}{\text{total number of seedling germinated}}$$

Statistical analysis

Data collected were analysed by one-way analysis of variance (ANOVA). The differences between means were separated using Duncan's multiple range test (DMRT) at 5% significance level. Statistical analysis was completed using SAS software version 9.4.

Results and Discussion

Seed germination rate

The highest GP was recorded for Eksotika with 70% followed by P13, P90, and P248 (Table 1). All seeds from selected lines had GP of approximately 50%. A study by Macedo et al. (2013) showed that physiological quality of seeds of three hybrid combinations and their reciprocals was higher when compared to their selfed parents. Five out of six hybrids recorded GP above 90% in their study. Self-pollination method within the same hermaphrodite flower or autogamy may not be suitable for all selected lines in order to produce true to type papaya seeds. Pollination by different flowers within the same individual tree or geitonogamy may be suitable to enhance seed germinability of the selected lines. Study by Srijanchom et al. (2015) on inbreeding of 'Koko No. 1' stated pollen viability and seed germinability decreased with increasing selfing generations. The trend of GP was followed for GC where the highest GC was recorded for Eksotika significantly. The GC was defined as qualitative attribute of the germination process presented as binary number of germinated over ungerminated seeds (Ranal and Santana, 2006). The higher value of GC means the better germinability of seed batch since both GC and GP were calculated using number of germinated seeds of the selected seed batch.

The highest MGT and T50 were recorded on P248 followed by Eksotika and P90, which were statistically the same, and the lowest was recorded on P13. MGT and T50 were both calculated to indicate germination rate of seed. However, MGT reflects germination speed of a seed lot while T50 reflects on the time taken for 50% germination. Higher values of MGT and T50 indicate slower germination. Although GP and GC values for P13 were second after Eksotika, P13 had the shortest MGT and T50 indicating fastest germination. Higher GP did not guarantee faster germination. Slower germinating lots of maize indicated by higher value of MGT demonstrated a greater spread of germination over time thus produced smaller and more variable seedlings in the laboratory (Demir et al., 2008). In the case of this study, lower MGT of P13 implied that the seed of P13 germinated faster or within shorter period of time. Faster germination, i.e. over shorter period of time, suggests more synchronized seed germination.

Table 1: Seed germination rate and seed vigour for selected lines of papaya tolerant to PRSV.

Treatment	GP (%)	GC	T50 (days)	MGT (days)	GI
Eksotika	70^{a}	2.28^{a}	10.71^{b}	10.93^{b}	29.32^{a}
P13	58^{b}	1.38^{b}	9.69^{c}	10.25^{c}	28.98^{a}
P90	47^{bc}	0.90^{bc}	10.70^{b}	11.21^{b}	18.16^{b}
P248	39^{c}	0.63^{c}	11.86^{a}	12.52^{a}	10.30^{c}

Means with the same letter in the column do not differ at $p<0.05$ based on Duncan's multiple range test (DMRT). GP = germination percentage, GC = germination capacity, T50 = time to 50% germination, MGT = mean germination time, GI = germination index.

Seed vigour

GI is calculated to describe seed vigour. GI is an adaptation of the daily counting method to evaluate seedling vigour which is represented by seed lot vigour more precisely. GI predicts the relative vigour of samples with the same number of seeds. High value of GI signifies higher seedling vigour of one sample in relation to another seed sample (Ranal and Santana, 2006). The values of GI for Eksotika and P13 were statistically not significant compared to P90 and P248 (Table 2). P13 had lower GP but faster MGT and T50 when compared to Eksotika. Since GI was calculated based on relation between number of daily seed germination and day of germination; which is, in this case, related to speed of germination, therefore GP and MGT played important roles affecting value of GI. Thus, insignificant values of GI for Eksotika and P13 were due to high value of GP for Eksotika and high value of MGT for P13.

The lowest seedling FW was recorded for P248 followed by Eksotika, P13, and P90 while the lowest seedling DW was recorded for P13 followed by P248, Eksotika, and P90 (Table 2). The highest ratio of DW/FW was observed on P248 and the lowest was on P13. The evaluation of FW is associated with the ability of the genotype to accumulate water in a given condition (Macedo et al., 2013). The lowest value of DW/FW on P13 may be related to its ability to accumulate high water content during seedling germination.

Table 2: Papaya seedling FW and DW of selected lines for tolerance to PRSV disease.

Treatment	Seedling FW (mg)	Seedling DW (mg)	Ratio DW/FW (%)
Eksotika	94^{bc}	6.5^{b}	6.86 ^{b}
P13	101^{b}	6.2^{b}	6.10 ^{c}
P90	117^{a}	7.5^{a}	6.38 ^{b}
P248	90^{c}	6.3	7.02^{a}

Means with the same letter in the column do not differ at p<0.05 based on Duncan's multiple range test (DMRT). FW = fresh weight, DW = dry weight.

Conclusions

Eksotika papaya produced the best physiological quality of papaya seed based on its germination rate and seed vigour followed by P13, P90, and P248. Within selected lines of papaya with tolerance to PRSV disease, P13 showed the best seed quality. Further study on self-pollination method of geitonogamy, instead of autogamy, may enhance seed germinability of all selected lines.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Seed Germination of an Urban Tree, *Xanthostemon chrysanthus* (F. Muell.) Benth., in Different Sowing Media

Ahmad Nazarudin, M.R.[1,*], Nur'Ain Syafiqah, N.Z.[2], Siti Salwana, H.[3], Rosfarizal, K.[1] and Abdul Razak, S.[3]

[1]Forestry and Environment Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
[2]Faculty of Agriculture, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia.
[3]Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: nazarudin@frim.gov.my*

Introduction

Xanthostemon chrysanthus (F. Muell.) Benth. or golden penda belongs to the family of Myrtaceae. Locally known as jambu kuning, this species is native to tropical northern Australia, New Caledonia, New Guinea, Indonesia, and the Philippines. Jambu kuning is a medium-sized rainforest tree that grows up to 20 m in height in its natural habitat but usually smaller in cultivation (ANPSA, 2020; Figure 1A). The lance-shaped leaves are glossy green, approximately 15 cm in length and 5cm in width (Ahmad Nazarudin, 2016). The bright yellow inflorescence occurs at the ends of the branches and measures about 8-10 cm in diameter. The stamens are the principal feature that creates uniqueness of the bloom (Ahmad Nazarudin et al., 2012). The blooms also attract nectar-feeding birds and insects. In landscaping, *X. chrysanthus* can be used as screening plant, windbreaker, and specimen plant. This species becomes a prime candidate to beautify parks, roadsides, pocket spaces around buildings, golf courses, and house lawns. Jambu kuning is considered as one of the good options for urban landscape because it is a hardy species, growing well in full sun, tolerates acidic soil, and resists pollution and tropical heat (Anonymous, 2010). Previous investigation carried out in selected locations of Kuala Lumpur showed that this species was able to tolerate the improper condition of urban soil with high penetration resistance of 2.911-2.954 MPa and a low cation exchange capacity of 3.40-3.67 $cmolkg^{-1}$ (Ahmad Nazarudin et al., 2014).

Previous phenological study by using BBCH-Scale (Biologishe Bundesanstalt, Bundessortenamt and Chemical Industry) demonstrated that the flowering stage of this species was 40 days starting from inflorescence bud swelling until senescence, offering its unique landscape feature (Ahmad Nazarudin et al., 2018) (Figure 1B). A single fruit of *X. chrysanthus* is about 10-12 mm in diameter, spherically shaped and woody, containing 50-100 tiny seeds of approximately 3 mm each (Figure 1C). The inedible fruits are green at young stage and turn into dark brown when mature. The fruits require 2 to 3 months to reach full maturity stage (Ahmad Nazarudin et al., 2018). It was estimated that 1 kg of *X. chrysanthus* seeds is equivalent to 960,000 dry seeds (Sosef et al., 1998). Although the seeds are produced abundantly, there is no scientific evidence on the germination potential of the species. Thus, an experiment was carried out to assess the germination ability of the seeds in six different media under nursery condition. The best sowing media for germinating the seeds was also recommended.

Figure 1: *Xanthostemon chrysanthus* tree (A), inflorescence (B) and mature fruits with seeds (C).

Materials and Methods

The experiment was carried out in the nursery of Forest Research Institute Malaysia (FRIM), Kepong, Selangor. Mature fruits of *X. chrysanthus* were collected from existing trees grown at Metropolitan Batu Park, Kuala Lumpur (3° 12' N, 101° 40' E). The fruits were air-dried under room temperature prior to seed selection. Floatation method was used to test for viable seeds before sowing (Omokhua et al., 2015). The seeds of *X. chrysanthus* were soaked in a bowl of tap water for approximately 30 min. Seeds that floated were regarded as not viable and therefore discarded, while the ones that sank were used for experimentation. The chosen seeds were then placed into germination trays containing six different media as in Table 1.

Table 1: Different sowing media used for the experiment.

ID	Sowing media
M1:	100% top soil
M2:	100% sand
M3:	Mixture of top soil and sand (1:1)
M4:	Mixture of top soil and sand (1:2)
M5:	Mixture of top soil and sand (2:1)
M6:	Mixture of top soil, compost and sand (3:2:1)

One hundred seeds were sown into each germination tray. In total, 600 seeds were used in the experiment. The seeds were watered twice daily, i.e. in the morning and late afternoon. Growth performance of the seeds in terms of days to germinate, germination percentage (%), and seedling height (cm) were assessed daily. A seed was considered to have germinated when the shoot had emerged above the sowing media. The experiment was discontinued and considered completed at 50 days after sowing as there was no additional germination. Germination percentage was determined by using the following equation:

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Statistical analysis

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

Results and Discussion

Our observation found that *X. chrysanthus* seeds started to germinate at 11 days after sowing, regardless of the sowing media (Figure 2). The germination percentages were vigorously increased from day 11 to day 18 after sowing and then reduced gradually with time. At 18 days after sowing, the highest number of germinated seeds was recorded in M5, 84.8% in difference as compared to the lowest number of

germinated seeds observed in M2. Percentages of the germination of seeds in all media were relatively consistent from day 19 after sowing towards the end of the experiment.

At 50 days after sowing, the highest germination of 52% was recorded in M5, while only 9% germination was achieved in M2. M5 which was a mixture of top soil and sand (2:1) had a smaller amount of sand, less porosity, and higher moisture content as compared to M2 (100% sand). The moisture content in sowing media is one of the factors promoting seed germination. Seed germination starts with the uptake of water by the quiescent dry seed followed by the elongation of the embryonic axis (Bewley, 1997). This usually culminates in the rupture of the seed covering layers and emergence of the radicle, generally considered as the completion of germination (Finch-Savage and Leubner-Metzger, 2006).

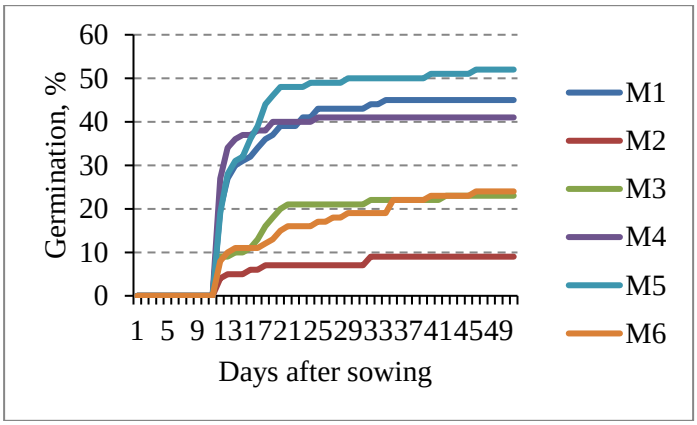

Figure 2: Germination percentage of *X. chrysanthus* seeds in different sowing media.

In terms of seedling height, seeds sown in M5 showed a better growth performance as compared to those in other media (Table 2; Figure 3). The highest seedling height was recorded in M5 while the lowest seedling height was observed in M2. At 50 days after sowing, seedling height in M5 was 38.8% higher than seedling height in M2 (Table 2). This result revealed that M5 is more suitable for growing *X. chrysanthus* seedlings as compared to 100% sand and other media.

Table 2: Height of *X. chrysanthus* seedlings in different sowing media at 50 days after sowing.

Sowing media	Seedling height (cm)
M1	0.82^{c}
M2	0.71^{d}
M3	0.99^{b}
M4	0.80^{c}
M5	1.16^{a}
M6	0.77^{c}

Means followed by the same letter within the same column are not significantly different by DMRT ($P<0.05$).

Figure 3: Seedlings of *X. chrysanthus* in different sowing media.

Conclusions

In conclusion, the seeds of *X. chrysanthus* were able to germinate at day 11 after sowing. Mixture of top soil and sand (2:1) (M5) was the best sowing media to promote germination of *X. chrysanthus* seeds. M5 also resulted in better growth of *X. chrysanthus* seedlings than other sowing media. Hence, this study recommends a mixture of top soil and sand (2:1) as the best sowing media for *X. chrysanthus* seeds.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Cellular, Structural and Physiological Differences Determine Seed Germinability and Storability

Tsan, F.Y.* and Mohamad Azril, H.
Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Jasin Campus, 77300 Merlimau, Malacca, Malaysia.
**E-mail: tsanfuiying@uitm.edu.my*

Introduction

Many plants depend on seeds for propagation in nature. Seeds that contain biparental combined genomes are important for adaptations and population growth, which are continuously threatened by extreme climatic episodes and human intervention in the agricultural development, urbanization, and industrialization progresses (Walck et al., 2011; Mezquida and Benkman, 2014; Arieira et al., 2016; Caughlin et al., 2016; Medeiros et al., 2018). Thus, seeds vary among species within and cross the ecological zones on the Earth. This paper is a brief review on the cellular, structural, and physiological differences in seeds. These attributes have widely been reported to affect the survival, germinability and storability of the seeds in ensuring biodiversity and ecosystem functioning.

Types of seeds

Seeds are produced by both angiosperms or flowering plants, and gymnosperms, the non-flowering plants. Angiosperms produce enclosed seeds as the seeds are protected in fruits. Some fruits are hard while others are fleshy or have both hard and fleshy tissues. Gymnosperms or non-flowering plants, for example the conifers, produce naked seeds. These seeds are developed on the bracts of cones, and just covered by the cone scales. Notwithstanding the differences in seed production, a seed generally consists of seed coat, embryo, and stored food.

Seeds of angiosperms

Angiosperms are divided into monocots and dicots. Most monocotyledous seeds are the grains. A grain denotes one having unseparated fruit and seed. There are two major types of grain, namely the rice grain and the maize grain. The rice grain is different from maize grain because it has structures called palea and lemmas. Palea and lemmas are bracts that make up the rice hull or rice husk. It is formed from hard materials of silica and lignin. Beneath the husk, there is a thin coat, of which the seed coat is fused to the fruit pericarp. It is also called bran. In a maize grain, seed coat and fruit pericarp are also fused to form the outer pale yellowish coat. In both maize and rice grains, the large portion of the grain is endosperm or stored food. The embryo is tiny. Its radicle is protected by coleorhiza whilst coleoptile protects the plumule. Scutellum is a single cotyledon. It is between the embryo and the endosperm. It absorbs food from the endosperm for the embryo to use during germination.

The other type of monocotyledous seed is the palm seed. It is usually enclosed in drupe type fruit with clearly seen exocarp, mesocarp and hard endocarp or the shell. Beneath the endocarp, there is thin testa or integument. The seed is dependent on the endocarp for protection. Therefore, the palm seed is also called a nut. The endosperm is stored food. In coconut, both the meat and milk are endosperm. The embryo is usually found near the end with fruit stalk at a structure called operculum. Operculum is made up of testa, thin layer of endosperm and cemented fibers.

For the dicots, the seeds are generally divided into exalbuminous and albuminous types. The exalbuminous seed is lacking endosperm, for example seed of garden pea. The stored food is the two fleshy cotyledons connected to the embryonic axis with radicle and plumule. The albuminous seed, for example the castor bean or rubber seed, has two thin cotyledons and the stored food is endosperm. It also has a structure called the caruncle at one end of the testa.

Seeds of gymnosperms

Gymnosperms do not have ovaries. The seeds produced are covered by the cone bracts in the cones. Gymnosperm seed is different from the angiosperm seed because the stored food is originated from the female gametophyte tissue and is haploid.

Seeds with special cellular features

There is a type of seed called the Eugenoid seed. An Eugenoid seed consists of two fleshy and partly fused cotyledons with no visible embryonic axis (van Wyk and Botha, 1984). Regenerative tissues spread within the whole seed (Silva et al., 2005; Delgado et al., 2010; Teixeira and Barbedo, 2012; Amadar and Barbedo, 2015; Prataviera et al., 2015; Calvi et al., 2017a; 2017b). New plants can be formed from any part of the seed.

There are also apomitic seeds. These seeds are produced without male fertilization. Apomixis is documented in more than 120 angiosperm genera (Hojsgaard and Hörandl, 2019). Apomictic seeds will germinate plants that are genetically identical to their mother plants. For example, mangosteen seeds are not true seeds because they are formed from integuments. When procambium is formed in the seed, the seed will germinate a plant. Sometimes, there are two or more procambiums in a seed (Lim, 1984; Ramage et al., 2004; Joshi et al., 2006; Pangsuban et al., 2009; Mohd Noor et al., 2016). This will give rise to two or more seedlings. The other well cited example of apomixis is with *Citrus*. Many *Citrus* spp. are polyembryonic with some embryos within the same seed being clones to their mother plant (Martinson, 1972; Andrade-Rodríguez et al., 2004). The apomitic seeds are gaining importance in plant breeding and propagation nowadays.

Variation in Size

Seeds of different species are very different in size (Leishman et al., 2000). The smallest seeds are the dust-like orchid seeds. They can be as small as only a few µm in size and weigh in µg or even less than 1 µg. The seeds are generally lacking stored food. They depend on mycorrhiza for nutrition during germination and early growth of seedling.

The largest seed is the *coco de mer* or "double coconut palm", *Lodoicea maldivica*. Each seed can weigh more than 20 kg.

Many annual plants produce great quantities of small seeds. They are the r-selected species. Perennials and woody plants, especially the climax species, often have larger seeds with more energy reserves for germination and seedling growth. They also produce larger and more established seedlings after germination.

Germinability

Germination marks the transition of growth from the stage of dependence on the stored food to autotrophic which is capable to photosynthesize. Due to the differences mentioned above, seeds are different in germination mechanic. Some seeds germinate readily while other are dormant when they remain viable but do not germinate when given conditions favourable for germination to happen (Baskin and Baskin, 2004; Wang et al., 2012; Barcelos et al., 2013; Lan et al., 2018). Seed germination can be species specific. For example, some leguminous seeds germinate rapidly but other legumes have seeds with dormancy.

Physiology of seed germination

In the first phase of seed germination, biomechanical weakening of fruit coat and testa is needed to allow water uptake. In nature, microbial degradation of fruit pulp or pericarp allows seed germination.

However, some seeds have water-impermeable testa and sealed chalaza and micropyle. It is due to palisade layer(s) of lignified macrosclerids with water-repellent phenolic and suberin-like substances. These seeds are said to have physical dormancy. They are also called hard seeds. These seeds need some cultural practices to overcome the impermeable testa before water uptake and germination can take place.

The following phase of seed germination is a plateau phase. Carbohydrates are oxidized and respiration increases after water uptake by the seeds. Enzymes of proteases and peptidases have been found in abundance in many germinating seeds (Bareke, 2018). Protease and amylase inhibitors are reduced in this stage. In oil seed germination, lipids are degraded in the glyoxylate cycle. In this cycle, stored lipids are converted into glucose for respiration. Nonetheless, the stored food mobilization during seed germination is still not well understood and needs more research work.

In the last phase of seed germination process, there is more water uptake, followed by radicle protrusion, which is the end of germination. Subsequent seedling growth involves cell wall loosening, cell elongation and expansion in the lower hypocotyl, transition zone, and radicle.

Control of seed germination

In general, seed germination is under hormonal control (Shu et al., 2016). The major hormones involved are gibberellic acids (GAs) and abscisic acid (ABA), but ethylene, auxins, cytokinins (CKs), salicylic acid, jasmonic acid, and brassinosteroids are also playing important roles in controlling the onset of germination (Chandler and Werr, 2015).

In some seeds, the endosperm can be a mechanical barrier for radicle protrusion. Testa and endosperm removal allows seed germination, for example in Arabidopsis seeds. It is a type of physiological dormancy. GA from the embryo is required to induce endosperm weakening. This signal can be replaced by external application of GA. Light and cold can also act as signals to break dormancy of seeds after water uptake and to promote seed germination by increasing GA levels. Endosperm weakening is inhibited in dormant seeds due to the presence of ABA.

Reactive oxygen species (ROS) are produced when germinating seeds face abiotic stresses like drought or extreme temperature (Suzuki et al., 2012). Hydrogen peroxide (H_2O_2) is commonly induced during stress. ROS cause cellular damage like lipid peroxidation and DNA oxidation. This will bring about loss of seed vigor.

In relation to control of seed germination, seed priming is a recent technique aimed to control imbibition by lowering the external water potential, so that the seed turns partially hydrated and becomes pre-germinated but the radicle protrusion is pending (Raj and Raj, 2019). This seed treatment technique allows improved rate and uniformity of germination. The compounds used for treating the seeds in this technique also provide added advantage; they improve stress tolerance in the seed.

Storability

After the seeds are shed from mother trees, they can be stored as future planting materials with space saving benefit. They are also saved in seed banks away from challenging environment and as germplasm collection, but they must be dried for successful storage (Hong and Ellis, 1996).

Tolerance to desiccation in seeds is classified into three groups (Roberts, 1973). Recalcitrant seeds are desiccation sensitive. Seeds of many tropical fruits are recalcitrant seeds (Asomaning et al., 2011; Luna and Chamorro, 2016; Mayrinck et al., 2016; Barbedo, 2018; León-Lobos and Ellis, 2018). They cannot be stored with no impairment. Orthodox seeds like those of mung beans, paddy and many vegetables survive very low moisture contents (MC) and they can be stored well for long periods (Ellis and Roberts, 1980). Intermediate seeds tolerate slight desiccation and can be stored for certain periods depending on

species. Many palms produce intermediate seeds, but a recent report has indicated that Judean date palm seeds that have survived for 2,000 years could successfully germinate in year 2005 (National Geographic, 2021). There are always exceptions.

Morphological and structural factor

Past research has proposed characteristics of seeds with regards to desiccation sensitivity. Many large seeds are recalcitrant seeds but there are also small seeds that cannot tolerate desiccation well (Chong et al., 2007; Hill et al., 2012; Lan et al., 2014). Seeds in fleshy fruits like those in durian and mango are recalcitrant seeds but seeds in juicy watermelon, for example, are desiccation tolerant.

Ecological factor

By ecology, many seeds from rainforests are recalcitrant seeds but there are also many orthodox seeds in this wet region, especially those of the annual plants (Pritchard et al., 2004; Daws et al., 2005; Donohue et al., 2010; Joët et al., 2013; Jordano, 2016; Gentallan Jr et al., 2018). However, species originated from arid or savannah habitats for sure do not form desiccation sensitive seeds.

Physiological factor

Orthodox seed is different from recalcitrant seed because it undergoes maturation drying during the last phase of seed development before it is shed from the tree. Maturation drying causes reduced vacuole volume followed by metabolic shutdown and sometimes, intracellular de-differentiation. Such seed development through which water is lost has caused oxidative stress due to the imbalance between the amount of reactive oxygen species (ROS) and antioxidant enzymes but the sugars, cyclitols and proteins, for example the heat shock proteins (HSP) and late embryogenesis abundant (LEA) proteins, in the desiccating seed are able to act as a protective mechanism to prevent damage of the seed (Boucher et al., 2010; Chen et al., 2011; Delahaie et al., 2013). Soluble sugars in the orthodox seed may also form glass during desiccation, resulting in molecular stabilization that can avoid damage.

Unlike orthodox seed, recalcitrant seed does not undergo maturation drying at the end of seed development process. It is shed at high MC. It remains metabolically active and therefore is sensitive to postharvest desiccation. The metabolites like sugars and proteins in recalcitrant seed also cannot act as a protective mechanism like what the orthodox seed experiences (Sahu et al., 2017).

Intermediate seed is a seed that is more tolerant to desiccation than recalcitrant seed but does not survive low temperature and extreme desiccation levels. It can only be stored for medium periods under certain conditions.

Future Work

Seeds as the primary propagation units for many plant species regulate the ecological stability whilst they are also of societal and economic importance. Studies and experimentations have continuously been carried out to better understand the differences among seeds, and the evolutions and adaptations in seeds for balanced community structure and subsequent ecosystem health.

Aknowledgement

The work is a part of the research funded by Universiti Teknologi MARA [500-BPD(BKK.14/5/4)(188427)].

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Chapter 4:
Postharvest Technology and Quality Control

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Paclobutrazol (PBZ) Application Induction on Vegetative, Reproductive Growth and Fruit Quality of Harumanis Mango under Greenhouse Conditions

Zul Helmey, M.S.[1,*], Muhamad Hafiz, M.H.[1], Wan Mahfuzah, W.I.[1], Shaidatul Azdawiyah, A. T.[2], Mohd Aziz, R.[2], Mohd Farid, A.F.[1], Muhamad Izzat, Z.[1], Siti Aishah, H.[1] and Siti Afiqah, J.[3]

[1]Horticulture Research Centre, MARDI Sintok, 06050, Bukit Kayu Hitam, Kedah, Malaysia.
[2]Agrobiodiversity and Environment Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400, Serdang, Selangor, Malaysia.
[3]Department of Crop Science, Faculty of Agriculture, 43400, UPM Serdang, Selangor, Malaysia.
**E-mail: zulhelmey@mardi.gov.my*

Introduction

Harumanis mango (*Mangifera indica*) is a mango variety that is economically important and classified as one of the sought-after mango variety in Malaysia (Farook et al., 2013). As recorded in 2019, there were about 6,373 hectares of mango cultivation in Malaysia with 15,766 metric tons production (Department of Agriculture, 2019). The demand for Harumanis mango is increasing yearly due to the exquisite taste and aroma of the fruit (Khalid et al., 2017). However, the plant is only cultivated in the northern region of Peninsular Malaysia such as in Perlis and some part of Kedah (Muhammad Hafiz et al., 2019; Rosidah et al., 2010). This area is located in zone 1 of Malaysia Agro-climatic zoning, characterized with significant drought during January to March followed by vagaries rainfall in the interim months to heavy rain in September to December (Mahmad Nor et al., 2014; Mohd Aziz et al., 2019). Mango is one of the recommended crops suitable for this agro-climatic zone. Furthermore, mango tree is categorized as a seasonal plant with fruit production that is limited once a year around May to June (Farook et al., 2012; Muhammad Hafiz et al., 2019). Thus, the fruit production does not fulfil the local market demand and the price has increased.

Mango flowering is an important physiological event that sets the start of fruit production. However, the knowledge about the requirements of mango for flowering and fruiting are questionable since both stages are highly influenced by the weather conditions. In natural, mango flowering occurs when the environment is drought and physiological of trees in the stress conditions (Muhammad Hafiz et al., 2019). These will inhibit the vegetative growth by inhibiting the gibberellin content in the mango trees. When gibberellin production is inhibited, the physiological trees will response to the reproductive system for the flowering. Thus, flower induction is necessary to increase the flowering and fruit production especially for seasonal or biennial bearing fruit crops. Traditionally, flower induction of mango can be done through mechanically by chop or girdling of the tree barks. Today, flower induction through chemical using plant growth regulator such as Paclobutrazol (PBZ) is more efficient and successful (Gollagi et al., 2019). Paclobutrazol has been commercially used throughout the tropics to control vegetative growth and stimulate flowering for several fruit crops such as apple (Zhu et al., 2004), pummelo (Phadung et al., 2011), apricot (Arzani and Roosta, 2004), and mango (Singh and Ram, 2000; Yaowara at el., 2017). In the recent years, many reports showed that application of PBZ by soil drenching or foliar spray to mango trees can cause reduction of vegetative growths, inhibits gibberellin biosynthesis, and induces water-stress tolerance as well as increases total non-structural carbohydrates (TNC) to induce flowering (Subhadrabandhu et al., 1997; Suranant et al., 1999; Gollagi et al., 2019). The application of flower induction is one of the key factors that may influence the growth, yield, and the quality of the mango trees. Therefore, this study was demonstrated with the objective to determine the application induction and rate of PBZ to induced vegetative, reproductive growth, and fruit quality of Harumanis mango in greenhouse conditions.

Materials and Methods

Study site and experimental design

The experiment was carried out in greenhouse at MARDI Sintok, Kedah that were planted with 54 grafted Harumanis mango started in November 2017 with planting distance 3 m x 3 m. All 54 trees were managed using proper and standard agronomic practices including fertilization, irrigation, pruning, and thinning, and pest and disease control. The type of soil at the experimental area is clay soil. The selected soil samples of the experimental area were analysed at the laboratory to identify the soil pH and moisture content. The soil pH was 5.63 and 6.30 at a depth of 0-15 cm and 15-30 cm, respectively. The soil moisture content was 17.90% and 19.38% at a depth of 0-15 cm and 15-30 cm, respectively. Portable weather station (WatchDog 2000 series) was installed in the greenhouse to record and monitor daily weather such as temperature (°C) and relative humidity (%) data for every hour started from the vegetative stage until the reproductive stage. The environmental condition of the greenhouse was extreme compared to the outside (difference temperature 2-3 °C and 8-10% relative humidity). Forty-eight trees of Harumanis mango trees were selected based on the same age and uniformity of growth for conducting the experiment. All the selected trees were divided and arranged with the experimental layout using Completely Randomized Design (CRD) with four treatments and three replications. Each replication consisted of four plants. The application induction treatments were applied using Paclobutrazol (PBZ) and it started when the age of the trees reached 22 months after planting. Before treatment was applied, the maturity of shoots was verified. The treatments of PBZ were as follows: T1- No Induction (control), T2- (soil drenching-2 mL/L PBZ), T3-(foliar spray- 1 mL/L PBZ) and T4- (soil drenching-2 mL/L PBZ) + (foliar spray-1 mL/L PBZ). The irrigation was stopped 1 week after the treatments were applied.

Measurement of vegetative growth

The measurement of vegetative growth started after 60 days of PBZ application induction to determine the plant's height, stem diameter, canopy size, internode length, number of shoots, and number of leaf. Measurement of plant height was taken from the surface of the soil 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 SCM DIGV-6) while canopy size and internode length was measured using a measuring tape. The number of shoots and leaf was manually counted based on fully expanded shoots and leaves. Chlorophyll content of leaf was measured using SPAD meter (Konica Minolta SPAD-502Plus).

Measurement of reproductive growth and fruit quality

The measurement of the reproductive growth started during the flowering stage until the fruiting stage. The data collected were percentage of inflorescence trees (%), time to inflorescence (days), number of panicles per tree, length of panicle (mm), and number of fruits set per panicle. The fruits were harvested after 12 weeks of fruit set for a quality assessment. Four fruits for each plant were selected and labelled for quality assessment. The harvested fruits were washed using water and soaked for 10 min in 0.2% Benomyl fungicide to prevent postharvest diseases. Then, it was dried in 25 °C and all the fruits were ripen using calcium carbide for 72 hours before being transferred to the laboratory for quality assessment. The fruits were recorded as fruit weight (g), skin weight (g), seed weight (g), and pulp weight (g) using analytical balance (AND ER 180A) while length (mm) and width of fruit (mm) were measured using digital caliper (MITUTOYO CD67). Total soluble solid (TSS) was measured using a digital handheld refractometer (ATAGO CO. LTD PAL-α) while the pH was taken using pH meter (HANNA Instrument HI2211). Total titratable acidity (TTA) content was measured by titrating 20 mL extract from sample with 0.1 M 1-1NaOH until it reached pH 8.2 while for ascorbic acid (vitamin C), 10 mL extract from 10 g and 100 mL 3% metaphosphoric acid were titrated with standard dye until the extract turn into faint pink.

Statistical analysis

The data obtained was analysed using ANOVA in SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Duncan Multiple Range Test Difference (DMRT) at P≤0.05%.

Results and Discussion

Measurement of vegetative growth

Table 1 below shows seven parameters evaluated on vegetative growth of Harumanis mango at 60 days after PBZ application induction. Vegetative growth response to PBZ was different between the treatments application. For parameter measured as height, T4 was significantly reduced growth compared to T1, T2, and T3, respectively. For internode length, T4 showed significantly reduced growth compared to T1, T2, and T3, respectively. However, there were no significant differences between each treatment for stem diameter, canopy size, chlorophyll content, and number of leaves. PBZ application induction significantly reduced number of shoots in treatment T4 compared to T1, T2, and T3, respectively. These results indicated that the height, internode length, and number of shoots reduction were affected by PBZ with combination applications (soil drenching + foliar spray) than with single application induction only. According to Gollagi et al. (2019), PBZ application induction inhibits gibberellins biosynthesis, which inhibits cell elongation, shoots growth and internode extension and ultimately retards plant growth. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate. That will result in reduced production of shoots, stunting of height, and internodes compressed into a shorter length.

Table 1: Effect of different PBZ application induction on vegetative growth of Harumanis under greenhouse conditions.

Treatment	Height (m)	Stem diameter (mm)	Canopy size (m)	Internode length (cm)	Chlorophyll content	Number of leaves	Number of shoots
T1 – No induction (control)	1.92^{b}	47.66^{a}	2.72^{a}	28.44^{a}	53.14^{a}	76.83^{a}	81.92^{a}
T2 – Soil drenching	1.89^{b}	45.21^{a}	2.57^{a}	26.83^{b}	49.46^{a}	74.22^{a}	63.67^{b}
T3 – Foliar spray	1.96^{b}	47.91^{a}	2.96^{a}	26.11^{b}	50.60^{a}	71.53^{a}	66.75^{b}
T4 – Soil drenching + Foliar spray	1.62^{a}	48.60^{a}	2.62^{a}	23.32^{ab}	52.82^{a}	70.97^{a}	51.67^{ab}

***Different letters in the same column indicate significant differences (P≤0.05) according to DMRT.*

Measurement of reproductive growth

In terms of reproductive growth measurements, five parameters were taken: percentage of inflorescence (%), time to inflorescence (days), number of panicles, length of panicle (mm), and number of fruits set per panicle after PBZ application induction. The percentage of inflorescence of Harumanis from Table 2 indicated that T2 produced highest percentage of inflorescence in case of soil drenching and significantly different compared to T3 and T4, respectively. However, T3 produced the lowest percentage of inflorescence in case of foliar spray compared to T2 and T4 respectively. No response of flowering was observed in treatment T1 (control). There were similar studies found by Suranant et al. (1999), the research conducted on Nam Dok Mai mango trees in Thailand where, the use of soil drenching produced the highest percentage of flowering compared to foliar spray. Application of PBZ in the soil has been commercialized for early and effectively enhanced flowering in some other fruit crops such as apple and grape (Gollagi et al., 2019). For time to inflorescence, treatment T4 produced the shortest period and significantly different to response for flowering compared to T2 and T3,

respectively. In term of number of panicles, treatment T2 produced the highest number panicles but not significantly different with T4. However, treatment T3 produced the lowest number of panicles and significantly different compared to T2 and T4, respectively. Application induction of PBZ also affected the length of panicles in the Harumanis mango. Treatment T4 produced the shorter length of panicles and compacted flowers compared to T2 and T3, respectively. Combination applications (soil drenching-2 mL/L + foliar spray-1 mL/L) of PBZ may cause overdose concentration of PBZ in the mango trees (Figure 1b-c). These might result in undesirable effects such as stunting of panicle and flower malformation. The stunting of panicle and flower malformation that can reduce the number of fruits set formation (Yaowarat et al., 2017). The number of fruits set in mango tree can be determined by counting the number of fruits in the inflorescence. In this experiment, the number of fruits set per panicle was highest and significantly different in T2 compared to T3 and T4, respectively.

Table 2: Effect of different PBZ application induction on reproductive stages of Harumanis under greenhouse conditions.

Treatment	Percentage of inflorescence (%)	Time to inflorescence (Days)	Number of panicles	Length of panicle (mm)	Number of fruits set per panicle
T1 – No induction (control)	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}	0.00^{c}
T2 – Drenching	75.00^{a}	108.00^{a}	10.33^{a}	308.20^{a}	8.54^{b}
T3 – Foliar spray	43.33^{b}	112.00^{a}	4.00^{b}	296.34^{a}	5.17^{a}
T4 – Drenching + Foliar spray	58.33^{b}	88.00^{b}	9.08^{a}	186.00^{b}	6.83^{a}

**Different letters in the same column indicate significant differences ($p \leq 0.05$) according to DMRT.*

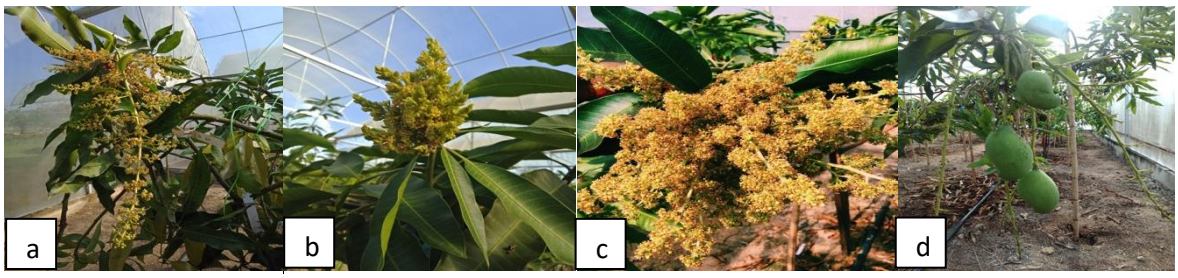

Figure 1: (a) The longest panicle of inflorescence produced in T2, (b)-(c) shorter and compact flower produced in T4 and (d) fruits set produced in T2 after application of PBZ.

Measurement of fruit quality

Fruit quality assessment was divided into two main sub-parameters as physical and chemical assessments. For physical fruits assessment, parameters such as fruit weight (g), fruit length (mm), fruit width (mm), skin weight (g), seed weight (g), and pulp weight (g) were measured. The physical appearance of Harumanis fruit from different treatments of PBZ application during fruit quality assessment is shown in Figure 2. In terms of fruit weight assessment from Table 3, T2 gives the highest value and significantly different compared to T3 and T4, respectively. The trend was also the same with fruit width where T2 gave the highest value and significantly different compared to T3 and T4, respectively. A similar result was observed by Jindal and Chandel (1996) on 'Santa Rosa' plum (*Prunus saliciana*) in which the soil drenching application of PBZ at 1 mL/L to 2 mL/L produced maximum fruit weight and volume of fruits. However, for the fruit length, skin weight, seed weight, and pulp weight there were no significant differences recorded in this study. Furthermore, for fruit quality assessment as total soluble solids in Table 4, T2 gave the highest value and was significantly different compared to T3 and T4, respectively. In terms of total titratable acidity and ascorbic acid content, T2 also gave the highest value and was significantly different compared to T3 and T4. These results showed that application of PBZ by soil drenching significantly increased the yield and fruit quality of Harumanis mango compared to other treatments. There were similar studies conducted on Dashehari mango where,

the use of PBZ by soil drenching produced the highest fruit yield, high total soluble sugar (TSS), and total titratable acidity (TTA) compared to foliar application (Sing et al., 2005). A significant improvement in the fruit quality of mango cv. Langra, in terms of total soluble solids (TSS), total titratable acidity (TTA), and ascorbic acid content in comparison to foliar spray and control, was reported by Singh and Saini (2001). For the pH values, no significant differences were recorded for all the treatments in this study.

Table 3: Effect of different PBZ application induction on physical fruit assessment of Harumanis mango under greenhouse conditions.

Treatment	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Skin weight (g)	Seed weight (g)	Pulp weight (g)
T1 – No induction (control)	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{b}
T2 – Drenching	416.00^{b}	125.00^{a}	86.00^{a}	52.50^{a}	32.00^{a}	305.50^{a}
T3 – Foliar spray	295.50^{a}	108.50^{a}	69.50^{b}	48.50^{a}	29.50^{a}	224.00^{a}
T4 – Drenching + Foliar spray	344.08^{a}	120.20^{a}	72.15^{b}	45.41^{a}	27.63^{a}	253.89^{a}

**Different letters in the same column indicate significant differences (P ≤ 0.05) according to DMRT.*

Table 4: Effect of different PBZ application induction on chemical fruit assessment of Harumanis mango under greenhouse conditions.

Treatment	Total soluble solid (TSS) ^{o}Brix	pH	Total titratable acidity (TTA)	Ascorbic acid (Vitamin C)
T1 – No induction (control)	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}
T2 – Drenching	18.93^{b}	5.56^{a}	3.52^{b}	0.18^{b}
T3 – Foliar spray	16.78^{a}	5.04^{a}	1.40^{a}	0.11^{a}
T4 – Drenching + Foliar spray	16.91^{a}	6.28^{a}	1.97^{a}	0.10^{a}

**Different letters in the same column indicate significant differences (P≤0.05) according to DMRT.*

Figure 2: The appearance of Harumanis fruit from different treatments of PBZ application during fruit quality assessment as T2 (a), T3 (b), and T4 (c).

Conclusions

Based on the results, the combination application of PBZ by soil drenching plus foliar spray was effective to reduce vegetative growth while application of PBZ by soil drenching only was most effective in producing higher percentage of flowering and improvement in the fruit quality.

Acknowledgements

This research work was supported by the Malaysian Government under 11th Development Fund Project (P-RH405). The authors would like to acknowledge their deepest thank to MARDI and to the team members for giving generous support to this research.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Nanofertilizer-nitrogen, Phosphorus, and Potassium (NPK) Uptake by *Carica papaya* var. Sekaki Seedlings

Khalisanni, K.*, Muhammad Najib O.G., Noorhapizah, N. and Hartinee, A.
Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: sanni@mardi.gov.my*

Introduction

Supply of eleven fruits in 2018 was sufficient to fulfill the local demands with self-sufficiency rate (SSR) of more than 100 per cent. Watermelon recorded the highest SSR (168.1%) followed by papaya (150.2%) and starfruit (146.2%). It is important to us to ensure the continuity of sufficient SSR especially for papaya driven by local and international market demands (DOSM, 2018).

Papaya, which belongs to family *Caricaceae*, is a large perennial plant with rapid growth and categorized as one of climacteric fruit. The '*Sekaki*' cultivar is one of the Malaysian's favourite commodities with a great tropical taste. Papaya is a plant that continuously absorbs large amounts of nutrients especially during its seedling stage in the first year (Silva et al., 2016).

Despite the nutritional requirements, it is necessity to provide the optimized quantity of fertilizer to promote maximum plant growth rate through slow release- or targeted release-nanofertilizer. In this present study, the effects of colloidal nanofertilizers on nutritional uptake of nitrogen, phosphorous and potassium (NPK) has been studied to determine its efficiency compared to conventional fertilizer.

Materials and Methods

Papaya (*Carica papaya* var. Sekaki) seeds were sown in polybag contains the mixture of topsoil, rice husk and cocopeat to allow the germination of the seeds. After 1 month of growth, the seedlings were transferred and monitored in the shaded area for 8 weeks with the treatment of nanofertilizers. Five types of colloidal nanofertilizer namely trade secret registered nanofertilizer T1, T2, T3, commercial Khazra nanofertilizer (T4) and commercial NPK 15:15:15 (T5) were sprayed for every two weeks interval. The experiment was designed in Randomised Complete Block Design (RCBD) with four replications.

Established protocol was carried out to determine NPK content (Pequerul et al., 1993). Leaves were collected from all the five treatments of potted plants for every 2 weeks of growth after treated with the nanofertilizers. The leaves in an oven were air-dried for 24 hours at 70 °C. For all treatments, acid digestion has been completed with 100 mg of dried leaves. In a glass vial, 5 mL of HNO_3 was added to 100 mg of each dried sample and the mixture remained for 24 hours. On a hot plate, the acidified sample test was heated for 1 hour at 120 °C. The sample was then air-dried for 48 hours in an oven at 80 °C. The dried specimens were cooled and dissolved for 2 hours in 3 mL of 10% HCl (v/v). This solution was diluted with distilled water and analysed by Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) for P and K while N was quantified using an Elementar analyzer 6 (LACHART Instruments, Model Quickchem IC+FIA 8000 Series). Data were subjected to Analysis of Variance (ANOVA) using IBM SPSS Statistics for Macintosh, Version 25.0. The means were compared by the Tukey's Multiple Comparison Test.

Results and Discussion

In the present study, the top fully expanded leaf samples were harvested and washed with deionized water at 70 °C for 72 hours every 2 weeks after transplanting. The concentrations of nutrients were measured using the method described by Pequerul et al., 1993. Figures 1 to 3 show the effect of different treatments of nanofertilizers on selected macronutrients content in *C. papaya* leaf.

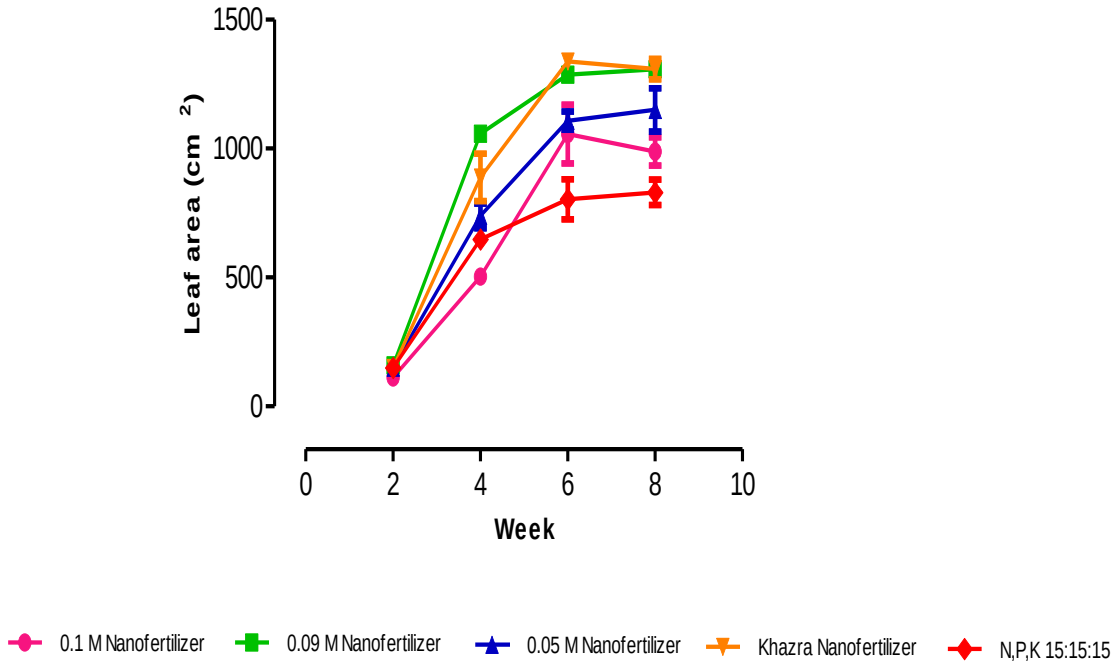

Figure 1: Effects of fertilizers on the concentration of nitrogen (N) in the leaf of *Carica papaya.*

Different nanofertilizer treatment did not have significant effects on the nutrient content of the *C. papaya* leaf. In contrast, the nutrient compositions reported in this study were found in healthy leaf tissue of different plant species in the normal range Awang et al. (2009). The concentration of total N ions in leaf was not significantly affected ($P>0.05$) by different types of treatment concentration measured (Figure 1). Treatment week from week 4 to 6 showed a constant total N and was declined on week 8 by reduction of 44.71- 42.88% respectively. This condition occurs during vegetative growth when there is inadequate nutrient supply in the soil; the matured leaf becomes sources for N to support the growth of new organs (Malagoli et al., 2005), that contributes to the reduction of total N in each treatment. From Figure 1, treatment with 0.09 M nanofertilizer showed a rapidly decreased amount of N at 42.29% compared to others. This suggested the treatment efficiency as stated by Avice and Etienne (2014), which indicates the remobilization of nutrients often associated with foliar senescence, allowing nutrients accessible to younger plant organs and contributing to the efficiency of nutrient usage.

The concentration of P increases after week 2 for all treatment of fertilizers with no significant differences between treatments (Figure 2). From the graph, the amount of P reached maximum and maintained until week 8 at 0.641-0.628 %. This result is similar to the finding of Supriya, (2013) on integrated nutrient management for papaya var. *Ranchi* dwarf. P is essential for enhancing seed maturity and seed development (Ziadi et al., 2008). P is required in the first 5-6 weeks after emergence of crop as a research conducted by (Elemike et al., 2019) proved about 75 % of P uptake by a cereals sp. in the early stages of crop production.

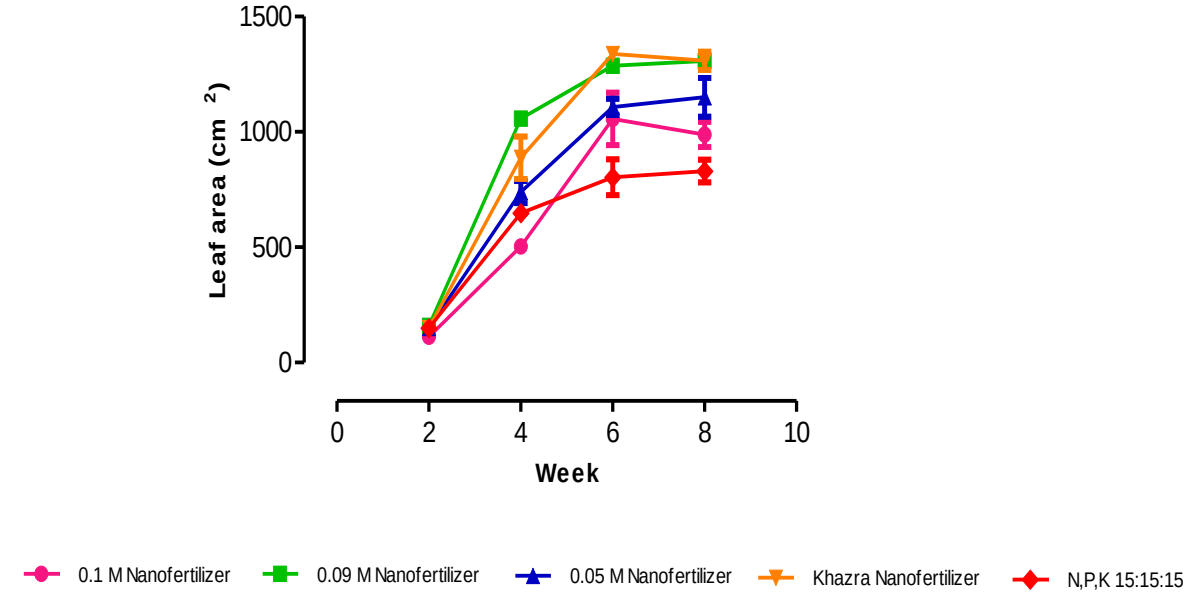

Figure 2: Effects of fertilizers on the concentration of phosphorus (P) in the leaf of *Carica papaya*.

Meanwhile, the content of K in papaya leaf at seedling stage show a continuous K uptake throughout 8 weeks of treatment, i.e. at a constant rate of all treatments with no significant different (Figure 3). Treatment with 0.1 M nanofertilizers contributes to 3.547% of K from week 2 to week 4. The study from India Institute of Horticultural Research concludes the same amount of K required for papaya at the vegetative growth stage. K function importantly after fertilization of flowers to provide higher levels of sugar and total soluble solids to produce bigger and better quality fruit. K is also of particular importance due to its active role in the plant's biochemical functions, such as the activation of different enzymes, protein production, carbohydrates and fat concentration, drought tolerance and frost resistance, lodging, pests, and disease assault (Gosavi et al., 2017).

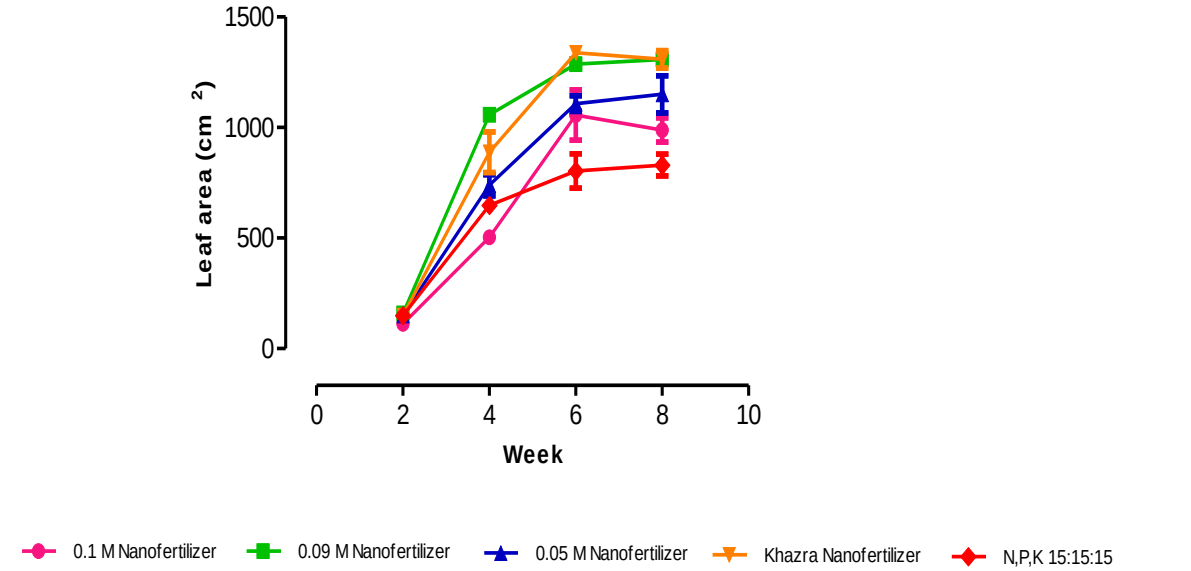

Figure 3: Effects of fertilizers on the concentration of potassium (K) in the leaf of *Carica papaya*.

Conclusion

The results showed that spraying colloidal NPK nanofertilizer at 0.09 and 0.10 M on plants resulted in the best nutritional uptake of N, P, K in leaves of *C. papaya*. Total N was declined on week 8 by reduction of 44.71-42.88 % respectively as the major source to support the growth of new organs. The amount of P reached a maximum and was maintained until week 8 at 0.641-0.628 % due to the seed development (Ziadi et al., 2008). The treatment of foliar nanofertilizers contributes to 3.547% of K from week 2 to week 4 due to its active role in the plant's biochemical functions. Therefore, the modified colloidal NPK nanofertilizers will be an exceptionally choice to promotes the growth of *C. papaya* at seedlings stage.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Performance of Different Forage Sorghum Varieties as Affected by Mechanised and Semi-mechanised Planting Density and Harvest Cycle in Mineral Soil

Muhammad Najib, O.G.[1,*], Nurhazwani, M.[1], Halimah, H.[1], Faizah Salvana, A.R.[1], Hanim, A.[1] and Thiyagu, D.[2]

[1]Industrial Crop Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400, Serdang, Selangor, Malaysia.
[2]Industrial Crop Research Centre, Kampung Aur, Mukim Jalan Kandis, 16310, Bachok, Kelantan, Malaysia.
**E-mail: najibo@mardi.gov.my*

Introduction

The productivity and availability of good quality feed and fodder are crucial for the development of livestock. Malaysia imports almost 100% of livestock feedstuff every year with import value of RM 7.01 billion (Sharif and Shanmugavelu, 2016). The low productivity of livestock is primarily due to insufficient fodder and feed resources. Forages are the mainstay of animal wealth and their production. The scarcity of green forages and grazing resources in the country has made the livestock to suffer continuously with malnutrition resulting in their production potentiality at sub optimum level as compared to many developed nations.

Sorghum (*Sorghum bicolor*) is an indigenous crop to Africa which belongs to the grass family, *Graminea* (Brink and Belay, 2016). It can be used to produce energy, fibre, or paper, as well as for syrup and animal feed (Steduto et al., 1997). It has great ability to produce high forage biomass yields (Rooney et al., 2007). Sorghum is ranked as the fifth most-cultivated cereal worldwide and is widely used in the production of grains, ethanol, and forage.

As forage, sorghum may be cut several times (multicut) because of its regrowth habit (ratoon). Sorghum is a new crop in Malaysia and still not well adapted to the local climate. Agronomic measures planting density and harvest cycle have not been tested under climatic conditions of Malaysia yet. These factors can affect biomass as well as chemical composition of sorghum. The optimum seedlings per hill ensure the plants to grow in their both aerial and underground parts through efficient utilization of light, water, and nutrients (Ahmad and Hasanuzzaman, 2012). Hence, it is dire need to optimize these various agronomic practices. A study was conducted in MARDI Serdang, Selangor to determine the effect of different planting density (for semi-mechanised and mechanised) and harvest cycle on growth and yield of selected sorghum varieties cultivated in mineral soil.

Materials and Methods

Plant materials

Four forage sorghum varieties (3 hybrids - Brown Mid Rib Rocket (BMR), Mega Sweet (MS), and Sugar Graze (SG); 1 inbred - SPV422) were used in this study. The hybrids were obtained from Advanta Seeds, India while the inbred was supplied by local company, Rimbun Group Sdn. Bhd. All seeds were kept in temporary storage at 5 °C prior to planting.

Land preparation and agronomic practices

Field experiments were carried out during January - July 2020 at the MARDI Headquarters (2° N, 101° E). The soil (Serdang series) is categorized as low-grade metamorphic mineral with characteristics of deep profiles having sandy loam to sandy clay loam textures (DOA, 2008). The experimental plots were ploughed, harrowed and the layout was demarcated using rope, pegs, and tape. Each experimental unit

has a dimension of 5 m x 5 m. Organic fertilizer was applied 7 days before planting at the rate of 3 tonnes ha^{-1}. Chemical fertilizer; 15:15:15 (N: P: K) and urea was applied after 14 days of planting at rate of 50 $kgha^{-1}$ each and after each cut with rate of 75 $kgha^{-1}$ each. The plots were irrigated daily using 6-feet sprinkler system. Weeds were controlled manually at three-week interval using a hoe.

Planting density treatments

The seeds from each variety were sown at a depth of 3-5 cm with four different spacing designed for semi-mechanised and mechanised planting systems; 45 x 10 cm, 45 x 20 cm, 75 x 9.3 cm, 75 x 11.3 cm to achieve 500, 250, 318, and 264 plants per plot equivalent to 222222, 111111, 143369, and 117994 plants per hectare, respectively.

Determination of plant growth and yield

Data pertaining to growth characters and green forage yields were recorded at 50 and 100 days after sowing. From each plot, randomly ten sorghum plants were selected. Measurement of plant height was taken from the surface of the soil to the highest leaf collar by using a measuring tape. Stem diameter was measured 15 cm from the lowest part of stem using an electronic digital caliper (Model SCM DIGV-6, Mitutoyo, Japan) while the leaf number was manually counted based on fully expanded leaves. Leaf length and leaf width were measured from the petiole to the tip and the mid-section of the fifth leaf, respectively using a measuring tape. All the plants in each experimental unit were cut 10-15 cm above the ground and the fresh weight was recorded and converted into tha^{-1}.

Experimental design and data analysis

Factorial experiment comprising of four planting density, four varieties and two harvest cycles were arranged in three replications split-plot design with varieties as the sub-plot. The data obtained was analysed using ANOVA in the SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatment means were compared using Tukey's Honest Significant Difference (HSD) at $P \leq 0.05\%$.

Results and Discussion

Growth and yield for semi-mechanised planting system

BMR variety had significantly ($P<0.05$) lower value for leaf width, leaf length and stem diameter compared to the others, regardless of harvest cycle and density. Results also showed that plants from first harvest had significantly more leaf number than second harvest regardless of variety and density (Table 1).

Significant interactions ($P<0.05$) between variety and harvesting cycles indicated that plant height and yield in each variety were highly influenced by harvesting cycle, regardless of planting density. At first harvest, MS and BMR were significantly taller compared to SG and SPV. However, at second harvest, no significant differences were recorded between height of the varieties (Figure 1A).

In terms of yield, at first harvest, MS had higher total yield as compared to SPV and SG. No significant differences were recorded between total yield of SG and SPV. However, at second harvest, no significant differences of total yield between the three hybrid varieties were recorded (Figure 1B). Total yield in each variety was also influenced by planting density, regardless of harvesting cycle. Densely planting density (45 x 10 cm) resulted in no significant difference of yield between the varieties. Less populated planting density (45 x 20 cm) however resulted in significantly lower yield of SG and SPV as compared to BMR and MS (Figure 2). The variation in various sorghum forage varieties plant height and yield may be attributed to the difference in genetic makeup of these cultivars. Different sorghum cultivars differed in plant height (Hussain et al., 2011).

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Table 1: Main and interaction effects of harvest cycle and variety on growth and yield for semi-mechanised planting density.

	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Stem diameter (mm)	Yield (t/ha)
Harvest cycle, H						
First	158.99^{a}	10.28^{a}	90.11^{a}	8.20^{a}	20.62^{a}	33.56^{a}
Second	156.53^{a}	7.89^{b}	81.62^{a}	6.01^{a}	14.44^{a}	24.92^{b}
Plant density, P.D						
45 x 10 cm	157.71^{a}	9.11^{a}	83.59^{a}	6.73^{a}	17.47^{a}	32.49^{a}
45 x 20 cm	157.80^{a}	9.06^{a}	88.14^{a}	7.48^{a}	17.59^{a}	25.99^{b}
Variety, V						
BMR	164.12^{ab}	9.07^{ab}	78.89^{b}	4.83^{b}	14.19^{c}	33.66^{a}
MS	179.47^{a}	9.24^{a}	91.02^{a}	7.42^{a}	17.18^{b}	36.14^{a}
SG	148.63^{cb}	9.73^{a}	82.88^{b}	8.09^{a}	19.87^{a}	25.32^{b}
SPV422	138.80^{c}	8.28^{b}	90.67^{a}	8.07^{a}	18.87^{ab}	21.83^{b}
Harvest cycle, H	ns	**	ns	ns	ns	**
Plant density, P.D	ns	ns	ns	ns	ns	**
Variety, V	**	*	**	**	**	**
H*P.D	*	ns	ns	ns	ns	ns
H*V	**	ns	ns	ns	ns	*
P.D*V	ns	ns	ns	ns	ns	*
H*P.D*V	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).*

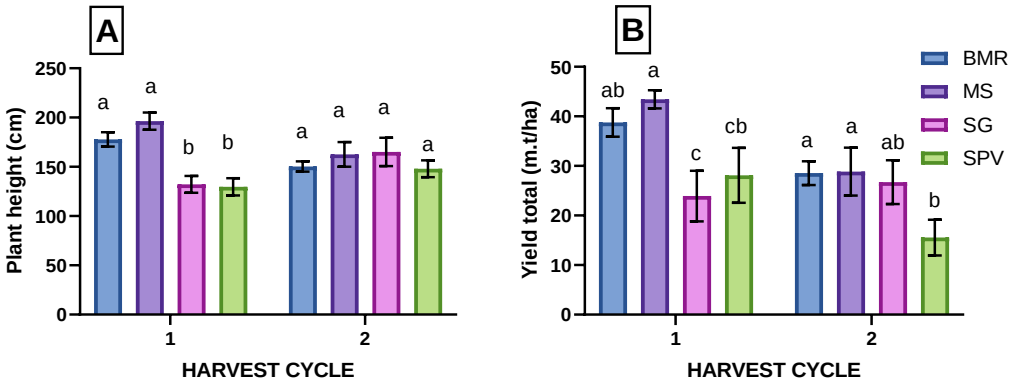

Figure 1: Plant height (A) and yield (B) of different sorghum varieties at different harvest cycle. Means in each graph with the different letters indicate significant differences at $P \leq 0.05\%$ level according to Tukey's HSD. (Mean ± S.E; n=3).

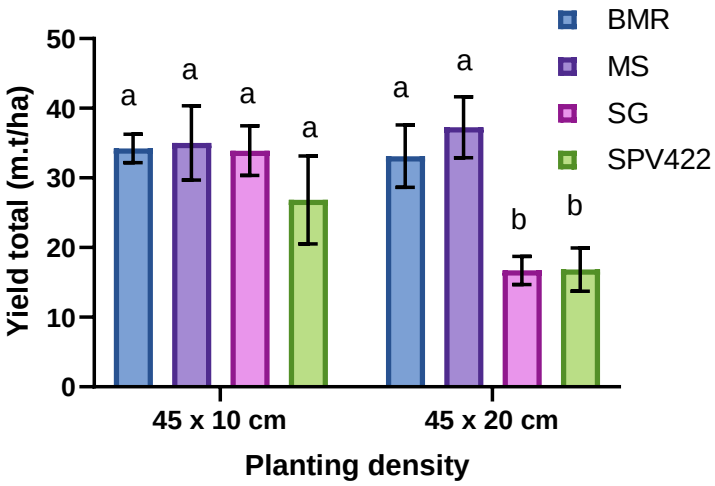

Figure 2: Yield of different sorghum varieties at different planting density. Means in each graph with the different letters indicate significant differences at $P \leq 0.05\%$ level according to Tukey's HSD. (Mean ± S.E; n=3).

Growth and yield for mechanised planting system

Results showed that BMR variety had significantly ($P<0.05$) shorter and smaller leaf with smaller stem diameter as compared to other varieties at any given harvest cycle and planting density. In terms of main factor harvest, first harvest had significantly ($P<0.05$) larger stem diameter and wider leaf compared to second harvest regardless of variety and planting density. Regardless of any harvesting cycle and variety used, densely planting density of 75 x 9.3 cm gave significantly higher yield (32% more) as compared to 75 x 11.3 cm (Table 2).

These results are in line with the findings of Ahmad et al. (2007) who reported that narrow planting patterns produced the highest yield and other related parameters as compared to wider planting. Furthermore, Ayub et al. (2010) also reported an increase in yield of var. Sorghum-211 as planting density increased.

Significant interactions between variety and harvesting cycle indicated that plant height, leaf number and yield in each variety was highly influenced by harvesting cycle, regardless of planting density. At first harvest, MS was significantly taller (216.2 cm) compared to others whereas at second, MS and SG were ranked the same while the others significantly shorter (Figure 3A).

For leaf number, MS had more leaf than others at first harvest but during second harvest, it has the lowest number of leaves (Figure 3B).

In terms of yield, MS had highest yield compared to others at first harvest, followed by BMR, SPV, and SG. However, at second harvest, no significant differences of total yield between BMR and MS were recorded (Figure 3C). Difference in yield of various cultivars of sorghum might be due to variation in seed viability, diversity in seed weight or genetic ability of these cultivars (Salahuddin et al., 2002).

Table 2: Main and interaction effects of harvest cycle and variety on growth and yield for mechanised planting density.

	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Stem diameter (mm)	Yield (t/ha)
Harvest cycle, H						
First	167.80[a]	9.93[a]	96.10[a]	8.21[a]	20.11[a]	32.71[a]
Second	158.31[a]	8.00[a]	85.39[a]	6.54[b]	13.98[b]	22.55[a]
Plant density, P.D						
75 x 9.3 cm	167.32[a]	9.27[a]	89.21[a]	7.33[a]	17.37[a]	31.47[a]
75 x 11.3 cm	158.79[a]	8.66[a]	92.28[a]	7.42a	16.72[a]	23.79[b]
Variety, V						
BMR	168.24[b]	9.41[a]	81.29[b]	4.88[b]	14.36[b]	33.79[a]
MS	193.46[a]	9.16[a]	94.42[a]	7.98[a]	16.91[a]	35.79[a]
SG	154.44[b]	9.29[a]	84.72[b]	8.18[a]	18.52[a]	20.28[b]
SPV422	136.07[c]	8.00[b]	102.56[a]	8.45[a]	18.38[a]	20.88[a]
Harvest cycle, H	ns	**	ns	**	**	**
Plant density, P.D	ns	ns	ns	ns	ns	**
Variety, V	**	*	**	**	**	**
H*P.D	ns	ns	ns	ns	ns	ns
H*V	**	*	ns	ns	ns	*
P.D*V	ns	ns	ns	ns	ns	ns
H*P.D*V	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).*

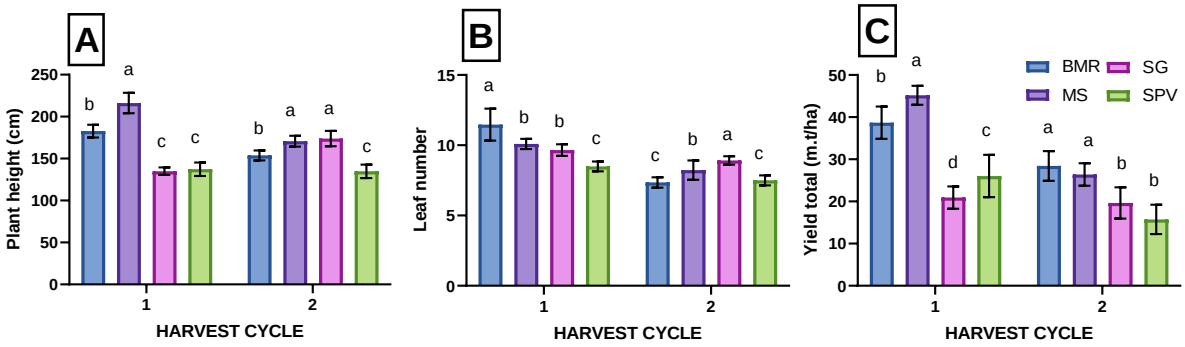

Figure 3: Plant height (A), leaf number (B) and yield (C) of different sorghum varieties at different harvest cycle. Means in each graph with the different letters indicate significant differences at *P*≤0.05% level according to Tukey's HSD. (Mean ± S.E; n=3).

Conclusions

In conclusion, Megasweet (MS) variety is recommended in both planting system as it gave significantly higher yield. For semi mechanised system, planting distance of 45 x 20 cm is recommended as it incurred less cost for seed without compromising the yield significantly while for mechanised, 75 x 9.3 cm is recommended as it gave significantly higher yield as compared to 75 x 11.3 cm.

Acknowledgements

This research was funded by MARDI RMKe11 project, PGBA08. The authors would like to thank staff of MARDI Industrial Crop Research Centre, Engineering Research Centre and Livestock Research Centre for helping the research work.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effect of Packaging Materials on the Postharvest Quality of Winged Beans (*Psophocarpus tetragonolobus*)

Nor Elliza, T.* and Nur Syahirah, A.R.
Faculty of Sustainable Agriculture, UMS Sandakan Campus, 90000 Sandakan, Sabah, Malaysia.
**E-mail: ellizatajidin@ums.edu.my*

Introduction

Psophocarpus tetragonolobus or winged bean contains 32-38% protein, similar to soybeans (Raai et al., 2020). Fresh pods of winged beans have a starchy crunchy texture, nutty flavour, and delightful taste, hence its popularity for use in vegetable salads and as a flavourful addition to stir-fries and grills (Tanzi et al., 2019). The beans are usually harvested before it reaches physiological maturity stage. The pods lose sweetness and crispness as well as begin degreening and experience meal development shortly after harvest, which increases throughout the postharvest supply chain (Logegaraya et al., 2010). The degradation of the quality characteristics of freshly harvested beans is due to its intense respiration rate that is partly attributed to the vigorous metabolic activity of the immature seeds inside the pods. Conversion of sugar to starch results in loss of the sweet taste and water content in the pods during the ripening stage, which leads to the pods becoming too fibrous to eat (Lucera et al., 2011). El-Mogy and Kitinoja (2019) also noted that shrivelling due to moisture loss, decay due to microbial growth and fibre development due to over-maturity are often associated with unacceptable quality characteristics of fresh bean pods.

Given the above situations, more attempts are necessary to find ways to maintain the quality of fresh bean pods. It is noted that suitable packaging materials that extend the shelf-life of fruits and vegetables, especially fresh produce with high respiration rate, have been proposed in the past. Kader (1997) reported that in order to achieve a decrease in respiration rate the oxygen level should generally be maintained at less than 5%. Appropriate packaging methods also offer possibilities to extend the shelf life of fresh produce, by retarding produce respiration rates and delaying enzymatic degradation of complex substrates (Caleb et al., 2013). As Mangaraj et al., (2009) claimed, the packaging principles are to protect fresh goods from outside influences and damage, to contain food and to provide ingredients and nutritional information to customers.

Polyethylene (PE) plastic bags and printed newsprint paper packaging are the most commonly used packaging materials in Malaysia. PE bags are the most inexpensive plastic material that provide an excellent barrier to water vapour. PE bags comprise soft, flexible and strong material that are resistant to chemicals and recyclable (Mangaraj et al., 2009). These criteria have made PE bags the most popular packaging film used in the food packaging industry (Mangaraj et al., 2009; Allahvaisi, 2012;). Meanwhile, according to Marsh and Bugusu (2007), the use of paper for food packaging dates back to the 17th century with increased usage in the latter part of the 19th century. Printed newsprint paper is a low-cost material, readily available, light in weight and easy to recycle. However, paper type packaging materials do not have the capability to protect fresh produce for long periods of time as they have poor barrier properties and are not heat sealable (Marsh and Bugusu, 2007). Additionally, the printing ink on printed newsprint paper contain cancer-causing agents that can cause severe health issues (Zhou et al., 2012). Jeenusha and Amritkumar (2020), on the other hand, state that banana leaves packaging has potential to protect food from deterioration inquality. Additionally, this packaging material provides pleasant flavour and odour characteristics (Forero-Cabrera et al., 2017). Banana leaves are also decomposable, which makes it more sustainable to be used as packaging material, and can help reduce the single-use plastic packaging materials currently going to landfills (Forero-Cabrera et al., 2017; Ahmadi et al., 2019). Furthermore, banana plants are commonly found all around Malaysia. Plastic pollution is no longer a novel issue in this era and is now one of the problematic challenges that humans have to face and resolve. The objective of this study was to determine the effects of different packaging materials on the postharvest quality of winged beans, *P. tetragonolobus*.

Materials and Methods

Freshly harvested immature beans were purchased from the Batu 8 Wet Market, in Sandakan, Sabah. The beans were then immediately transported to the Postharvest Laboratory at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (UMS), Sandakan Campus, where the experiment commenced on the same day. The pods were selected based on uniformity in size and free from damage. Immediately after selection, the pods were washed with tap water containing 1% sodium hypochlorite solution to remove field heat, soil dust and reduce the microbial population. The pods were then air-dried for 10 min at room temperature (~25 °C) and subsequently packed in the different types of packaging materials chosen, as described in Table 1.

Table 1: Descriptions of different packaging materials used in this experiment.

Packaging materials	Descriptions
Control	Without packaging
Perforated PE bags	Size 15 × 22 cm and 0.08 mm thickness with four holes (0.5 cm), distributed evenly
Printed newsprint paper	Obtained from a recycle centre and cut into pieces measuring 20 × 20 cm
Banana leaves	Banana leaves were harvested from the banana plant plot at UMS. Leaves that had uniform colour and free from radiation damage and pest attack were chosen. The leaves were pretreated by heating in an oven at 80 °C for 3 min in order to avoid spread of fungi from the leaves (Forero-Cabrera et al., 2017)

The bean pods, wrapped in packaging and without packaging (control), were subsequently stored in the refrigerator at 5±2 °C (85-90% RH). Each wrapped package and the control contained four bean pod samples. Weight loss, firmness, hue colour value and ascorbic acid and chlorophyll contents were measured at 4 days interval from 0 day until 12 days of storage. To determine weight loss, the pods were weighed using a digital balance, before storage and after each 4 days interval until 12 days in storage, and the results were expressed as percentage loss of initial weight. Pod firmness was measured using a hand penetrometer (Fruit pressure tester, FT 327) and the results were expressed in kg cm^2. Hue colour value was determined by a Minolta chromameter (CR 300, Minolta Corp., Japan). For ascorbic acid analysis, 5 g pod tissues were homogenized with 3% HPO_3 using a mortar and pestle. The homogenized sample was then filtered and titrated against 2,6-dichlorophenolindophenol dye. The results were expressed in mg/100 g. The total chlorophyll content (nmol/cm^2) was measured using a portable SPAD meter (Minolta, Japan) at every 4 days interval.

The study was conducted using the CRD in a factorial arrangement of treatments (four packaging materials × four storage days) with four replications. The obtained data were analyzed using the ANOVA and means were separated by LSD test at $P \leq 0.05$. When there were significant interaction effects between the two factors (packaging materials and storage days), the effects of the 2-way interactions were partitioned into single degree freedom using proc glm (SAS, version 9) and means were compared by LSD test at $P \leq 0.05$.

Results and Discussion

There was significant interaction effect between packaging materials and storage days on the quality attributes studied (Table 1). The highest weight loss was for the control treatment. No significant differences were observed between the weight loss of beans packed with printed newsprint paper and banana leaves for the 12 days storage. The perforated PE bags showed significant differences in weight loss of beans at 8 and 12 days of storage as compared to the other packaging materials (Figure 1a). Cheng et al. (2019) reported that withering and shrivelling of fresh produce was due to water loss via transpiration. The moisture loss was attributed to vapour phase diffusion, which is driven by the gradient of the water vapour pressure in- and outside of the horticultural commodity (Suseno et al., 2014). Beans wrapped in PE plastic bags showed lowest weight loss (Thompson, 2001) and retained freshness of beans longer.

Table 1: Interaction effects of packaging materials and storage days on quality attributes of winged bean.

Attribute	Packaging materials (PM)	Storage days (SD)	PM × SD
Weight loss (%)	73.94***	431.54***	***
Firmness (kg.cm^2)	25.34***	73.41***	**
Hue (*h*°)	15.58***	66.06***	**
Ascorbic acid (mg/100 g)	32.74***	1326.58***	***
Chlorophyll content (nmol/cm^2)	26.92**	19.23**	***

, * *Significant at P≤0.01 or P≤0.001, respectively.*

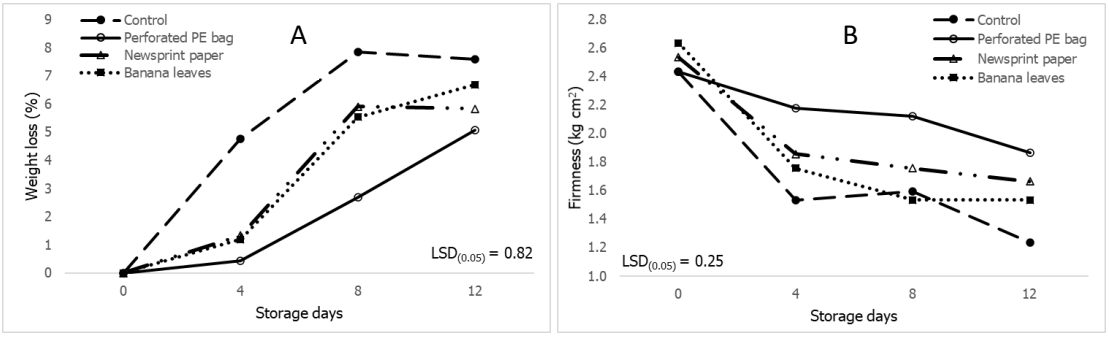

Figure 1: Effect of packaging materials (control, perforated PE bags, printed newsprint paper and banana leaves) and storage days (0, 4, 8, and 12 days) on (A) weight loss and (B) firmness of the winged beans.

The firmness of beans in PE bags was significantly higher as compared to other treatments during 12 days of storage, while there was no difference in firmness between the packaging treatments on 0 day of storage (Figure 1b). Reduction of firmness is due to changes in the lamella and cell wall constituents such as pectic polysaccharides depolymerization and solubilization, water and turgidity loss during ripening (Esua et al., 2019). The results indicated that PE bags maintained bean firmness better other packaging materials. This effects of the packaging materials may be attributed to their retardation effects on ripening and reduction of water loss during storage (Manrique and Lajolo, 2004).

The beans packed in the perforated PE bags recorded significantly higher hue of colour values as compared with the other packaging materials at 8 and 12 days of storage (Figure 2). The results indicate that the beans packed in the perforated PE bags had a more vibrant greenish colour. In contrast, reduction of *h*° value for the other packaging materials at 12 days of storage resulted in yellowish-green bean pods. Hue is considered as a qualitative feature of colour which is used to differentiate particular colours. Furthermore, the higher the hue angle the lesser the yellow character appearence in the peel of horticultural produce (Pathare et al., 2013). According to the results shown in Figure 2, the hue values of beans packed in perforated PE bags from day 4 to day 12 were shown to have dropped rapidly. In this case, it means that the hue angle was lower, and the yellow characteristic can be spotted on the skin of the bean pods.

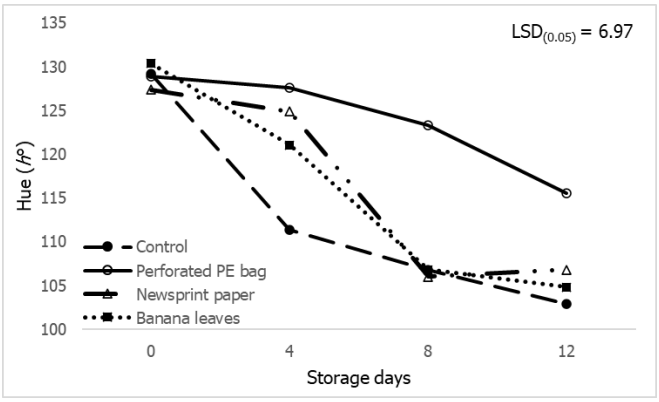

Figure 2: Effect of packaging materials (control, perforated PE bags, printed newsprint paper and banana leaves) and storage days (0, 4, 8, and 12 days) on hue colour value of the winged beans.

On days 4, 8, and 12, the ascorbic content of the bean pods packed in the perforated PE bags were higher than that in the control. The beans without packaging tended to have the lowest ascorbic acid content at 4, 8, and 12 days of storage while the printed newsprint paper packed beans had higher ascorbic acid content compared to the control (Figure 3a). The possible reduction in internal O_2 and decrease in ethylene concentration might explain the presence of higher value of ascorbic acid content in the packaged beans, through delay in respiration and ripening of the packaged beans (Azene et al., 2014).

There was no significant difference in the chlorophyll content of the beans packed in perforated PE bags and printed newsprint paper at 12 days of storage (Figure 3b) However, the beans without packaging material had significantly lowest chlorophyll content as compared with the beans packed with in the perforated PE bags. The reduction in chlorophyll content was due to the process of chlorophyll degradation with the loss of green pigment in the beans during storage, due to environmental factors such as light, temperature and humidity (Wu et al., 2016). It indicated that the freshness of the beans in the control sample had deteriorated as chlorophyll content was one of the evaluation methods to determine the freshness of produce (Tay and Teo, 2019). The barrier properties of packaging materials are important in maintaining the physiological and biochemical activities where the quality of produce can be preserved better (Caleb et al., 2013). The results showed that the control and banana leaves packaging material were not able to delay the chlorophyll degradation over a longer period of storage.

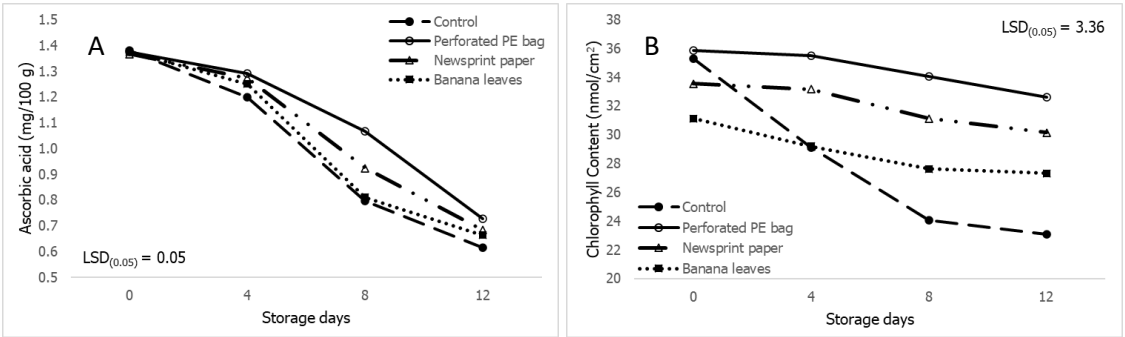

Figure 3: Effect of packaging materials (control, perforated PE bags, printed newsprint paper and banana leaves) and storage days (0, 4, 8, and 12 days) on ascorbic acid (a) and chlorophyll contents (b) of the winged beans.

Conclusion

Packaging materials and storage days had significant interaction effects on the postharvest quality of the winged beans. Winged bean pods packed in perforated PE bags maintained better fresh weight over the

storage period. Further, as the storage time advanced, the bean pods packed in perforated PE bags showed more firmness, better greenish hue value, and ascorbic acid and chlorophyll contents. Overall, the perforated PE bags were more effective compared to printed newsprint paper and banana leaves packaging materials and the control in maintaining quality as well as prolonging the shelf life and marketability of the winged beans.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Effects of UV-C Intensity on Postharvest Quality of *Ficus carica* During Storage

Aimi Fatini, M.M. and Ding, P.*
Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
**E-mail: phebe@upm.edu.my*

Introduction

Fresh fig is one of a delicate climacteric fruit. To obtain optimum flavour, fresh fig fruits should be harvested when they are almost fully ripe. Unfortunately, softening, and post-harvest diseases are the limiting factors when storing fresh fig fruits (Mat Jusoh et al., 2019). Therefore, retaining quality and prolonging shelf-life of fresh fig fruit is of great importance. At present, the methods used to preserve fruit and vegetables quality are 1-methylcyclopropene (1-MCP) fumigation (Amornputti et al., 2014), chitosan coating (Kerch, 2015; Zhang et al., 2019), cold storage, modified atmosphere packaging (MAP) (Allende et al., 2007), high temperature stimulation (Nasef, 2018), and biological control agents (Droby et al., 2016). Previous studies showed that ultraviolet (UV) light could improve the postharvest quality of various fruit such as banana (Nuratika et al., 2018), strawberry (Araque et al., 2018), grapes (Maurer et al., 2017) and pineapple (Sari et al., 2016). However, the application of UV-C in retaining postharvest quality of Malaysian grown fresh fig is almost nil. Therefore, this study was carried out to evaluate the effects of UV-C irradiation on postharvest quality of fresh fig during storage.

Materials and Methods

Fruit source

On-tree ripened fig fruits var. Ipoh Blue Giant were obtained from Selangor Fruit Valley in Rawang, Selangor and then transported to the Postharvest Laboratory, Faculty of Agriculture of Universiti Putra Malaysia on the same day. The fruits were selected according to the uniformity of size, shape and free from any diseases.

UV-C irradiation treatment

A stainless-steel chamber equipped with UV-C irradiation facility was used in this study. The chamber contained a low-pressure mercury vapor discharge lamp (9.0 cm long, 220 V, and 36 W) and emitted 254 nm of UV-C light (VER Bright, Vision Scientific, Korea). The interior of chamber was lined with reflective mirrors which designed to minimize any shadowing effect on irregularly shaped samples. Before application, UV-C light was switched on for 30 min to stabilize the emission of radiation. Initially, the UV-C dose rate was obtained using a digital radiometer (UVC-254, Lutron Electronic Enterprise, Taiwan) and calibrated to read specifically at 254 nm before being used. The dose rate at eight areas inside the chamber was measured and averaged and 0.336 W/m^2 dose rate was obtained. Fig fruits were irradiated with UV-C intensity of 0 (control), 0.01, 0.02, 0.03, and 0.04 kJ/m^2. After UV-C exposure, fruits were kept at 26±2 °C. At day 0 and 2 after UV-C irradiation, colour, firmness, soluble solids concentration, titratable acidity and pH of fruits were taken and a total of 8 fruits were used for each treatment with two replications each.

Determination of colour

Changes of the fig fruit skin colour were measured using a Minolta CR- 400 Chroma Meter (Minolta Corp., Osaka, Japan). The values of L*, a*, b*, C*, and h^o were taken randomly at three positions of a fruit. L* value indicated lightness of the colour, which ranged from 0 (dark) to 100 (white). The positive value of a* indicated red colour, while negative value of a* indicated green colour. The positive value

of b* indicated yellow colour, while negative value of b* indicated blue colour. The C* values indicated the saturation of the colour. The ho values of 0°, 90°, 180° and 270° corresponded to the red, yellow, green, and blue colour, respectively.

Determination of firmness

The firmness of the fruits was evaluated using a bishop penetrometer FT 327 (Italy) with an 11-mm-diameter plunger. Forces from constant penetration of the plunger were applied perpendicularly to the 1-cm thick of fig fruit which was cut from equatorial region of a fruit with a smooth motion in 2-3 s. The readings in kilograms forces were made at two opposite directions of every slice of the fruit and were converted to newton (N).

Determination of soluble solids concentration (SSC)

The SSC of fruit was measured using a digital refractometer (Model N-1 a Atago Japan). An amount of 10 g fig fruit sample was measured and homogenized with 90 mL distilled water using a hand blender for 1 min and filtered through cotton wool.

Determination of titratable acidity

The remaining filtrate from SSC determination was used to measure titratable acidity of fig fruit using titration method (Ranggana, 1977). The titre volume was recorded in mL and the results were expressed as percentage of anhydrous citric acid by using following equation.

Citric acid (%) =

$$\frac{Titre\ (mL)\ x\ NaOH\ normality\ (0.1\ M)\ x\ Vol\ made\ up\ (100\ mL)\ x\ citric\ acid\ equivalent\ weight\ (64.04\ g)\ x\ 100}{volume\ of\ sample\ used\ for\ titrate\ (5\ mL) x\ weight\ of\ sample\ (10\ g)\ x\ 1000}$$

Determination of pH

The remaining filtered from titratable acidity determination was used to measure the pH of fig fruits using a glass electrode pH meter (Crison Instruments, S.A., Barcelona). The pH meter was calibrated with pH buffers at pH 4.0 and 7.0.

Experimental design and statistical analysis

The experimental design was conducted in a completely randomized with a factorial arrangement of treatment (4 levels of UV-C intensity x 2 levels of storage duration). Data were analysed using analysis of variance (ANOVA) (SAS version 9.4, 2010). Comparison of means was performed using Tukey test at the 5% significance level.

Results and Discussion

The L*, C*, and h* values of fig peel did not affect by interaction between UV-C intensity and storage day (Table 1). Similarly, main effects of UV-C intensity and storage day also did not affect fig colours. This indicates the colour of fig did not change after detached from mother plants. Fruit firmness is a main index of quality and it is important as quality evaluation in fruit. Firmness was not affected by UV-C intensity but as storage day progressed, the texture turned softer (Table 2). Most probably water and turgidity loss from fruit during storage while pectic polysaccharides depolymerized and solubilized which affects lamella and cell wall constituents, thus firmness decreased during storage (Nambi et al., 2016).

In the present study, UV-C intensity did not affect the SSC of fresh fig but as storage day progressed fruit became sweeter (Table 2). Similar finding was also reported in apple (Hagen et al., 2007) and tomato (Liu et al., 2009) where UV-C irradiation did not affect SSC of these fruit. In this study titratable acidity of fresh fig was not affected by UV-C intensity but decreased as storage day advanced (Table 2). A decrease in total acidity is typical during postharvest storage and has been attributed to the use of organic acids (such as citric acid) as substrates for the respiratory metabolism (Gol et al., 2013). Unlike TA, the pH of fig fruits decreased as UV-C intensity increased, but storage day did not affect fig pH (Table 2).

Table 1: Main and interaction effects of UV-C intensity and storage duration on fig colour.

Factor	L*	a*	b*
UV-C Intensity (UV), kJ/m^2			
0	54.62^a	6.74^a	25.27^a
0.01	54.37^a	5.19^a	24.62^a
0.02	48.43^a	8.61^a	20.05^a
0.03	50.38^a	1.31^a	29.79^a
0.04	54.82^a	5.21^a	25.42^a
Storage duration (SD), day			
0	54.34^a	3.67^a	28.10^a
2	50.70^a	7.15^a	21.96^a
Interaction			
UV x SD	ns	ns	ns

ns = non-significant at P >0.0

Table 2: Main and interaction effects of UV-C intensity and storage duration on firmness, soluble solids concentration, titratable acidity, and pH.

Factor	Firmness (N)	Soluble solids concentration (%SSC)	Titratable acidity (%)	pH
UV-C Intensity (UV), kJ/m^2				
0	1.88^a	15.11^a	0.18^a	5.49^a
0.01	4.58^a	13.04^a	0.19^a	5.24^{ab}
0.02	4.87^a	14.11^a	0.22^a	5.09^b
0.03	4.44^a	13.28^a	0.17^a	5.10^b
0.04	4.11^a	15.28^a	0.14^a	5.04^b
Storage duration (SD), day				
0	6.57^a	12.48^b	0.25^a	5.19^a
2	1.37^b	15.85^a	0.11^b	5.19^a
Interaction				
UV x SD	ns	ns	ns	ns

ns = non-significant at P>0.05.

Conclusion

The findings of this study reveal the quality of fresh fig fruit was not affected by UV-C irradiation except pH. However, the quality of fresh fig was affected storage period. Therefore, optimum storage temperature should be used to slow down quality deterioration of fresh fig.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Alluvial Soil and Pepper Gas Exchange Properties Enhancement with Compost, Fermented Juices and Biochar

Kevin, M.T.S.[1,*], Ahmed, O.H.[2,3,4], Khew, C.Y.[1], Lai, P.S.[1], Zehnder, J.A.M.[1] and Mohd Effendi, W.[5]

[1]Research and Development Division, Malaysian Pepper Board, 93916 Kuching, Sarawak, Malaysia.
[2]Department of Crop Science, Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia (UPM), Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia.
[3]Institute of Tropical Agriculture and Food Security (ITAFoS), Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia.
[4]Institut of Ekosains Borneo, Universiti Putra Malaysia (UPM), Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia.
[5]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.
**E-mail: kevinmuyang@mpb.gov.my*

Introduction

Due to continued utilization of chemical compound fertilizers, many black pepper farms are compromising soil health or quality, even on a fertile soil such as the alluvial soil (Paulus, 2011). The gradual and continued decline in soil health reduces the yield and lifespan of pepper vines. The use of organic amendments or organic farming is being advocated as a way to sustain the health and fertility of soils (Doran and Zeiss, 2000). Organic or natural agriculture is a farming structure that does not include the application of chemical insecticides and fertilizers (Higa, 2012). The use of organic amendments in pepper farming is a present and ongoing work to improve sustainability through lower production cost, socially acceptable high-value products, and reduced pollution of the environment. Plant physiological characteristics including photosynthetic rates are a determining component for crop development and productivity as optimal carbon assimilation rate generally led to favourable plant yield, as it is the most fundamental, yet significant physiological process instantly related to pepper productivity, especially at the ripened fruit stage (Paulus, 2011). Kho and Chen (2017) stated that crop productivity capability is enhanced by elevating gas exchange rates particularly photosynthesis as it is in one and another associated to yield. A study was conducted on a typical black pepper farm grown with mature vines under organic soil amendments. The objectives were to compare certain characteristics of soils following introduction of compost, fermented juices, and biochar in mature pepper cultivation and to assess the gas exchange properties of mature vines under compost, fermented juices, and biochar.

Materials and Methods

Based on United States Department of Agriculture (USDA) classification system, the alluvial Bemang series in the experimental site is a Typic Dystroepts, silty/fine loamy, mixed/siliceous, acidic, iso-hyperthermic, from sedimentary rocks (Soil Survey Staff, 2000). The research site was a full-grown pepper establishment with about 0.5 hectare in size. Fully-matured *Piper nigrum* var. Kuching aged four years old were selected for the experiment as it was extensively cultivated in Malaysia. The study was initiated in the month of July 2017 and completed in the month of February 2019.

Design of experiment

A completely randomized block design (CRBD) with five treatments replicated five times, giving a sum of 25 experimental plots was used for this research. Treatments used were as shown: (i) FNPK - 12:12:17 compound fertilizer (control), (ii) FPJ - Fermented Plant Juice, (iii) FPJBC - Fermented Plant Juice combined with compost and biochar, (iv) FFJ - Fermented Fruit Juice, and (v) FFJBC - Fermented Fruit Juice combined with compost and biochar. The FPJ and FFJ were concocted monthly to make sure

solely new group of fermented juices were sprayed to soils. The FPJ and FFJ treatments were sprayed monthly at start of the study.

Determination of soil characteristics

The soils were studied for soil texture, porosity, bulk density, total nitrogen (N), and total organic carbon (TOC) following the method by Edwards (2010).

Measurement of pepper physiological traits

Determination of pepper Normalized Difference Vegetation Index (NDVI), transpiration (*E*), net photosynthesis (*A*), leaf chlorophyll concentration, and leaf stomatal conductance (*gs*) were done prior to the harvesting of fruits according to the protocol of DiCristina and Germino (2006).

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze the data and the statistical software used was the SPSS software (version 15). To compare treatment means, the Tukey's Honest Significance Difference (HSD) Test, at α=0.05 level of significance was done. Using trend analysis, the correlations between pepper foliar chlorophyll concentration and soil total nitrogen, pepper NDVI and soil total nitrogen, and pepper photosynthetic rates and soil total nitrogen were studied.

Results and Discussion

Physical characteristics of soils

The soil porosity, bulk density, total organic C, and total N of under different treatments in farm planted with full-grown vines are shown in Table 1. The soils treated with compost and biochar (FPJBC and FFJBC) demonstrated bulk densities which were lower when compared to the fermented juices only treated soil (FPJ and FFJ). Soils under the NPK fertilization (FNPK) gave the highest value for bulk density. The findings also indicate that the porosities and bulk densities of the soils were related inversely. As observed, the reduction in soil hardness after compost and biochar amendments instantly led to a rise in soil porosities. Bulk densities for soils treated with compost and biochar (FPJBC and FFJBC) were the lowest whereas its porosities were the highest. At the end of the study, the NPK fertilized plot recorded porosity values which were low. Several literatures agreed with these findings. Mensah and Frimpong (2018) reported that the correlations of soil porosity and bulk density is correlative whereas one decreases, the other increases. Schulz and Glaser (2012) stated that among the factors for soil showing considerably high porosities and low bulk densities was the presence of biochar and compost as organic amendments. Kumar and Swarupa (2017) mentioned that compost application impacted the structure of soils by reducing soil density caused by admixture of lighter-density organic matter in soils. This favourable result was seen in other findings as well and is related with high porosity due to the interactions between inorganic and organic fractions (Agegnehu et al., 2016).

Therefore, biochar and compost application presumably reduce soil hardness via the dilution or mixing reaction. Sim and Paulus (2011) were of the opinion that the increased soil porosity caused by biochar and compost application can have positive implications for the movement of plant roots, water, heat, and gases in the soil. As expected, TOC for soils amended with compost and biochar (FPJBC and FFJBC) gave considerably higher values compared to the other treatments (FNPK, FPJ, and FFJ). The increase in soil TOC were caused by the incorporation of more carbon source with incorporation of compost and biochar. This was mentioned by Schulz and Glaser (2012) as well who suggested that rise in soil carbon in the treatments amended with organic fertilizer was caused by addition of materials such as compost and biochar from root biomass, farmyard manure, and crop residues. Total nitrogen of soils under organic amendments (FPJ, FPJBC, FFJ, and FFJBC) were notably low compared to that of the control treatment (FNPK) (Table 1). This outcome indicates that NPK fertilizer elevated soil total nitrogen

significantly. Kumar and Swarupa (2017), and Yap (2012) reported that because of considerable proportion of nitrogen from the 12:12:17 chemical compound fertilizer that was liberated to soils, hence led to high soil nitrogen availability for the crop optimum growth. On the other hand, Doran and Zeiss (2000), and Rajkovich et al. (2011) stated that limited N might be due to nitrogen immobilization caused by microbe movement that happen in soil treated with amendments which are organic in nature.

Table 1: Selected soil properties under different organic amendments.

	Value acquired				
Characteristic	FNPK	FPJ	FPJBC	FFJ	FFJBC
Texture of soil	Sand 60%	Clay 25%	Silt 15%	=>	Sandy clay loam
Bulk density (%)	1.32 ± 0.10^{a}	1.25 ± 0.06^{bc}	1.18 ± 0.07^{cd}	1.27 ± 0.07^{ab}	1.17 ± 0.04^{d}
Porosity (%)	50.21 ± 3.72^{d}	53.01 ± 2.22^{bc}	55.57 ± 2.76^{ab}	52.23 ± 2.64^{cd}	55.95 ± 1.45^{a}
TOC (%)	1.06 ± 0.10^{b}	1.11 ± 0.11^{b}	3.68 ± 0.18^{a}	1.19 ± 0.06^{b}	3.75 ± 0.15^{a}
Total N (%)	0.85 ± 0.05^{a}	0.17 ± 0.03^{b}	0.16 ± 0.04^{b}	0.14 ± 0.02^{b}	0.15 ± 0.01^{b}

Note. Treatments are FNPK - compound fertilizer control, FPJ - Fermented Plant Juice, FPJBC - Fermented Plant Juice incorporated with biochar and compost, FFJ - Fermented Fruit Juice, and FFJBC - Fermented Fruit Juice incorporated with biochar and compost (mean±S.E., n=15). Note. Means with same letter superscript within rows are not statistically different using Tukey's at P>0.05 probability level.

Physiological characteristics of pepper

The mature vines chosen physiological traits under 12:12:17 fertilization and organic amendments are shown in Table 2. Treatments FPJBC and FFJBC showed greater foliar chlorophyll concentration values followed by treatments FPJ and FFJ. Treatment FNPK depicted the lowest foliar chlorophyll concentration values. Based on the result in Table 2, a quite similar trend was detected in result for Normalized Difference Vegetation Index (NDVI) where at the end of the experimental period, the compost, fermented juices, and biochar treatments (FPJBC and FFJBC) depicted higher values than the fermented juices alone treatments (FPJ and FFJ) and the control treatment (FNPK). The correlation between foliar chlorophyll and soil nitrogen in the farm with full-grown pepper vines under various soil ameliorations are presented in Figure 1. The negative correlation between both parameters irrespective of treatment was better represented as a straight regression line with an R^2 of 0.73. This consequence demonstrated strong inverse relationship among both factors where foliar chlorophyll concentration reduced with rising total nitrogen in soils in a mature pepper farm. The FNPK plots showed significantly higher soil total N but at the same time lower chlorophyll concentration. Paulus (2011) stated that longer period and wide use of inorganic fertilizers which have higher nitrogen applied around full-grown vines seems to have detrimental outcome to soils resulting in decreasing nutrient exchange and water holding capability hence influencing foliar chlorophyll development.

Table 2: Photosynthesis (*A*), Normalized Difference Vegetation Index (NDVI), transpiration (*E*), leaf stomatal conductance (*gs*), and foliar chlorophyll concentration of full-grown pepper vines under different organic amendments.

	Value obtained				
Property	FNPK	FPJ	FPJBC	FFJ	FFJBC
Chlorophyll (µmol per m^2 of leaf)	55.59 ± 6.03^{c}	74.62 ± 3.32^{b}	86.99 ± 2.07^{a}	77.50 ± 2.95^{b}	86.51 ± 1.15^{a}
NDVI	0.76 ± 0.03^{c}	0.89 ± 0.03^{b}	0.91 ± 0.03^{ab}	0.90 ± 0.03^{b}	0.94 ± 0.03^{a}
Photo (*A*) (µmol $CO_2m^{-2}s^{-1}$)	7.87 ± 0.27^{c}	8.01 ± 0.13^{bc}	8.35 ± 0.87^{ab}	8.11 ± 0.13^{abc}	8.49 ± 0.20^{a}
Cond. (*gs*) (mol $H_2Om^{-2}s^{-1}$)	0.09 ± 0.02^{b}	0.14 ± 0.03^{a}	0.15 ± 0.02^{a}	0.15 ± 0.02^{a}	0.15 ± 0.10^{a}
Trans. (*E*) (mmol $H_2Om^{-2}s^{-1}$)	1.85 ± 0.22^{b}	3.64 ± 0.18^{a}	3.76 ± 0.27^{a}	3.67 ± 0.16^{a}	3.81 ± 0.22^{a}

Note. Treatments are FNPK - compound fertilizer control, FPJ - Fermented Plant Juice, FPJBC - Fermented Plant Juice incorporated with biochar and compost, FFJ – Fermented Fruit Juice, and FFJBC - Fermented Fruit Juice incorporated with biochar and compost (mean±S.E., n=15). Note. Means with same letter superscript within rows are not statistically different using Tukey's at P>0.05 probability level.

Similarly, the correlation between pepper NDVI and total nitrogen of soils from the selected farm with mature vines under different soil treatments were negative with an R^2 of 0.76 (Figure 2). The correlation among the two parameters irrespective of treatments was ideally explained by a straight regression line describing a value of 76% of the differential in NDVI. This outcome suggests strong inverse correlation between both factors where NDVI reduced with elevating soil nitrogen in farm grown with mature pepper vines. Nitrogen is commonly related to the green colour of the leaves, where abundance of nitrogen presence in soils influences foliar growth and development, particularly chloroplasts structuring and build-up of chlorophyll in the leaf (Khaliq et al., 2016; Shanmugapriya et al., 2012). Nevertheless, for the study of the mature vines, although with high nitrogen availability in the soil will not cause healthier and greener foliage as shown in Figures 1 and 2.

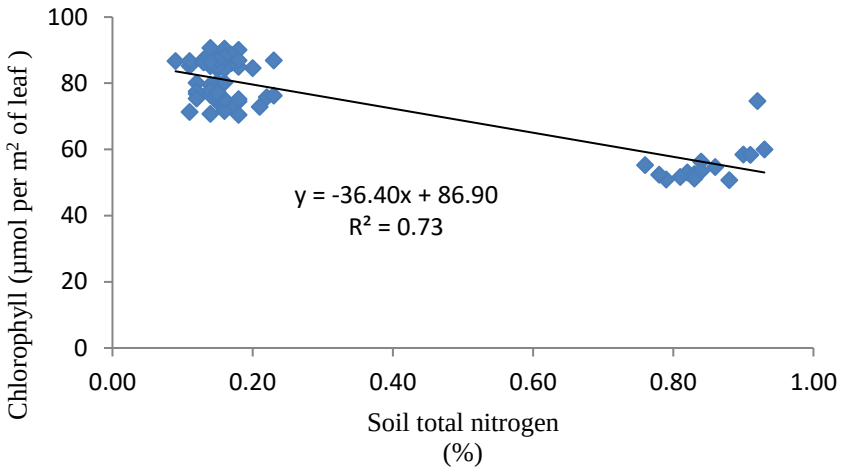

Figure 1: Correlation between pepper foliar chlorophyll concentration and soil total nitrogen in a full-grown pepper vines farm.

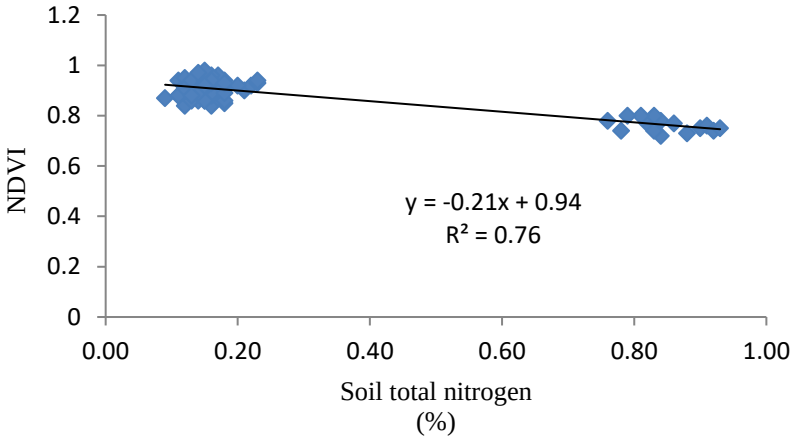

Figure 2: Correlation between pepper Normalized Difference Vegetation Index (NDVI) and soil total nitrogen in a full-grown pepper vines farm.

The outcome was in agreement with Chen et al. (2016) who mentioned that poor soil properties diminished NDVI and chlorophyll of the foliage, however, it was not because of low nitrogen in soils. Throughout the research, few hostile soil health properties were recorded in plots subjected to 12:12:17 fertilization but the properties directly associated to chlorophyll concentration in the foliage apart from soil nitrogen were porosity and bulk density. This result was consistent with McGrath and Henry (2016) who stated that soil with low porosities and high bulk densities incline to limit root expansion to explore for moisture leading to drought hence reduced NDVI and foliar chlorophyll concentration. Bianco et al.

(2005) studied the result of lack of moisture because of less porous and hard soil on the structure of bundle sheath chlorophylls and mesophyll of crops. Bianco et al. (2005) went on to mention that the loss of moisture might be due to mesophyll cells that are removed farther from the vascular supply of water than the bundle sheath cells, and thus resulting in significant leaf-cell moisture deficiency which led to a considerable depletion of chlorophyll. Meanwhile, McGrath and Henry (2016) reported that lighter and permeable soil enable roots of the crop to search and infiltrate deeper under the ground to acquire water. Due to this, crops could function significantly healthier in chlorophyll and growth development even when subjected to sparse nitrogen availability provided that segregation of nitrogen within the crop and coherent use of nitrogen at the leaf cellular level (Hawkesford and Griffiths, 2019). Additionally, Mensah and Frimpong (2018) discovered a relation ($r = 0.65$, $P \leq 0.001$) of total organic carbon with soil water concentration after incorporation of compost and biochar, verifying that abundance carbon level in the soil elevates the water-retention capability due to the consequence of organic matter on soil structure. This increased supplies available moisture to crops and also assisting with resistance to water stress.

As discovered in Table 2 in which organic amendments reduced soil nitrogen when compared to that of the NPK compound fertilizer, it was the enhancement in soil porosities, bulk densities, and total organic carbon that had caused the photosynthetic rates to elevate. A report by Bianco et al. (2005) documented that crop's photosynthesis improvement due to presence of soil water from soil organic amendments usage such as compost and biochar. Atiyeh et al. (2000) reported that soil's moisture holding capability corresponds with soil porosity, total organic C, and density in a positive way. Black pepper photosynthesis and soil nitrogen in the full-grown pepper vines farm under various soil amendments exhibited inverse relationship with a correlation of $R^2=0.52$ (Figure 3). The relation between both parameters irrespective of treatments was clearly portrayed by a straight regression line which describes a value of 52% of the differential in photosynthetic rates. This demonstrated standard correlations in both factors where photosynthetic rates reduced a little with elevating soil nitrogen in a full-grown pepper farm. The FNPK plots recorded considerably higher soil total nitrogen while at the same time lower photosynthesis rates. A comparable result by Sim and Paulus (2011) reported that although soil total nitrogen is elevated, pepper photosynthetic rates are reduced under extensive and heavy application of NPK chemical compound fertilizers, suggesting soil health declination with longer farm duration. Sim and Paulus (2011) added that a declining soil environment due to extensive application of chemical-based farm inputs will hamper the capability of soils to preserve water and nutrient hence reducing yield of pepper vines.

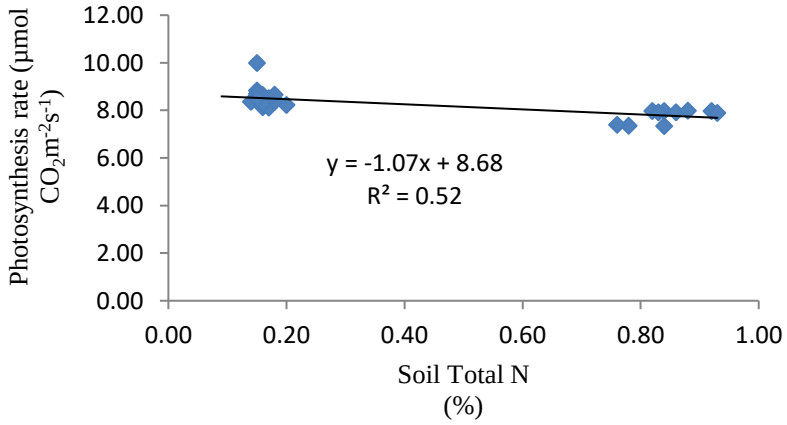

Figure 3: Correlation between pepper photosynthesis rates and soil total N in a full-grown pepper vines farm.

As for outcome of pepper transpiration and stomatal conductance rates in Table 2, it was discovered that both rates follow an almost the same pattern. All the organic amendment treatments (FPJ, FPJBC, FFJ, and FFJBC) exhibited significantly higher foliar stomatal conductance and transpiration than that of the NPK compound fertilizer (FNPK). The usage of biochar and compost did not give higher leaf transpiration and stomatal conductance rates as the rates in treatments with fermented juices alone (FPJ and FFJ) were similar with that of FPJBC and FFJBC. Thus, these outcomes indicate that long period of NPK fertilization in a full-grown pepper farm significantly decreased both transpiration and leaf stomatal conductance rates. Ghasemzadeh and Jaafar (2011) reported that extended NPK-based fertilizers usage led reduced soil water holding ability because of the depletion in pores of soils and the elevated soil hardness. This led to crop's response to reduce presence of soil moisture by limiting opening of the stomatal thus reducing transpiration and conductance rates.

Therefore, it is worth to mention that in this experiment on full-grown pepper vines, usage of NPK compound fertilizer has detrimental consequence as observed in the profound decreased of pepper NDVI, foliar chlorophyll concentration, stomatal conductance, transpiration, and photosynthesis. On the other hand, soil treated with compost, biochar, and fermented juices depicted better pepper physiological characteristics due to the enhanced water and nutrient holding ability of these organic amendments (International Biochar Initiative, 2012). Hence, the increase in pepper physiological properties because of organic amendments was consistent with few soil enhancements which were related to moisture retention capability including porosity, bulk density, and total organic carbon.

Conclusion

In this research, enhancement of several soil properties suggests that soils treated with incorporation of compost, fermented juices, and biochar functioned better when compared to the NPK treated soil. In the outcomes for soil physical characteristics, plots ameliorated with the incorporated compost, fermented juices, and biochar showed elevated porosities and reduced bulk densities. Selected soil chemical traits subjected to compost, fermented juices, and biochar soil amendment exhibited positive outcome for total organic carbon. Meanwhile, for *P. nigrum*'s physiological traits, treatments with incorporation of compost, fermented juices, and biochar demonstrated positive findings for NDVI, chlorophyll, and gas exchange rates such as transpiration, stomatal conductance, and photosynthesis. It was observed that photosynthesis rate, NDVI, and foliar chlorophyll were associated negatively with soil nitrogen where these physiological properties of pepper reduced with rising soil nitrogen. From the results, it was concluded that implementing organic practice in full-grown pepper planting can assist in enhancing several soil properties to a level which is better than using chemical fertilizers and also provide pepper growers with a sustainable planting alternative.

Acknowledgments

We appreciate and recognize the contribution of Malaysian Pepper Board and Universiti Putra Malaysia in producing this manuscript.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Quality Aspect of Lowland Cauliflower During Storage

Nur Syafini, G.[1,*], Joanna, C.L.Y.[1], Azhar, M.N.[1], Mohd Nazrul Hisham, D.[2] and Mohd Lip, J.[2]

[1]Horticulture Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
[2]Technology Commercialisation and Business Centre, Malaysian Agricultural Research and Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: syafini@mardi.gov.my*

Introduction

Cauliflower (*Brassica olearaca* var. *botrytis*) has greatly increased in demand, well recognized as a highly market demand and highly imported from China (RM279.56 Million in year 2018) (Anon, 2018). In Malaysia, cauliflower is produced from very limited highland areas in Cameron Highlands and Ranau. The technology of lowland cauliflower cultivation has also been emphasized to cater for the high local demand of this temperate vegetable thus, enabling to reduce the extreme usage of limited land areas. In general, consumers prefer cauliflower that is white, sweet, and crisp, same as cauliflower imported from China that sold in our local market. According to Ryall and Lipton (1979), slightly yellowish curds are not necessarily a sign of inadequate postharvest handling but are rather a consequence of incomplete shading and exposure of the curd to sunlight during maturation on the plant, resulting in increased chlorophyll synthesis. Although slightly yellow curds are not as acceptable in terms of appearance, they are still good for consumption and contributed higher nutritional values (Ryall and Lipton, 1979). On the other hand, coloured cultivars are increasing in popularity due to their attractive colour, better taste (sweeter and less intense flavour), and higher nutritional values (Gajewski and Radzanowska, 2003; Cebula et al. 2006). Cauliflower is a highly perishable vegetable and compositional quality deteriorates very quickly if handled improperly. Dark spotted, riciness (loose or protruding floral parts), or fuzziness curds are signs of over mature and start to senescence. Thus, this study was conducted to gain the new information for lowland cauliflower in postharvest aspects and determined the postharvest quality during storage and health-promoting phytochemicals.

Materials and Methods

Determination of curd development

Lowland cauliflower was Down using fertigation system under rain shelter. To determine the curd growth, cauliflower's curd was tagged after 55 to 60 days after transplanting (DAT). Data of curd development and harvesting time was taken at 70 DAT until 90 DAT. Postharvest quality evaluation included physical and chemical analysis characteristics.

Determination of suitable temperature on quality

After harvested at optimum day, cauliflower was selected, leaves trimmed and packed with film plastic wrapping with thickness of 0.008 mm and stored at 5 °C and 10 °C for 4 weeks, respectively. The quality of cauliflower was visually judged, and the criteria used were retention of original colour, freshness, and severity of discoloured. 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 cauliflower 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. Antioxidant activity for cauliflower was studied

through the evaluation of free radical scavenging effect on the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical (Yen and Hsieh, 1997). A 0.5 mL sample of the extract was added to 1 mL methanolic solution of DPPH radical (0.2 mM). The mixture was shaken vigorously and left for 30 min. The absorbance was then measured at 517 nm. The antioxidant activity was reported as the percentage of radical scavenging as follows: % radical scavenging = (1-Asample/Acontrol) x 100 where Asample is the absorbance of the mixture of the sample extract and DPPH, Acontrol is the absorbance of the mixture of DPPH and acidified methanol. Total phenolic content was determined using the Folin-Ciocalteu method and gallic acid was used as standard (Sunita and Dhananjay, 2010). All the samples were analyzed in weekly intervals.

Statistical analysis

The experimental design was a completely randomized design (CRD) with four replications. The obtained data was analysed using analysis of variance (ANOVA) and mean comparison was conducted on the data collected using the Statistical Analysis System (SAS 9.0). The means 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 curd development

Best quality for lowland cauliflower has a creamy-yellowish colour, with medium compact, firm, and relatively smooth curds. This is opposite colour and compactness compared to white cauliflower that imported from China. Optimum harvesting day around 75 to 85 DAT with good marketable quality. Lowland cauliflower at 80-85 DAT at this stage can be served as eating quality. Development of curd and appearance of lowland cauliflower showed mature curds are at least >15 cm in diameter and 400-450 gm in weight (Figure 1). Curd at 90 DAT easier to have riciness (loose or protruding floral parts), bolting, and fuzziness that may reduce the quality appearance and acceptability in consumption (Figure 1). In the current study, lightness (L*) value did not give significant among harvesting day (Table 1). However, chroma (C*) and hue value (h°) value showed high significantly among difference harvesting day. Curd became less white and more saturated; showed creamy-yellowish coloured (lower L*, high C* and high hue values) during increasing day of curd development (Table 1). Floret texture did not show significantly, however stem texture was significantly increased during development of curd (Table 1). Stem texture became harder at 90 DAT. Harvesting at 80-85 DAT gave higher value in SSC, ascorbic acid, percentage inhibition of DPPH, and total phenolic compound (TPC) compared to harvesting at 75 and 90 DAT.

Determination of suitable temperature on quality

Lowland cauliflower stored at 5 °C maintained acceptable visual quality during 3-4 weeks storage. However, after 4 weeks storage, the curd appeared less compact and firm. A brownish discoloration developed in some of the florets. Storage at higher temperatures (10 °C) rapidly cause deterioration of cauliflower quality and shelf life reduced less than 2 weeks. Yellowing of the curd and development of brownish spots were rather faster in cauliflower stored at 28 °C (ambient condition) (data not shown). On day 4-5, the cauliflower firmness reduced. The curd became less white and more yellow (lower L* values), while some brown discoloration was visually apparent after 4 weeks storage duration (Table 2).

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

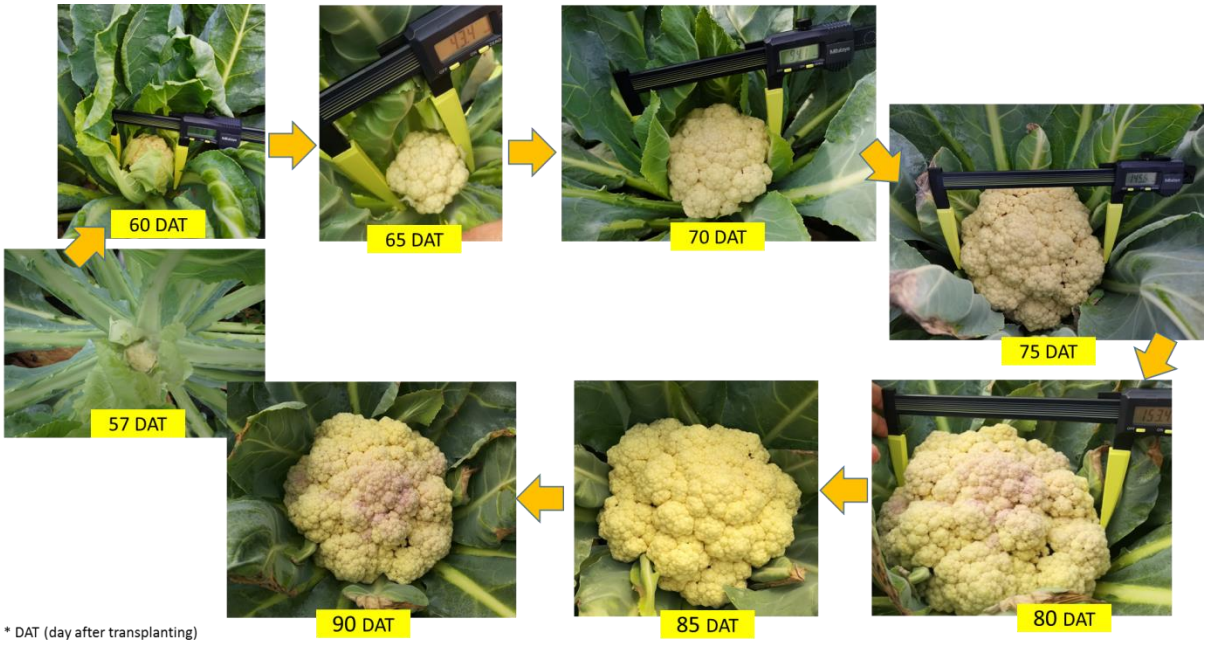

Figure 1: Development of lowland cauliflower's curd.

Table 1: Changes in curd colour, texture curd, soluble solids content (SSC), ascorbic acid content (AAC), % inhibition of DPPH and total phenolic content of lowland cauliflower during development of curd and different harvesting day.

Factor	Curd colour			Floret texture (N)	Stem texture (N)	SSC (%)	Ascorbic acid content (mg/100g)	% DPPH inhibition	GAE (mg/100 g fresh fruit)
Day (D)	L*	C*	h°						
75 DAT	84.70	26.79	91.57^{b}	4.02	6.09^{c}	7.16^{a}	49.79^{b}	63.63^{b}	0.064^{b}
80 DAT	84.04	29.22	92.45^{ab}	4.13	7.67^{b}	7.13^{a}	62.78^{a}	73.77^{a}	0.076^{a}
85 DAT	83.48	30.67	93.72^{a}	4.46	7.24^{b}	7.29^{a}	65.47^{a}	74.53^{a}	0.077^{a}
90 DAT	81.02	30.84	93.69^{a}	4.35	9.25^{a}	6.54b	48.11^{b}	61.60^{b}	0.060^{b}
F-Test significant	ns	*	*	ns	**	*	*	**	**

Means separation within columns and main effect by Duncan's Multiple Range test at p≤0.05. L= lightness; C*= chroma and h° = hue angle; ns, *, ** Non-significant or significant or highly significant at p≤0.05, respectively.*

Table 2: The effect of different temperature and storage duration on physio-chemical properties (curd colour, floret texture, stem texture, soluble solids content (SSC), ascorbic acid content, pH and total titratable acidity (TTA) of lowland cauliflower.

Factor	Curd colour			Floret texture (N)	Stem texture (N)	SSC (%)	Ascorbic acid content (mg/100g)	pH	TTA (% citric acid)
Temperature (T)	L*	C*	h°						
5 °C	83.32	25.02	89.91	4.74	8.32	6.03	66.02a	6.64	0.130
10 °C	83.61	23.87	89.10	4.79	7.90	6.04	59.37b	6.62	0.136
F-Test significant	ns	ns	ns	ns	ns	ns	*	ns	ns
Storage (W)									
0	87.21^{a}	21.88^{b}	91.25^{a}	5.00^{a}	9.40^{a}	6.63^{a}	68.81^{a}	6.57^{cd}	0.143^{a}
1	83.64^{b}	24.09^{ab}	93.23^{a}	5.42^{a}	8.79^{b}	6.27^{ab}	66.22^{a}	6.52^{d}	0.131^{a}
2	82.40^{bc}	25.71^{a}	88.32^{b}	5.06^{a}	8.21^{b}	6.13^{ab}	63.83^{b}	6.63^{bc}	0.134^{a}
3	82.23^{bc}	25.59^{a}	88.86^{b}	4.82^{a}	7.20^{b}	5.81^{bc}	54.82^{c}	6.66^{b}	0.136^{a}
4	81.84^{c}	25.08^{a}	87.92^{b}	3.53^{b}	5.62^{c}	5.38^{c}	53.53^{c}	6.73^{a}	0.105^{b}
F-Test significant	**	*	*	**	**	*	**	*	*
Interaction (T x W)	ns	ns	ns	*	ns	ns	ns	ns	ns

Means separation within columns and main effect by Duncan's Multiple Range test at P≤0.05; L= lightness, C*= chroma and h° = hue angle; ns, *, ** Non-significant or significant or highly significant at P≤0.05, respectively.*

During storage period, there were showed highly significant and decreased gradually in floret and stem texture. This is related to weight loss increased during storage and is significantly affected by temperature and storage duration (Romo-Parada et al., 1989). Ascorbic acid content was highly significant in 5 ℃ compared to 10 ℃ and gradually decreased during storage duration (Table 2). Similar to the finding of Albrecht et al. (1990), ascorbic acid content of cauliflower decreased after 3 weeks, which corresponds to retention of about 97% during storage at 2 ℃ and 95-100% relative humidity. Storage at different temperature did not affected physicochemical quality accept ascorbic acid content and visual appearance. During storage week, we found that all physicochemical quality decreased consistently after 2 weeks storage and showed significantly affected (Table 2).

Conclusion

In summary, the combined optimum harvesting and suitable storage temperature promotes edible quality of lowland cauliflower. Cauliflower store at 5 ℃ maintains better visual quality for longer periods of time (3-4 weeks) compared to storage at higher temperatures. Storage at 10 and 28 ℃ reduce postharvest life of cauliflower to 1-2 weeks and 4 days, respectively, due to wilting, yellowing of the curd and leaves and development of brown spotting.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Chapter 5:
Biotechnology and Nanotechnology

eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Establishment of *In vitro* of Plantlets Production of White Dragon Fruit (*Hylocereus undatus)* using Nodal Segments

Jessica, J.J.A.[1,*], Nurul Fitriyani, M.[1], Noorasmah, S.[1] and Sreeramanan, S.[2]

[1]Department of Crop Science, Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia. Bintulu Sarawak Campus, Nyabau Road, P.O. Box 396, 97008 Bintulu, Sarawak, Malaysia.
[2]School of Biological Sciences, Universiti Sains Malaysia (USM), Georgetown, 11800, Penang Malaysia.
**E-mail: jessica@upm.edu.my*

Introduction

White pitahaya or pitaya blanca has its scientific name that is *Hylocereus undatus* that comes from the botanical family of *Cactaceae*. In Malaysia, it is called '*buah naga*' which means dragon fruit. *Hylocereus* is characterised as a climbing plant with aerial roots that bare a huge berry-like large scales fruit. Moreover, pitahaya tree has columnar and climbing vine. The fruit has white flesh with pink or red skin. The name of undatus is originally come from Latin and it means wavy that referred to the margins of dragon fruit's stem ribs (Eggli and Newton, 2004). Where else, the fruit is called as pitaya which give the meaning ''the scaly fruit'' because of the bracts or scales on the fruit skin (Perween et al., 2018).

Propagation of dragon fruit through direct regeneration by seeds and cuttings is susceptible to disease such as damping-off (Hua et al., 2014). Moreover, traditional propagation of these cacti plants is time consuming as compared to tissue culture protocols. Hence, *in vitro* micropropagation of dragon fruit is appliable to produce disease-free plants for large scale production from relatively small stocks and is the fastest way of propagation than the traditional propagation methods (Lema-Rumińska and Kulus, 2014; Thinesh and Seran, 2014; Qin et al., 2017).

This study was carried out to determine the effect of nodal stem segments size and optimal MS (Murashige and Skoog, 1962) media components for production of multiple plantlets of white dragon fruit.

Materials and Methods

Plant material

Before *in vitro* multiple plantlets of white pitahaya was established, the explant was regenerate *in vitro* in standardised MS media from seeds and grew in culture room. The seeds were taken from white pitahaya fresh fruit purchased in Tai Kiong supermarket in Bintulu, Sarawak. The fruit was brought to Tissue Culture Laboratory of Universiti Putra Malaysia, Bintulu Campus, Sarawak, where all the experiment was carried out. The fresh white dragon fruit were cut off, were brought into laminar flow, and inoculated on magenta boxes containing MS media (1-MS) with 30 g/L sucrose and 1 mg/L BAP. The medium pH was kept between 5.7 to 5.8 prior to autoclaving. The seeds were incubated in culture room at 25 ^{o}C with a 16-hour photoperiod for 4 weeks.

Effect of explants sizes

Nodal stem segment of white pitahaya was selected and excised from 4 weeks old *in vitro* raised seedlings. Explants were excised into two different sizes (3-4 mm and 5-6 mm) inside a laminar flow using sterilized apparatus and explants were cultured in standard full MS media supplemented with optimum concentration of sucrose (30 g/L) and BAP (1 mg/L) and incubated for 2 weeks in a culture room at 25 ^{o}C and a 16 hours photoperiod. The regeneration ability was observed based on growth index analysis.

Effect of MS media strength

The optimal explant sizes were selected and cultured in different strength of 1/2, 1, and 2 MS (Murashige and Skoog, 1962) media supplemented with 20 g/L sucrose and 1 mg/L BAP. The cultures were incubated for 2 weeks in a culture room at 25 ºC with a 16-hour photoperiod. The regeneration ability was observed based on growth index analysis.

Effect of sucrose concentration

Sucrose was used as a source of carbon for *in vitro* multiple plantlets of white pitahaya regeneration. The optimal size of stem node was selected and cultured in optimal full MS supplemented with five different sucrose concentrations 0 (control treatment), 10, 20, 30, and 40 g/L (Zahara et al., 2017) and 1 mg/L BAP. The incubation period was 2 weeks in a culture room at 25±2 ºC and 16 hours photoperiod. The regeneration ability was observed based on growth index analysis.

Effect of cytokinin

Cytokinin was used as shoot induction hormone. The optimal size of stem node was selected and cultured in optimal MS media. The optimum size of explant was cultured on media supplemented with standardized sucrose concentration from previous experiments with various BAP concentrations, 0.0 (control treatment), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L. Cytokinin was added in the medium based on Dahanayake and Ranawake (2011) with modifications. The incubation period was 2 weeks in a culture room at 25±2 ºC for a 16 hours photoperiod. The regeneration ability was observed based on growth index analysis.

Data collection and analysis

The regeneration ability of *in vitro* multiple plantlets of explants were observed and analysed. After 2 weeks of incubation, the growth index analysis was calculated using the following formula (Atawia et al., 2016):

Growth index = ([final weight - initial weight] / initial weight)

Statistical analysis

All experiments were conducted in a completely randomized design (CRD). Each treatment comprises three replicates with five explants. The growth index results were reported as mean ± standard error (SE) from all three replicates. Experimental data were analysed using one-way analysis of variance (ANOVA). Significance difference ($P<0.05$) between means of treatments were identified using Duncan's multiple range test (DMRT). Statistical analysis was performed using SPPS statistical software package.

Results and Discussion

Effect of explants sizes

Explants with the size of 5-6 mm resulted in the highest growth index compared to those of 3-4 mm size (Figures 1 and 2). The growth index for 5-6 mm explant size after 2 weeks of incubation in culture room was highest with the mean value of 3.42 ± 0.40^{a} compared to 3-4 mm explant size, 1.24 ± 0.16^{a}.

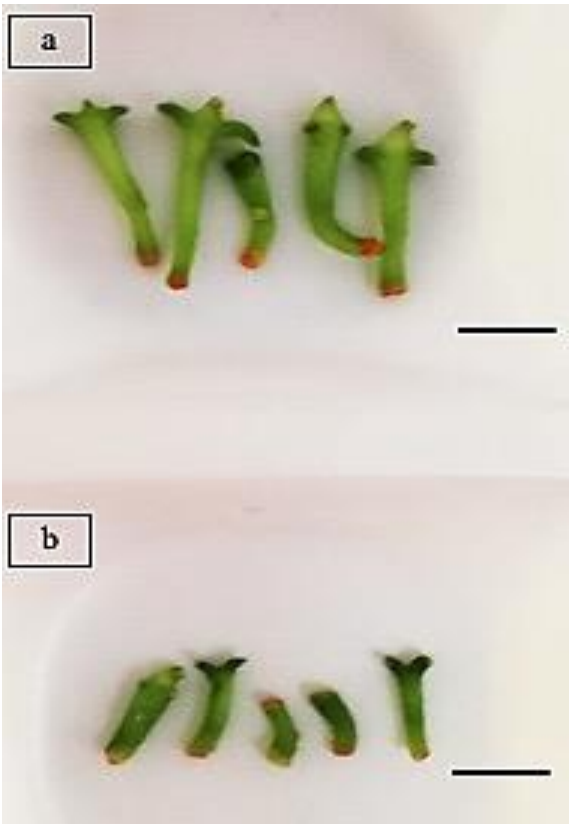

Figure 1: The explants showing shoot formation after 2 weeks of incubation; a) 5-6 mm and b) 3-4 mm explant sizes. Scale bar = 1 cm.

The size of explant plays a vital role towards the growth of multiple plantlets *in vitro* culture medium. Explants with size 5 mm shoot tips were reported to be highly efficient *in vitro* propagation in *Coryphantha elephantidens*. The nodal explant is one plant part that is suitable to regenerate multiple plantlets. The structure of nodal stem segment consists of meristematic cells containing undifferentiated cells capable of cell division and totipotency of cells that are able to generate into whole plantlets (Earle, 1974). In addition, previous studies on shoot regeneration of *Spilanthes mauritiana*, three explants viz. shoot tip, nodal segments and leaf explants were found to significantly influenced direct shoot regeneration *in vitro* (Sharma et al., 2009).

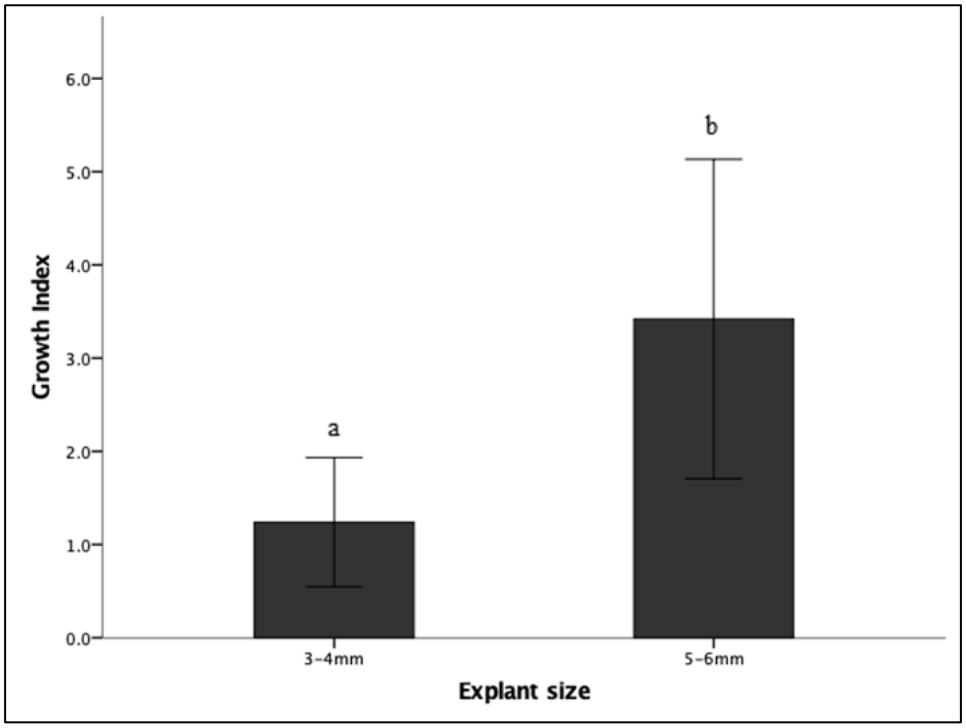

Figure 2: The growth index of *Hylocereus undatus* from different explant size.

Effects of MS media strength on growth index and shoot length

Media strength of 2 MS resulted with the highest growth index following 2 weeks of incubation of explants under 16 hours photoperiod. In contrast, both full and half MS media strength resulted with the lowest growth index at 1.81±0.33 and 1.72±0.14, respectively (Figure 3).

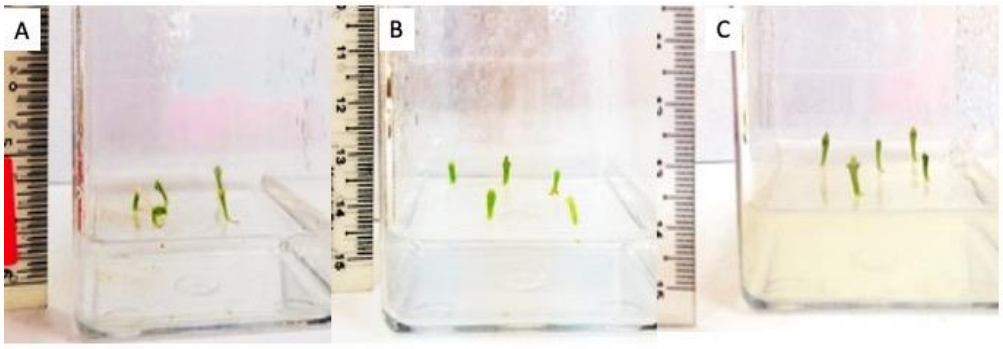

Figure 3: The shoot regeneration of nodal stem segment on different MS strengths; (A) ½ MS (B) 1 MS and (C) 2 MS of *Hylocereus undatus* after 2 weeks incubation.

MS media is composed of salts and is vital in plant tissue culture. The strength of MS media influences the growth and development of plant species. The nodal stem segment taken from *in vitro* germinated seedlings of *H. undatus* survived well in double MS strength as it grew larger after 2 weeks of incubation compared to half and full MS, although all explants had equal standardized initial size (5-6 mm). The *H. undatus* species showed that it could tolerate high salt concentration. As reported by Rahman et al. (2015), several plant species grow well in MS media that contained high amount of salt concentration.

Effects of sucrose concentration on growth index and shoot length

The results on the effect of different sucrose concentration on the growth elongation of nodal stem segments were taken after 2 weeks of incubation. Explants cultured on 2 MS medium strength supplemented with 30 g/L of sucrose exhibited the highest number of growth index at 4.23±0.15. On the other hand, explants cultured on 2 MS media supplemented with 20 g/Lof sucrose and without sucrose exhibited lowest growth index valued as 1.94±0.34 and 1.27±0.10, respectively (Figure 4). Medium supplemented with 30 g/L of sucrose was the best concentration of carbon source to produce multiple plantlets in white dragon fruit due to vigorous growth of explants compared to other sucrose concentrations tested.

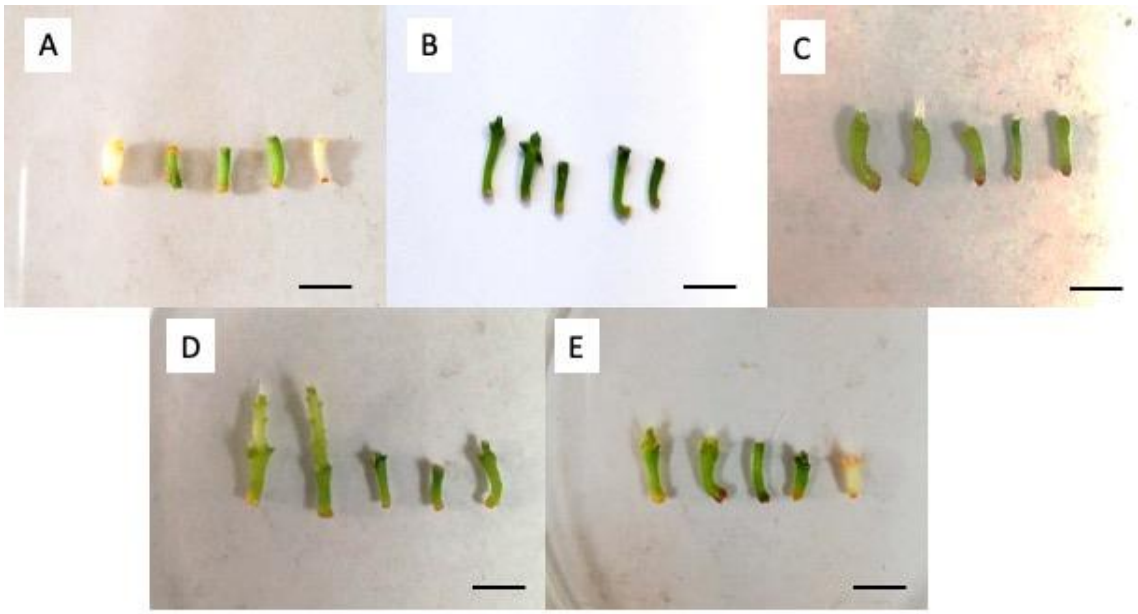

Figure 4: The shoot regeneration of nodal explant on different sucrose concentration. Results taken after 2 weeks of culturing; (A) 0 g/L (B) 10 g/L (C) 20 g/L (D) 30 g/L (E) 40 g/L. Scale bar = 1 cm.

Sucrose is a very important component in any nutrient medium used in plant cell, tissue, and organ culture. The sucrose acts as carbon source and its addition are vital for ensuring optimal development of explant (Zahara et al., 2017). The amount of sugar concentration chosen like sucrose or glucose is dependent on the type and age of explant, for instance very young embryos need a relatively high sugar concentration. However, for some plant species, increasing the concentration of sucrose in nutrient media above the adequate level will decrease the rate of shoot growth and development, as well as biomass accumulation of cultured cells will be retarded (Zahara et al., 2017). Therefore, sugar concentration in MS medium depends on plant species requirements for carbon source.

Effects of BAP concentration on shoot length

The nodal segments cultured on medium supplemented with 2.5 mg/L BAP exhibited significantly highest growth index at 2.06±0.17 followed by 1.0 mg/L BAP and 2.0 mg/L BAP with 1.38±0.22 and 1.45±0.20, respectively (Figure 5). Meanwhile, the lowest mean growth index response was obtained in explant cultured on standardised medium supplemented with 0.0 mg/L BAP (control treatment) with growth index valued at 0.29±0.22.

The optimal amount of cytokinin is essential in enhancing the cells to divide properly. For this study, BAP was used for induction of multiple plantlets. Bhau and Wakhlu (2015) reported that BAP was a better choice for multiple shoots induction and proliferation for cacteceae family as compared to kinetin.

Meanwhile, in a previous study on *in vitro* of *H. costaricensis*, the addition of BAP concentrations was essential for explant bud sprouting and shoot growth and proliferations with optimal concentrations of 15 and 30 μM, respectively (Vinaz et al., 2012). The explants cultured on MS media added with 2.3 mg/L of BAP lead to high frequency of shoot formation of *Citrullus lanatus* after 6 weeks of incubation (Kavitah and Fahrul, 2010). In this study, 2.5 mg/L of BAP exhibited highest growth index of the cultured nodal stem segments of *H. undatus* following 2 weeks of incubation.

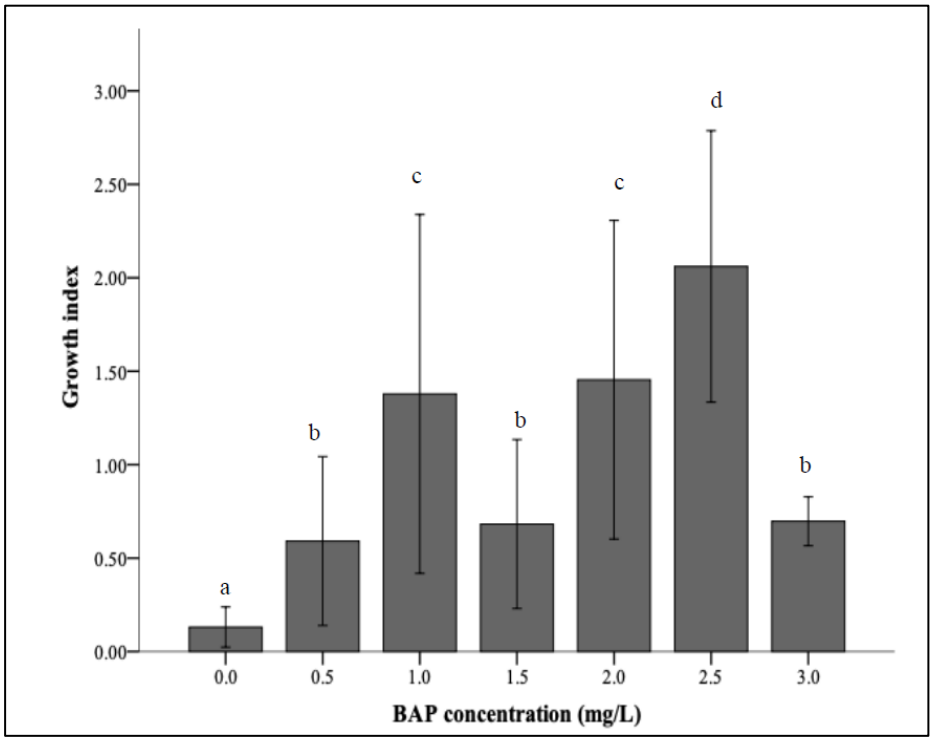

Figure 5: The effects of difference BAP concentrations on growth index of *Hylocereus undatus* nodal stem segment.

Conclusions

The results showed that optimal size of nodal stem segments that produced maximum growth for *in vitro* culture of *H. undatus* was 5-6 mm. The most effective MS medium components for enhancing the development and growth of nodal segment explants were with 2 MS media supplemented with 30 g/L of sucrose and 2.5 mg/L BAP.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Preliminary Study on *In vitro* Propagation of *Intsia palembanica* (Merbau)

Nor Rashidah, M.*, Nashatul Zaimah, N.A., Nadiah Salmi, N., Nor Asmah, H., Noraliza, A., Fadzlinah, Z. and Muhammad Alif Daniel, M.S.

Biotechnology Programme, Forestry Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: norrashidah@frim.gov.my*

Introduction

Intsia palembanica or Merbau is undoubtedly one of the most highly valued trees in the western Pacific and Indo-Malaysian region, both in terms of its traditional cultural importance and its value for commercial timber. Its durability, favorable physical and mechanical properties, ease of machining, and attractive dark red-brown color are especially favoured for use in house construction, furniture, canoe making, pavilions, stairs, parquet flooring, outdoor furniture, weathering boards, and woodcarving of valuable cultural artifacts such as kava bowls and weapons. The heartwood of Merbau is extremely dense (641-961 kg/m^3), has low shrinkage movement over time, and good insect repellent property. However, it has a disadvantage in some applications. Merbau is quite an oily timber where its extractives is water soluble and can be readily leached out to stain adjacent materials.

Conventional propagation of *I. palembanica* is through seeds and vegetative cuttings. The propagation rate through seed germination is unreliable due long generation cycle of seed production. An alternative approach to overcome the propagation limitation is the mass propagation through tissue culture using the available seeds as explants. The viability of the seed lot was determined by the germination tests done according to International Seed Testing Association standard (ISTA, 2006) at the Seed Technology Laboratory, FRIM.

Plant tissue culture is a system of growing plant cells, tissue, or organs, that have been separated from the mother plant (called explants) in artificial medium under aseptic condition (Omamor et al., 2007). Although aseptic conditions are usually employed, plant cultures may not stay aseptic *in vitro*. Microbes can be present in the explants (endophytic) or can be reintroduced from poor aseptic handling, unhygienic conditions in the laboratory or from laboratory instruments. Microbial contamination is a common problem, which often compromises development of *in vitro* cultures (Webster et al., 2003). These microbes compete adversely with plant tissue cultures for nutrients, and their presence often results in increased culture mortality or can also result in variable growth, tissue necrosis, reduced shoot proliferation, and reduced rooting (Oyebanji et al., 2009).

Instia palembanica is generally propagated by seeds which can cause genetic variations which, as a result, influence the content of the active principles present in plants. Micropropagation was a valuable instrument for collecting more standardised seedlings on a broad scale in less time and region for many forest species of economic importance or at risk of extinction (Dousseau et al., 2008). Through micropropagation *in vitro* crops is a viable way to multiply various native species, to form homogeneous crop populations and hence, to allow highly healthy and vigorous seedlings to be produced (Souza and others, 2007). The problem is exacerbated when explants are sourced directly from field grown plants (Odutayo et al., 2007).

The aim of this study was to investigate and identify the most effective sterilization technique for *I. palembanica* obtained from the field using seed explants.

Materials and Methods

Surface sterilization of seed

Fruits of *I. palembanica* were collected originally from selected mother trees in Forestry Research Institute of Malaysia (FRIM). The fruit of *I. palembanica* is flat, oval, smooth, and dark brown when ripe and contains 3 to 4 flat and thick seeds measuring 3.0-4.5 cm long and 1.5-3.0 cm wide.

The seeds were first washed with 0.1% (w/v) Benomyl with 1 drop of Tween 20 for 30 min. They were then surface sterilized using commercial bleach, Clorox with 1 drop of Tween 20 at three different concentrations [Method 1: 70% (v/v), Method 2: 50% (v/v) and Method 3: 30% (v/v)] for 30 min and rinse 3-5 times with sterile distilled water. The seeds are cut into smaller pieces without injuring the embryo and were cultured on basal half strength of Woody Plant Medium, ½ WPM (Lloyd and McCown, 1981) and maintained at 24± 2 °C in the growth chamber. In each treatment, the experiment had 10 replicates. They were kept in a growth chamber and maintained for 8 weeks. Observations on the percentage of clean cultures, germination, and survival rate were recorded.

Shoot induction media

The best sterilization technique was repeated for shoot induction experiment. Two different basal media at half strength were prepared to determine the suitable medium for *I. palembanica* culture; ½ WPM (half strength concentrations of the major and minor salts of the WPM medium) basal and ½ MS (half strength concentrations of the major and minor salts of the MS medium) basal media. WPM and MS (Murashige and Skoog, 1962) basal medium (stock powder) were obtained from Duchefa Biochemie (WPM Product No: M0220.0050; MS Product No; M0222.0050) were used. These media were solidified with 3% (w/v) gelrite agar and 30 g/L of sucrose as carbon source. The pH was adjusted to 5.8 prior to autoclaving at 121 °C for 15 min. *Instia palembanica* cultures were kept at 24±2 °C with 16 h light and 8 h dark. The morphology of explants in each medium was observed and recorded after 4 weeks.

Medium preparation for shoot multiplication

Half strength WPM basal medium supplemented with two different concentrations of benzyl-amino-purine (BAP: 0.1 mg/L and 0.5 mg/L) were tested for shoot multiplication. WPM basal medium without an addition of plant growth regulator were used as a control. Each treatment was repeated two times. All media were solidified with 3% (w/v) of gelrite agar and 30 g/L of sucrose as carbon source. The pH was adjusted to 5.8 prior to sterilization at 121 °C for 15 min. Cultures were maintained at 24±2 °C with 16 h light and 8 h dark. Number of new shoots, plant height, and root formation were observed and recorded after 4 weeks.

Results and Discussion

Seed surface sterilization

Seeds of *I. palembanica* were used as source of explants materials. Several criteria are important for the establishment of *in vitro* cultures, such as type of explant and the nutrient medium. While technically, any tissue may be used as an explant source, certain aspects of the most effective morphogenic processes of interest must be considered and checked (Grattapaglia and Machado, 1998). In micropropagation, the use of effective methods of disinfestation and germination, *in vitro*, of seeds, enables the development of aseptic plants that provide contaminant-free propagules which can be used for multiplication and eventual rooting *in vitro* or *ex vitro* (Grattapaglia and Machado, 1998). Different surface sterilization methods were used to determine the most effective method for culturing of *I. palembanica* seeds *in vitro*. Three different concentrations of commercial bleach, hypochlorite-sodium (NaOCl) Clorox® were tested. The 70% (v/v) hypochlorite-sodium and 0.1% (w/v) benomyl combination showed the highest

percentage of clean cultures and responses were observed. After 6 days in culture, the seeds started to germinate. Some of the seeds broke their seed coats and emergence of radicle were also observed. After 14 days in culture, the explants developed into normal and complete seedlings.

Table 1: Survival rate of *Instia palembanica* explants in the ½ WPM media.

No.	Treatments	Survival rate (%)	Day(s) taken for seeds to germinate
1	70% hypochlorite-sodium (NaOCl) + Tween 20	90	6
2	50% hypochlorite-sodium (NaOCl) + Tween 20	50	6
3	30% hypochlorite-sodium (NaOCl) + Tween 20	20	6

Shoot induction media

Suitable medium for micropropagation of *I. palembanica* need to be determined. Explant in both media maintained clean. However, *I. palembanica* explants cultured in MS medium become browning and eventually died after 8 weeks and showed no response of shoot multiplication. From the observation, half strength WPM basal medium showed positive responses on *I. palembanica* survivability based on the length of shoots and number of the leaves as well as the colour of the leaves. Therefore, it is more suitable for *I. palembanica* cultures compared to half strength MS basal media. The difference between the WPM basal medium and the MS basal medium is the concentration of the nutrient or salt. The content of nutrient or salt in the medium was an essential element in the differentiation of cells, development, elongation, and other processes of metabolism. The salt and sugar concentrations determined the media's osmotic properties. MS basal medium is commonly used for many plant species but *I. palembanica* did not respond well on MS. WPM basal contained less salt, 25% of nitrate and ammonium ion, but higher potassium and sulphate ion than MS basal media.

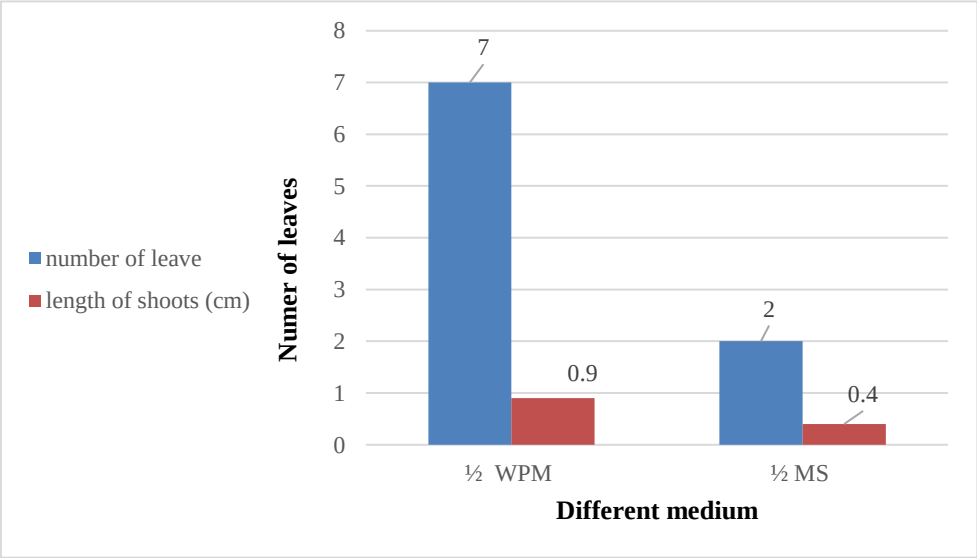

Figure 1: Effect of MS and WPM culture media for shoot induction of *I. palembanica*, after 8 weeks of culture.

Shoot multiplication

Half strength WPM, medium supplemented with BAP at the concentrations of 0, 0.1, and 0.5 mg/L were used for further experiment on shoot multiplication. Due to very limited explant sources, only two BAP concentrations were used. Figure 2 showed overall results obtained from shoot multiplication of *I. palembanica* at different concentrations of BAP. Results showed that at 0.1 mg/L BAP, 50% of the explants were induced to produce single new shoot per explant after 2 weeks in culture. However, it was

also observed that WPM medium without plant growth regulator showed healthier plantlets without any shoot initiation. No roots were observed in any media used.

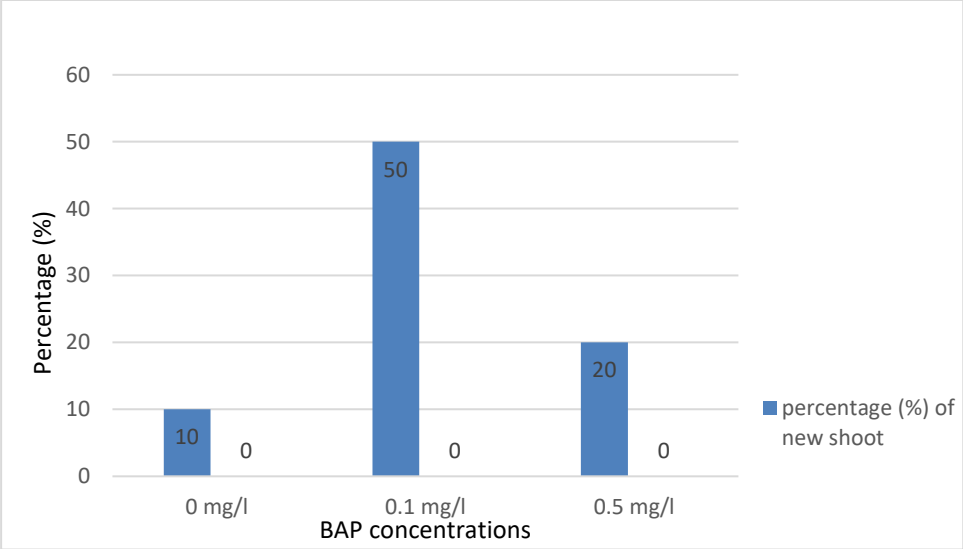

Figure 2: Effect of ½ WPM medium supplemented with different concentration of BAP on shoot and root development of *I. palembanica.*

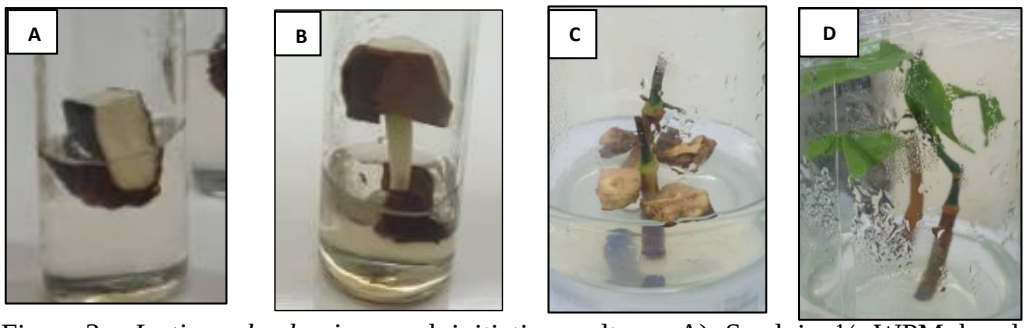

Figure 3: *Instia palembanica* seed initiation culture; A) Seed in ½ WPM basal media; B) Germinated seed with elongated radicle; C) Shoot development with the seed coats detached and D) Well developed shoot in ½ WPM + 0.1 mg/L BAP.

Conclusion

This is the first successful attempt to establish suitable surface sterilization method and direct shoot development of *I. palembanica.* The multiplication medium has also been determined. However, the next crucial step is the acclimatization of the plantlets in the nursery. The acclimatization experiment is needed to complete the *I. palembanica in vitro* propagation protocol.

Acknowledgement

The authors are thankful to Forest Research Institute Malaysia (FRIM) for providing the facilities and funding for this study. We also greatly acknowledge the effort and support from all the staff of the Seed Technology Laboratory, Biotechnology Division and Forest Research Institute Malaysia in completing this study.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Survival of *Eusideroxylon zwageri* (Belian) Shoot-tips in Liquid Nitrogen using Encapsulation-dehydration

Nashatul Zaimah, N.A.*, Rosdi, K., Nor Asmah, H., Noraliza, A., Nadiah Salmi, N., Nor Rashidah, M., Muhammad Alif, D. and Fadzlinah, Z.

Forest Biotechnology Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia.
**E-mail: nashatul@frim.gov.my*

Introduction

Eusideroxylon zwageri is a monotypic species in its genus *Eusideroxylon*. The tree is evergreen, tall with straight bole, and naturally found in Indonesia (Kalimantan and Sumatera), Philippines, and Malaysia's low land mixed dipterocarp forests in Sabah and Sarawak (Yong et al., 2011). In the Peninsula Malaysia, this species is known as Belian.

Also known as "Borneo Ironwood" this official tree of Sarawak produces heavy, hard, strong, and water-resistant timber, making it of high market value. No doubt its timber is popular and mostly sought for outdoor construction as well as in marine construction. As a result, this species is still decreasing in natural forests. *Eusideroxylon zwageri* has been assessed as vulnerable by the IUCN Red List of Threatened Species ver. 2.3, 1998 due to habitat degradation, habitat loss, and over exploitation of its timber (IUCN, 1998) in which in the medium-term future this species may have been extinct in the wild (Gibson and Rebicca, 2016).

Data gathered from a phenological observation conducted in Forest Research Institute Malaysia (FRIM) shows that flowering period of this tree is short, usually lasts for 3 to 4 months. Flowering of this tree starts in the beginning of a dry season between March and June. The fruiting season begins in July extended until December and continued to early of the following year. Mature *E. zwageri* fruits are brown with ovoid or globular shape and each fruit contains one seed. Seeds are large with hard seed-coats. Germination is slow, usually 6 to 12 months and this is caused by the seeds surface impermeability (Purba et al., 2019) and high accumulation of endogenous abscisic acid in mature seeds which also inhibits seed germination (Lee et al., 2007). On the other hand, our data shows 60% of *E. zwageri* seeds germinated after 3 months by extracting and cracking the seed out from the fruit (unpublished data).

Research study on the application of *ex-situ* conservation of *E. zwageri* is still limited. Cryopreservation is the preservation of genetic resources in liquid nitrogen, at ultra-low temperature of -196 °C offers an alternative and efficient method for the long-term storage of this species. At this temperature, all metabolic processes are stopped (Dereuddre et al., 1988) and thus is an ideal tool that contributes to phenotypic and genotypic stability. In addition, it requires minimum space, no sophisticated equipment needed and low-cost maintenance (Suzuki et al., 2008). In cryopreservation, the encapsulation-dehydration is widely used as it is easy to perform, less time consuming (Sakai, 2000). This technique has been successfully applied to shoot-tips of various plant species such as Malus (Niino and Sakai, 1992; Paul et al., 2000), Morus (Niino and Sakai, 1992; Gupta, 2011), *Pyrus communis* (Dereuddre et al., 1990), Rubus (Wang et al., 2005), Apricot (Soliman, 2013), Eucalyptus (Pâques et al., 2002), Raspberry (Wang et al., 2005) and *Hladnikia pastinacifolia* (Ciringer et al., 2018) but lack on research for tropical forest tree species.

In tissue culture, Murashige and Skoog medium (MS) (Murashige and Skoog, 1962) is mostly used as a basic medium for callus and shoot induction for most herbaceous plants. However, there are other recipes such as Woody Plant Medium (WPM) developed by Lloyd and McCown (1980) which was specifically formulated for woody species. Basal Woody Plant Medium (WPM) was found suitable for *in vitro* propagation of blueberry (Gonzalez et al., 2000), *Myrica esculenta* (Bhatt and Dhar, 2004) and *Shorea robusta* (Singh et al., 2014).

Therefore, the aim of this present study is to evaluate the possibility of utilizing cryopreservation of an endemic *E. zwageri* by using the encapsulation-dehydration technique and also, to identify a suitable culture medium for encapsulated *E. zwageri* shoot-tips.

Materials and Methods

Plant materials and surface sterilization

Samples of *E. zwageri* young shoots were obtained from healthy branches of matured *E. zwageri* trees located in FRIM. They were rinsed under running tap water and left overnight. Surface sterilization was carried out by immersion in 70% (v/v) ethanol for 5 min followed by 50% (v/v) Chlorox ® for 15 min and washed five times with sterile distilled water. Shoot-tips (size 3-5 mm) were excised and placed on moistened filter paper prior encapsulation.

Cryopreservation (encapsulation-dehydration)

Encapsulation was carried out by placing the excised shoot-tips in 2% (v/v) Na-alginate solution supplemented with 0.4 M sucrose prepared in Ca-free liquid MS (Murashige and Skoog, 1962) medium. A sterile 2 mL pipette was used to dispense a droplet of bead solution containing one shoot-tip into 0.1 M $CaCl_2$ solution and allowed to polymerize for 30 mins. The obtained beads were rinsed thrice with sterile distilled water and surface dried on sterilized paper filter.

To determine the optimal dehydration time, the beads were arranged on sterilize filter paper in sealed 9 cm glass petri dishes containing 30 g sterile silica gel and dried for 0, 2, 4, and 6 hours at room temperature. After each desiccation time, five beads were weighed individually, dried in an oven at 103 °C for 17 hours and re-weighed for moisture content determination. The moisture content was calculated using a formula: MC = [(Fresh weight – dry weight)] / Fresh weight] x 100%. For cryopreservation, ten dehydrated beads were placed in 2 mL polypropylene sterile cryotubes (Nalgene, five beads per tube) and plunged directly from room temperature into liquid nitrogen and left for 1 day. The cryopreserved beads were thawed rapidly in a 40 °C water bath for 10 min. For control, the beads were directly transferred to culture mediums after each desiccation time. To identify suitable culture medium, the beads were placed on two different medium; Murashige and Skoog (MS) and Woody Plant Medium (WPM) (Lloyds and McCown, 1980) without additional plant growth hormone. Each treatment was repeated three times. The cultures were maintained at 25 ºC under 16-h photo period provided by cool-white fluorescent lamps (3000 lux). The viability of the encapsulated shoot-tips was assessed based on the greening of the shoot-tips after four weeks. The results are expressed as mean ± one standard error (SE) of three replicates.

Results and Discussion

Figure 1 shows the loss of moisture content of encapsulated *E. zwageri* shoot-tips at different desiccation time. There was a drastic reduction in moisture content after 2 hours desiccation where almost 50% of the moisture was lost; from 58.89% (initial moisture at 0 hour) to 24.28%. When further desiccated to 4 and 6 hours, gradual decrease in the moisture content of the bead was noted, to 22.34% and 16.34% respectively.

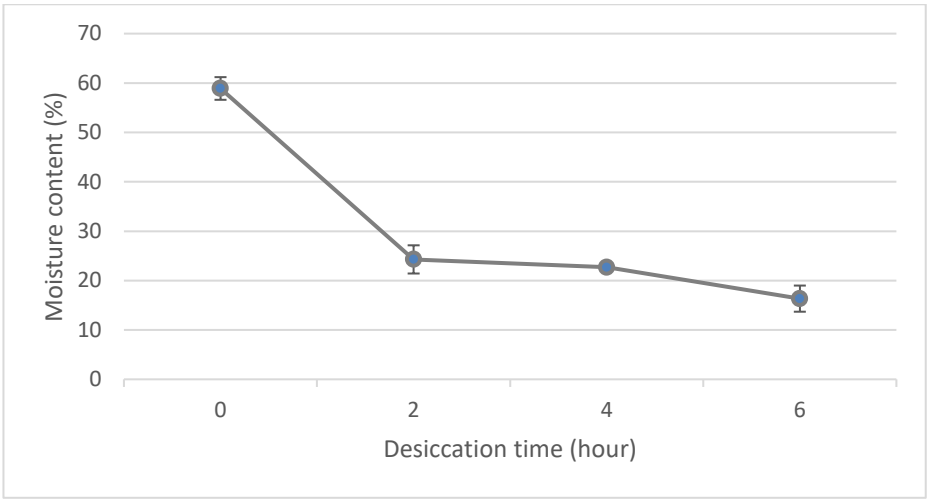

Figure 1: Changes in moisture content of encapsulated *E. zwageri* shoot-tips during desiccation in silica gel for 0-6 hours. Bars represent standard error.

Figure 2 shows the survival of non-cryopreserved beads of *E. zwageri* cultured on two different medium; Murashige and Skoog (MS), and Woody Plant Medium (WPM). When cultured on MS medium, all beads did not survive all desiccation times except at 4 hours but the survival was very low (10%). However, survival was high (80-100%) at all desiccation times when the beads were cultured on WPM. This observation indicates that WPM is more suitable than MS as a culture medium for encapsulated *E. zwageri* shoot-tips. Generally, formulation of WPM contains low total ion content; but high sulphate and magnesium content as compared to MS medium which has high ion concentration; mainly nitrogen, potassium zinc, and chlorine (Leifert et al., 1995). In this study, survival was observed in WPM which indicates that high concentration of macroelements in WPM are essential in supporting the tissue growth of *E. zwageri* shoot-tips. This also means that *E. zwageri* shoot-tips are sensitive to high concentration of macroelements as contained in the MS medium.

However, tissue culture studies on this species used MS as its basal medium (Gibson and Rebicca, 2016; Tarampak et al., 2019). It is suggested that further tissue culture work needs to be conducted mainly to compare the effectiveness of MS and WPM medium in establishing *in vitro* cultures of *E. zwageri.*

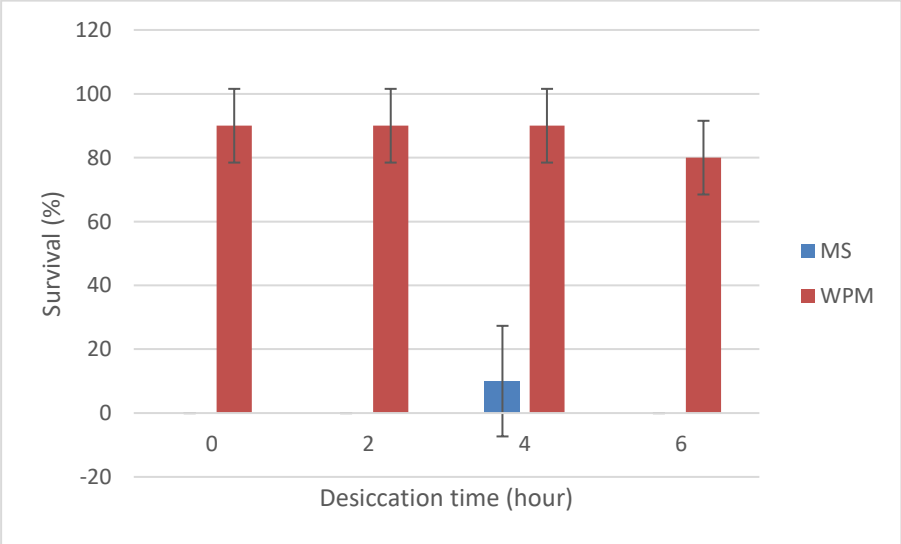

Figure 2: Survival of encapsulated non-cryopreserved *E. zwageri* shoot-tips on Murashige and Skoog (MS) and Woody Plant Medium (WPM). Bars represent standard error.

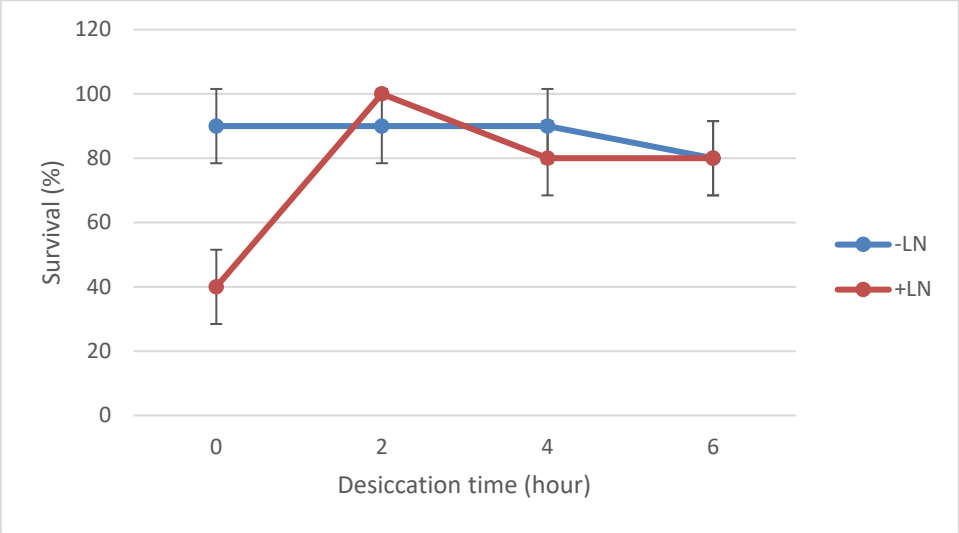

Figure 3: Survival percentages of non-cryopreserved (-LN) and cryopreserved (+LN) encapsulated *E. zwageri* shoot-tips after desiccation (0-6 hours) cultured on WPM medium. Bars represent standard error.

Survival percentages of encapsulated *E. zwageri* shoot-tips without (-LN) and with cryopreservation (+LN) cultured on WPM medium is shown in Figure 3. For non-cryopreserved (–LN) *E. zwageri* shoot-tips, 90% survival was observed after the beads were desiccated from 0 to 4 hours (22.70%) and 80% survival after 6 hours desiccation in which the beads attained 16.34%. For cryopreserved beads (+LN) the highest survival (100%) was obtained after 2 hours desiccation where the beads achieved 24.28% moisture content. High survivals (80%) were also obtained for cryopreserved beads desiccated for 4 and 6 hours in which the moisture content was at 22.70% and 16.34% respectively. In general, this finding shows that as the moisture content reduced to around 22 to 24%, the viability of cryopreserved beads increased.

Uragami et al. (1990) found that for explants to survive cryopreservation, the moisture content has to be in the range of 15 to 25% in order to avoid crystallization of the cells (Volk and Walters, 2006). According to Dereuddre et al. (1990) the optimum bead moisture content for shoot-tips preserved by the encapsulation-dehydration technique is normally about 20% in which at this moisture level vitrification occurs and stable glass was formed during thawing (Dumet et al., 2000). This explains the low viability (40%) of *E. zwageri* beads when cryopreserved at higher moisture content (>50%) where at this level crystallization of the water in the cells may have taken place and was harmful to the shoot-tips. Thus, it is important to identify the safest moisture content of the explant that survives cryopreservation. In our study, it was found that using encapsulation and dehydration technique survival of encapsulated *E. zwageri* shoot-tips in liquid nitrogen was achieved at the moisture content of 22 to 24%. Similar findings a study by Gupta (2011) shows encapsulated and cryopreserved Morus shoot-tip showed 30-35% regrowth when dehydrated to about 20% moisture content. In addition, Philippine taro shoot-tips, at about 20% of moisture, viability was between 28-33% after 3 days on regrowth medium (Acedo et al., 2018). Similar observations were observed when encapsulated pear shoot-tips at 25% moisture content survived cryopreservation with 43-55% regeneration rate (Jung et al., 2017).

Conclusions

This study demonstrated the potential for the use of cryopreservation in long-term storage of an endemic *E. zwageri* shoot-tips by utilizing the encapsulation-dehydration method. The results also showed that cryopreserved beads with 22-24% moisture content showed 100% viability and this was observed on WPM.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Cryopreservation of *In vitro* Grown Taro (*Colocasia esculenta*) Apical Meristem using a Droplet-vitrification PVS3 Technique

Noor Camellia, N.A.*, Abdul Muhaimin, A.K., Umikalsum, M.B. and Rozlaily, Z.
Pusat Penyelidikan Tanaman Industri, Ibu Pejabat MARDI, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.
**E-mail: camellia@mardi.gov.my*

Introduction

Taro (*Colocasia esculenta*) has great potential as source of carbohydrate. It is a staple food in the Pacific and many regions in developing countries. Almost all parts including the corms, leaves and stems can be eaten (Sant et al., 2006). It is immensely cultivated in Southeast Asia, East Asia, and the Pacific Islands and the corms are exported worldwide (Macharia et al., 2014). Symptoms resulting from disease infection include taro leaf blight (TLB) caused by the pseudo-fungus, *Phytophthora colocasiae*, attacks the leaves and stems of the taro leading to either stunting or failure to produce a corm. The disease incidence was reported infecting around 50% in Ghana. Severity of the disease was significantly higher during the rainy period (Abdulai et al., 2020). Serious outbreaks of taro leaf blight in Samoa in 1993 caused an almost 100% loss of the crop, threatening both food security and traditional cultural practices (Alexandra et al., 2020). This factor continues to have an impact on the genetic diversity. Development of resistant varieties through breeding via crossing or selection is required to conserve the genetic diversity. There is a growing need for stable long-term *in vitro* storage of the germplasm collections of taro (Taylor et al., 2010).

Cryopreservation seems a convincing alternative method to store vegetatively propagated crop species. Cryopreservation employs storage at ultralow temperatures using liquid nitrogen (-196 °C). At this temperature, all cellular division and metabolic processes are suspended, with minimal impact on genetic stability. Theoretically, plant material can thus be stored without any sub-culturing for an unlimited time. Moreover, cultures are stored in a small volume, protected from contamination, and require minimal maintenance (Withers and Engels, 1990; Maxted et al., 1997). Cryopreservation offers the safest, cost-effective option for long-term storage of tuber crop. Thus, cryopreservation seems to be a promising method for virus elimination. Plum Pox Potyvirus (PPV), cucumber mosaic virus (CMV) and banana streak virus (BSV) are among the successful eliminated viruses by cryopreservation of shoot tips (Brison et al., 1997; Helliot et al., 2002). The key to success in cryopreservation is achievable through dehydration tolerance (Panis et al., 2005). Therefore, optimum dehydration stage in cryopreservation is an essential criterion that needs to be studied. The method described in this study is the first report of long-term storage of taro in Malaysia. Therefore, the main aim of this study was to optimize the survival percentage of cryopreserved apical meristem of taro using droplet-vitrification protocol.

Materials and Methods

Plant materials

In vitro stock plants of taro were initiated from 2-3 cm long sterilized shoot tips of the field-grown corm and were multiplied by sub-cultures performed periodically on solidified Murashige and Skoog (MS) (1962) medium supplemented with 2.0 mg/L BAP. The cultures were incubated under photoperiod 16/8 h at 25 °C (Noor Camellia et al., 2020).

Vitrification procedure

Apical meristems (0.8-1.0 mm) were excised from 3 months old in vitro plants using scalpel blades no: 11 and a stereomicroscope (x 80) under aseptic conditions (Figure 1). The shoot tips were precultured

on MS medium supplemented with 0.3 M sucrose in a petri dish for 16 hours (overnight) under dark conditions at 25 °C. The precultured explants were osmo-protected with four types of loading solutions in petri dishes for 20 min and untreated as control. The explants were then exposed to cryoprotective solutions PVS3 [50% glycerol and 50% sucrose] for 0 to 60 min. Explants were placed in a droplet of PVS3 on aluminium foil strips (1 x 5 cm) that were placed on ice. The foil strips were then plunged directly in liquid nitrogen prior to storage for at least 2 hours. For recovery, foil strips with explants were rapidly rewarmed to unloading solution (MS medium supplemented with 0.8 M sucrose) following Kim (2012) at 40 °C for 30 seconds and thawing with the same solution at 25 °C for 40 min or rewarmed and thawing in unloading solution (MS medium supplemented with 1.2 M sucrose) following Sakai et al. (1990) at 25 °C for 15 min. The explants retrieved from unloading solution were blotted on double disc sterile filter paper over solidified MS medium supplemented with 0.3 M sucrose in a petri dish for 2 days, the explants were sub-cultured to recovery medium containing MS medium supplemented with 0.1 M sucrose in petri dish. The cultures were kept under dark condition for 15 days after rewarming.

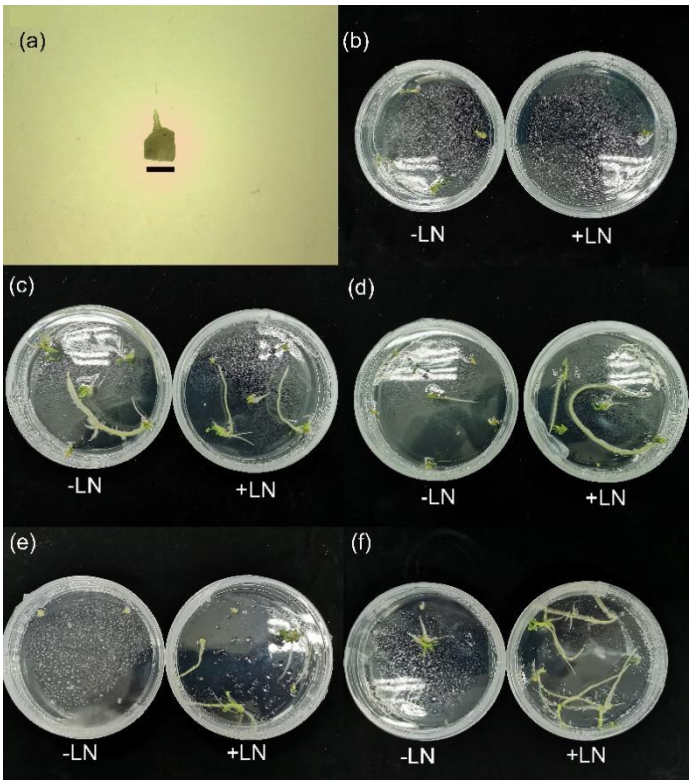

Figure 1: (a) Dissected apical meristem of taro consisting of meristematic dome and one or two leaf primordia (Bar =1 mm). Growth of cryopreserved (+LN) and non-cryopreserved (-LN) of taro apical meristem after treated in various loading solutions, (b) Control (untreated), (c) 2.0 M glycerol, (d) 2.0 M glycerol + 5% DMSO, (e) 1.5 M glycerol and (f) 1.5 M glycerol + 5% DMSO.

Recovery assessment and statistical analysis

Apical meristem that showed any signs of regrowth or greening within 4 weeks of incubation were considered to survive. Regenerated cryopreserved apical meristems were acclimatized 4 weeks on 100% peat moss media following cryopreservation and transferred to plastic pot to observe the growth. In all experiments, six apical meristems were used per experimental condition and experiments were replicated 3 times. The results were subjected to analysis of variance (ANOVA) and the means were compared using the Duncan's multiple range test (DMRT) using SAS 9.4. Prior to analysis, the original percentage data were transformed by arcsine transformation. Arcsine transformation is necessary to stabilize the variance of data that are proportions (binomial distribution).

Results and Discussion

Survivability was recorded when apical meristems were exposed to PVS3 solution for 0 to 30 min when unloaded with 0.8 M sucrose following Kim (2012) while 10 to 25 min exposed to PVS3 when unloaded with 1.2 M sucrose following Sakai et al. (1990) prior to cryopreservation (Table 1).

The survival percentage of cryopreserved apical meristems exposed to PVS3 (0 to 60 min) and unloading treatments (Kim, 2012) and Sakai et al. (1990) ranged from 0 to 41%. Survival of 0 to 22.3% was achieved by unloading explants with solution consisted of 0.8 M sucrose at 40 °C for 30 seconds and 40 min in room condition afterward. While 0 to 41% survival was achieved by unloading meristems with solution contained 1.2 M sucrose at 25 °C for 15 min (Table 1).

Table 1: Effect of combinations of dehydration duration (min) in PVS3 and two conditions of unloading on the survival of taro apical meristem.

Types and durations of unloading	Exposure time to PVS3 solution (min)	Survival (%) (Mean ± standard error)
		Cryopreserved
0.8 M sucrose; 40 °C 30 seconds, 25 °C 40 min (Kim, 2012)	0	5.6±5.7^{ab}
	10	22.3±5.3^{bc}
	30	5.6±5.7^{ab}
	60	0^{a}
1.2 M sucrose; 25 °C 15 min (Sakai et al., 1990)	10	41±8.5^{c}
	15	17±16.5^{abc}
	20	0^{a}
	25	8±8^{ab}

Percentage survival and regeneration data were transformed by arcsine square root and expressed as means ± standard error (SE) %. Mean separation by Duncan's Multiple Range Test (DMRT). Figures followed by the same letter within treatment are not significantly different (P≤0.05).

Exposure to PVS3 for 10 min and unloaded with 1.2 M sucrose treatment gave the highest survival rate of 41%. Thus, exposure to PVS3 for 10 min, in both unloading treatments resulted in the highest rate of survival. Regenerated apical meristem of taro formed direct organogenesis without intermediate callus formation. Regeneration of cryopreserved meristems that were exposed to PVS3 for more than 10 min was found stunted and died after four weeks of thawing regenerated cryopreserved apical meristem observed no morphological abnormalities in the growing plants (Figure 2).

Based on these results, a combination of dehydration for 10 min to PVS3 and unloading with 1.2 M sucrose for 15 min at room temperature was adopted in the following experiment on the effect of loading solutions. The survival of cryopreserved apical meristem treated to loading solutions was ranging from 55.5 to 77.8% (Table 2).

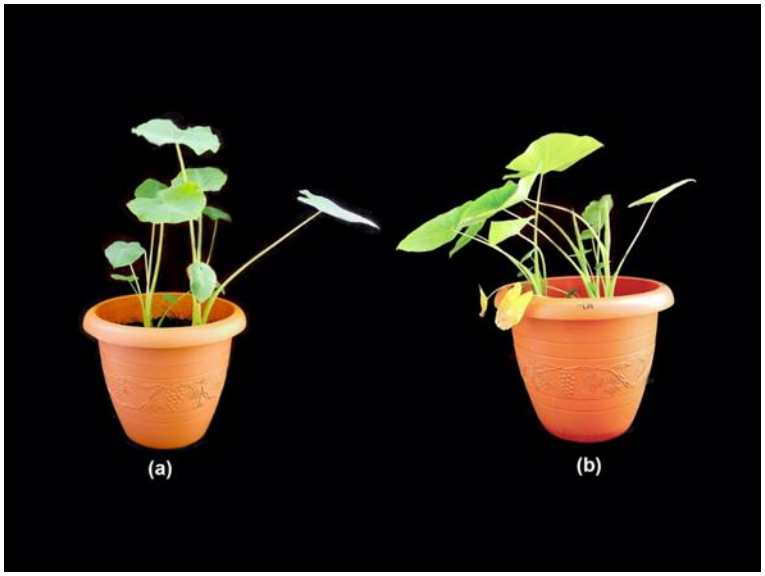

Figure 2: Acclimatized seven weeks old plantlet derived from (a) cryopreserved and (b) non-cryopreserved (control) taro apical meristem treated with PVS3 for 10 min.

Table 2: Survival of cryopreserved and non-cryopreserved apical meristem of taro following different types of loading solutions, 10 min dehydration in PVS3 and unloaded (1.2 M sucrose) at 25 °C for 15 min.

Loading solutions	Survival (%)	
	Cryopreserved	Non-cryopreserved
Untreated (Control)	5.6±5.6^{a}	58.9±4.9^{abc}
2.0 M glycerol + 0.4 M sucrose	61.1±14.7^{b}	72.2±5.5^{bc}
2.0 M glycerol + 5% DMSO + 0.4 M sucrose	55.5±14.7^{b}	83.3±9.6^{c}
1.5 M glycerol + 0.4 M sucrose	55.6±20.0^{b}	44.4±5.6^{ab}
1.5 M glycerol + 5% DMSO + 0.4 M sucrose	77.8±11.1^{b}	33.4±16.7^{a}

Percentage survival and regeneration data were transformed by arcsin square root and expressed as means ± standard error (SE) %. Mean separation by Duncan's Multiple Range Test (DMRT). Figures followed by the same letter within treatment are not significantly different (P≤0.05).

The four different loading solutions did not influence the survival rates of the meristems (Figure 1). Nevertheless, when compared to the control, that were not treated with loading solutions, survival on the cryopreserved meristems decreased to only 5.6%. The highest survival rate, 77.8% was observed in apical meristems treated with the loading solution consisted of 1.5 M glycerol and 5% DMSO, followed by the loading solution consisted of 2.0 M glycerol (61.1%).

The duration of the vitrification procedure is the most critical stage to be optimized in the cryopreservation protocol (Panis et al., 2011). The water extracted from the tissue is insufficient when the procedure was too short, leading to permanent freezing damage caused by ice-crystal forming. On the contrary, if the treatment was too long, the vitrification solution could produce an effect of toxicity that resulted in a reduction of survival after cryopreservation (Panis et al., 2011). The droplet-vitrification method used in this study was based on the one developed for banana meristems (Panis et al., 2005). Sant et al. (2006; 2008) examined the comparison between vitrification and droplet vitrification using PVS2 in taro. The regeneration rates increased dramatically from 21-30% by vitrification to 73-100% by droplet vitrification. Compared to normal vitrification, the use of aluminium foil strips in droplet vitrification resulted in a substantially higher survival rate (89.8%) also in sweet potato cryopreserved shoot tips (Park and Kim, 2015). The possible reason for this could be an ultra-fast cooling rate of 130 °C/min with a cryoprotectant droplet on aluminium strip compared to a cryo-vial freezing rate of about 6 °C/min (Towill and Bonnart, 2003).

Conclusion

This study has demonstrated that droplet-vitrification has potential for cryopreserving tropical taro (*Colocasia esculenta*). Selecting a suitable loading solution (1.5 M glycerol + 5% DMSO for 20 min), duration of PVS3 exposure (10 min) and unloading type (1.2 M sucrose for 15 min) is crucial for the survival of explants post cryostorage.

Acknowledgment

The authors would like to extend the highest gratitude to the Ministry of Agriculture and Agro Based Industry in providing financial assistance in Rancangan Malaysia ke Sebelas (RMK 11) for this project.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

Isolation of Genic Microsatellite Markers in *Mitragyna speciosa* (Ketum)

Lee, C.T., Nurul Farhanah, Z., Norliana Izzati M.R., Hazwani Humaira', Z.*, Nur Nabilah, A., Ng, K.K.S., Ng, C.H., Tnah, L.H., Norlia, B. and Lee, S.L.

Genetics Laboratory, Forestry Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.
**E-mail: hazwani@frim.gov.my*

Introduction

Mitragyna speciosa (Rubiaceae), a plant cultivated mostly for its leaves, is native to South-East Asian countries, mainly in Thailand, Malaysia, Indonesia, and Papua New Guinea (Kamarudin and Zoriah, 2012). Locally known as "kratom" in Thailand and "ketum" in Malaysia, leaves of this plant contain over 25 alkaloids, with mitragynine and 7-hydroxymitragynine being the most active compounds (Warner et al., 2016). In 2003, mitragynine, the major alkaloid in *M. speciosa* was listed in the Poisons Act 1952 by Malaysian government. Under this Act, a person found to possess or sell *M. speciosa* leaves or other *M. speciosa* preparations such as drinks and teas containing mitragynine may be fined to a maximum penalty of RM 10,000, sentenced 4 years jail or both (Poisons Act, 1952). The law was enforced in such way to curb and control the high misuse potential of *M. speciosa* among drug addicts (Chan et al., 2005).

On a positive perspective, this controversial plant has various useful medicinal values, mainly for its popular home remedy as a stimulant and analgesic when its fresh leaves were chewed or brewed into tea (Veltri and Grundmann, 2019). In addition, *M. speciosa* had been documented to be used as a wound poultice, cure for fever, and a substitute for opium (Kamarudin and Zoriah, 2012). Interestingly, reliance on *M. speciosa* as an alternative for opium substitution may have some basis. Apart from the pharmacological and animal studies pointing towards this direction (Babu et al., 2008; Vicknasingam et al., 2010), study by Boyer et al. (2008) also showed that this plant could attenuate opioid withdrawal symptoms based on molecular screening of mitragynine activity at the central nervous system receptors.

Due to the widespread benefits and increasing demands of *M. speciosa*, there is a need to carry out conservation genetic studies on this potential medicinal plant species. Microsatellites or simple sequence repeats (SSRs) are tandem repeats of 1 to 10 nucleotides that are extensively distributed throughout the genome and have been widely accepted as tools for measuring genetic diversity (Vieira et al., 2016). Hence, we aimed to develop microsatellite markers in this plant to facilitate future conservation and breeding programmes. We used next-generation sequencing (NGS) approach as it is much efficient and more cost effective compared to the conventional methods (Csencsics et al., 2010).

Materials and Methods

Sample collection and DNA isolation

Leaf samples from 32 *M. speciosa* trees were collected from FRIM Research Station at Jengka, Pahang. The samples collected were kept in liquid nitrogen prior to DNA isolation following a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980). The DNA samples were subsequently purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany).

RNA extraction and transcriptome sequencing

The same leaves samples from 32 *M. speciosa* were also used for RNA extraction. Total RNA was extracted from *M. speciosa* leaf using RNeasy Plant Mini Kit (Qiagen GmbH, Germany), followed by quality check using an Agilent 2100 Bioanalyser (Agilent Technologies, USA). Transcriptome sequence

data was generated via Illumina paired-end sequencing. Subsequently, the quality of the raw sequence data was checked and trimmed by using FastQC tools (Andrews, 2010) and Trimmomatic v0.32 (Bolger et al., 2014), respectively. Trinity-v2.4.0 (Grabherr et al., 2011) was used to assemble the sequence reads into contigs, scaffolds, and unigenes.

Isolation and characterization of microsatellites

Microsatellite-containing sequences were identified from the transcriptome unigenes by using MIcroSAtellite identification tool (MISA) (Thiel et al., 2003) while Primer 3 was used for primer design. Initially, 70 primer pairs for di-, tri- and tetranucleotide microsatellite-containing regions were screened for specificity using the 32 *M. speciosa* samples.

Polymerase Chain Reaction (PCR) amplification was performed using four *M. speciosa* samples, each with 10 µL reaction mixture, consisting of approximately 10 ng of DNA template, 1 X GoTaq Flexi Buffer, 1.5 mM $MgCl_2$, 0.3 µM of each primer, 0.2 mM of dNTPs and 0.5U of GoTaq Flexi DNA Polymerase (Promega Corporation, USA). The reactions were carried out using a GeneAmp PCR System 9700 (Applied Biosystems, USA) with the following programme: initial denaturing step of 4 min at 94 °C, 40 cycles of 94 °C for 1 min, 50 °C annealing temperature for 30 s, and 72 °C for 30 s, followed by 30 min at 72 °C. The PCR products were electrophoresed on 2% agarose gels with a 100 bp DNA ladder (New England Biolabs, USA) as size standard.

Upon screening, 40 primer pairs which yielded single-band of expected size were selected for fluorescent labelling at the forward primers. Thereafter, PCR using labelled primer was conducted with the same protocols and thermal cycling programme for the 32 *M. speciosa* samples. Fragment analysis was carried out by using an ABI 3130xl Genetic Analyzer with ROX 400 (Applied Biosystems, USA) as the internal size standard.

Data analysis

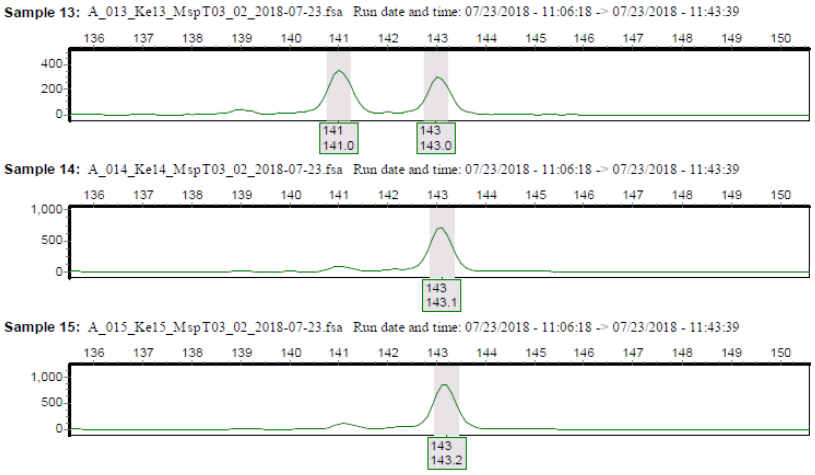
Allele genotyping was performed using GeneMarker v2.6.4 (SoftGenetics, USA). Data analysis was carried out using GDA version 1.0 (Lewis and Zeykin, 2001). Cluster analysis at the individual level was performed using PowerMarker version 3.25 (Liu and Muse, 2005), based on the proportion of shared alleles (Jin and Chakraborty, 1993).

Results and Discussion

The microsatellite peak patterns reflect that *M. speciosa* is a diploid. A total of 29 loci yielded consistent and scorable genotypes based on the fragment analysis results. Of these microsatellite loci, 21 loci were polymorphic while the remaining eight loci were monomorphic. Figure 1 shows the examples of electropherograms of two polymorphic loci (*Msp*T03 and *Msp*T22). Excluding the monomorphic loci, the observed number of alleles per locus ranged from two to three, except for *Msp*T29 (A = 6), with the mean number of alleles of 2.6 (Table 1). The mean observed heterozygosity (H_o = 0.436) is comparable with the mean expected heterozygosity (H_e = 0.439). The mean polymorphism information content (PIC) was 0.365.

The low genetic diversity reflected by the low number of alleles per locus could be because the samples examined are genetically closely related since *M. speciosa* can be easily propagated vegetatively. In other words, *M. speciosa* population in Jengka has a narrow gene pool. The dendrogram generated based on shared allele method (Jin and Chakraborty, 1993) revealed that some of the samples are genetically identical. For examples, samples Ke03, Ke04, and Ke05 shared the same multilocus genotype, as well as for Ke23 and Ke24, and Ke31 and Ke32 (Figure 2).

(A)



(B)

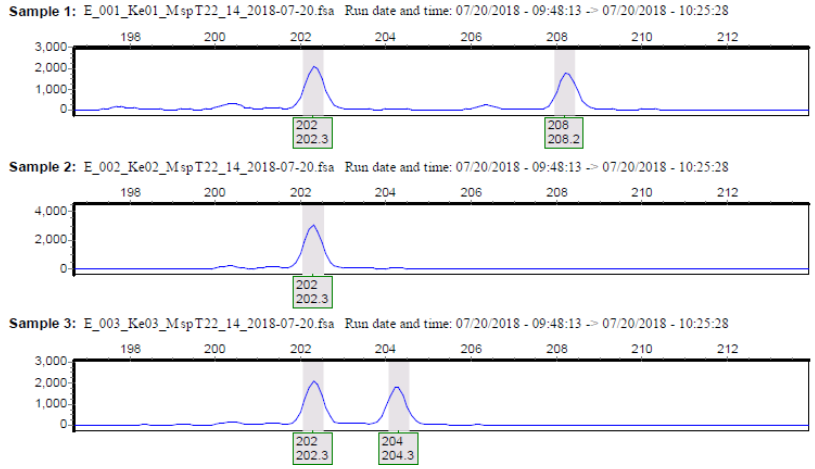


Figure 1: Electropherograms of two polymorphic *Mitragyna speciosa* microsatellite loci. (A) *Msp*T03 and (B) *Msp*T22, showing the genotypes of three of the 32 samples analysed. The peaks represent alleles of 141 and 143 bp for *Msp*T03 and 202, 204, and 208 bp for *Msp*T22, respectively.

Table 1: Genetic diversity measures estimated for the 21 polymorphic genic microsatellite markers in *M. speciosa* based on 32 samples. A = number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, PIC = polymorphism information content.

Locus	Repeat motif	A	H_o	H_e	PIC
*Msp*T02	$(TA)_8$	3	0.344	0.550	0.464
*Msp*T03	$(TC)_{11}$	3	0.563	0.474	0.408
*Msp*T08	$(AG)_{12}$	2	0.469	0.496	0.369
*Msp*T10	$(AT)_8$	2	0.344	0.448	0.344
*Msp*T13	$(TG)_8$	2	0.031	0.031	0.030
*Msp*T14	$(GA)_9$	3	0.656	0.627	0.544
*Msp*T22	$(GA)_{12}$	3	0.344	0.377	0.322
*Msp*T25	$(CT)_{10}$	2	0.438	0.476	0.359
*Msp*T27	$(CT)_{11}$	2	0.219	0.198	0.176
*Msp*T29	$(CT)_9$	6	0.656	0.736	0.678
*Msp*T30	$(AGA)_{12}$	3	0.563	0.568	0.468
*Msp*T35	$(TCA)_8$	2	0.313	0.268	0.229
*Msp*T36	$(AGA)_8$	2	0.563	0.411	0.323
*Msp*T40	$(GCG)_8$	2	0.281	0.246	0.212
*Msp*T41	$(AGA)_{11}$	2	0.313	0.381	0.305

*Msp*T44	$(CTC)_{11}$	3	0.500	0.520	0.409
*Msp*T50	$(GGA)_8$	3	0.563	0.617	0.530
*Msp*T56	$(TGG)_8$	2	0.563	0.506	0.374
*Msp*T59	$(AGAA)_6$	3	0.750	0.659	0.575
*Msp*T64	$(ATCT)_6$	3	0.688	0.569	0.496
*Msp*T69	$(TTAT)_6$	2	0.000	0.062	0.059
Mean		2.6	0.436	0.439	0.365

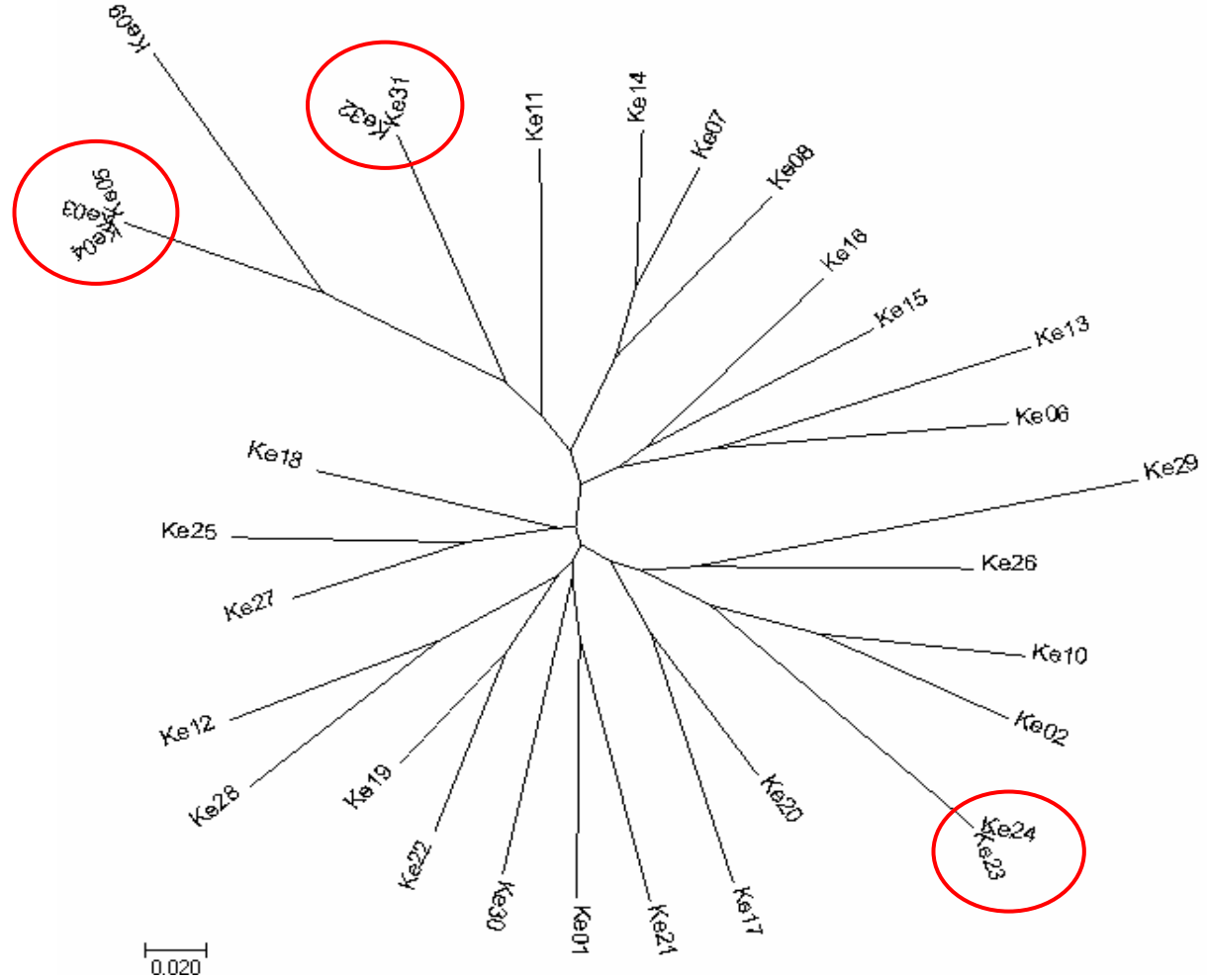


Figure 2: Dendrogram showing the genetic relatedness among the 32 *M. speciosa* individuals. Based on the microsatellite analysis, samples Ke03, Ke04, and Ke05 are genetically identical, as well as Ke23 and Ke24, Ke31, and Ke32, respectively.

Conclusion

To date, no microsatellite markers have been reported in *M. speciosa* yet. We have developed 29 genic microsatellite markers in *M. speciosa*, with 21 being polymorphic based on the 32 samples from Jengka. There is a possibility that the eight monomorphic loci might exhibit allelic variation when more samples from different locations are genotyped. These markers can be applied for clonal identification, genetic diversity assessment, outcrossing rate estimation, germplasm evaluation, and other genetic studies.

Acknowledgement

We would like to thank the supporting staff from the Genetics Laboratory, Forestry Biotechnology Division, FRIM (Ramli, P., Ghazali, J., Yahya, M., Yasri, B., Sharifah, T. and late Suryani, C.S.) for

their assistance in the field as well as the laboratory. This research was funded under the 11th Malaysia Plan, Ministry of Energy and Natural Resources.

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eISSN 2600-9595
Trans. Malaysian Soc. Plant Physiol. 28
First Published, 2021

HPLC Method for Mitragynine and 7-Hydroxymitragynine Determination in *Mitragyna speciosa*

Norliana Izzati, M.R.[1,2,*], Fauziah, A.[1], Ling, S.K.[1], Lee, S.L.[1], Ng, K.K.S.[1], Ng, C.H.[1], Ng, C.L.[2], Goh H.H.[2] and Lee, C.T.[1]

[1]Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia.
[2]Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.
E-mail: norlianaizzatimr@gmail.com

Introduction

Mitragyna speciosa or commonly known as Kratom belongs to the coffee family, Rubiaceae. It is a tropical medicinal plant from Southeast Asia which is indigenous to Malaysia and Thailand (Nations, 2007; Ingsathit et al., 2009) . Kratom leaf has been used as a traditional remedy by local residents to treat chronic pain, muscle pain and fever (Boyer et al., 2007; Carpenter et al., 2016; Kruegel and Grundmann, 2018). It was also reported that *M. speciosa* has many medicinal properties such as mood enhancer, analgesic, antitussive and opioid-like effects (Nations, 2007; McWhirter and Morris, 2010;). Mitragynine (9-methoxy-coryantheidine, MG) and 7-hydroxymitragynine (7-OH) are the pharmacologically active compounds in *M. speciosa* that can bind to opioid receptors with the latter being significantly more potent (Kruegel et al., 2016, 2019).

Mitragynine is the major alkaloid of *M. speciosa* that was found accumulated in the leaves and was not detected in the roots (Charoonratana et al., 2013). More than 25 alkaloids have been isolated by high-performance liquid chromatography (HPLC) from *M. speciosa* and MG was found as the primary active alkaloid (Chittrakarn et al., 2012). Metabolomics profiling of *M. speciosa* leaf using LC-ESI-TOF-MS also showed the presence of MG and other alkaloids such as 7-OH, mitragynaline and paynantheine (Veeramohan et al., 2018).

HPLC is a preferred technique to separate different chemical components in plants because of the efficiency and time-saving properties. Among the methods reported for *M. speciosa* alkaloids quantification, most have time-consuming extraction conditions, varied chromatographic conditions, and limited chromatographic resolution for the alkaloids (Chittrakarn et al., 2012; Charoonratana et al., 2013; Parthasarathy et al., 2013; Zuldin et al., 2013; Ranggasamy et al., 2015). Mudge and Brown (2017) have validated the HPLC method for determination of MG and 7-OH in *M. speciosa* for raw materials and finished products (Mudge and Brown, 2017). The method showed high resolution for MG and 7-OH, yet the earlier peak elution was not variably separated. Therefore, optimization of the HPLC method based on Mudge and Brown (2017) was initiated to reduce the extraction time duration, improve chromatographic condition and resolution of MG and 7-OH peak profiles.

Materials and Methods

Sample preparation

Fresh leaf samples from a *M. speciosa* plantlet were collected from Forest Research Institute Malaysia (FRIM) nursery. The leaves were collected from the plant at age 1 year and at 1 m tall. Approximately 2 g of the leaves were cut into small pieces and immediately immersed in liquid nitrogen. Subsequently, the frozen leaf sample was ground into fine powder with a 6875 Freezer/Mill (SPEX SamplePrep, USA) for 1 min before transferred into a pre-cooled Falcon tube and then freeze-dried. The dried sample was weighed 0.5 g into a glass vial added with 5 mL of 100% HPLC grade methanol (0.1 g/mL). Then, the mixture was sonicated for 15 min at room temperature using a 2800 CPXH Ultrasonic Cleaner (Branson, USA). Sediment was formed at the bottom after sonication. Sample extract on the top was then transferred into a new HPLC vial using a syringe with 0.45 µM PTFE-B syringe filter (Whatman, England). As the extract contained high concentration of mitragynine, the sample was diluted to 0.03 g/mL prior to HPLC analysis.

Calibration solution preparation

Calibration solutions were prepared according to Mudge and Brown (2017) based on the reference standards of 1 mg/mL of MG (Lipomed, Switzerland) and 100 µg/mL of 7-OH (Cayman, USA). Linearity determinants were prepared in seven different concentrations ranging from 1-500 µg/mL for MG and, 0.1-25 µg/mL for 7-OH, by mixing the reference standards MG and 7-OH in extraction solvent of 100% HPLC grade methanol. Calibration solution preparation is summarized in Table 1. The seven concentrations of reference standards were run in triplicates. Linearity 1-7 were analysed using the optimised chromatographic conditions as described below. The average peak areas of targeted analytes were used to construct the calibration curves.

Table 1: Calibration solution preparation.

Standard	1 mg/mL MG (µL)	100 µg/mL 7-OH (µL)	Extraction solvent	Total volume (mL)	MG concentration (µg/mL)	7-OH concentration (µg/mL)
Linearity 1	500	250	250	1	500	25
Linearity 2	250	200	550	1	250	20
Linearity 3	100	100	800	1	100	10
Linearity 4	50	50	900	1	50	5
Linearity 5	100 µL of Linearity 2		900	1	25	2
Linearity 6	100 µL of Linearity 3		900	1	10	1
Linearity 7	100 µL of Linearity 6		900	1	1	0.1

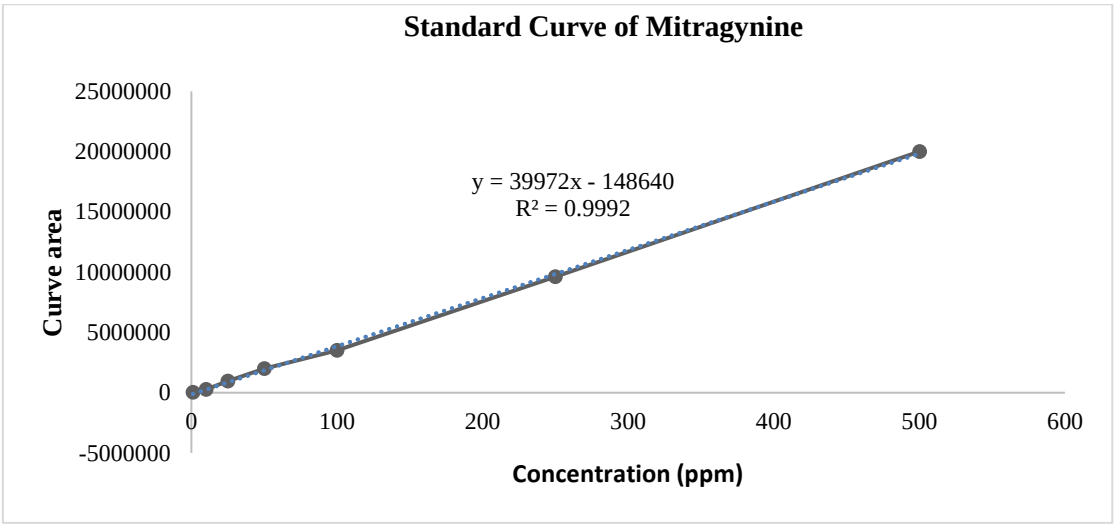
Chromatographic conditions

The presence of MG and 7-OH in the samples were verified based on the retention times and the UV absorption spectra of the samples and the reference standards (Parthasarathy et al., 2013; Ranggasamy et al., 2015). HPLC analysis was performed following the chromatographic conditions reported by Mudge and Brown (2017), with slight modifications. The HPLC system consists of 600 Controller, In-Line Degasser AF, 2707 Autosampler and 2996 Photodiode Array Detector (Waters, USA). The data were gathered and processed using Empower Software System. Chromatographic separation was achieved using column Kinetex 5 µM EVO C18 100 Å (150 x 4.6 mm, 5 µm particle size) (Phenomenex, USA) with a duration of 25 min in ambient temperature. The mobile phase consists of A: 5 mM ammonium bicarbonate buffer, pH 9.5, and B: 100% acetonitrile. Binary gradient elution was performed with 1 mL/min flow rate starting at 20% A (0-2 min) followed by 30% A (2-5 min); 60% A (5-10 min); 80% A (10-15 min); 80% A (15-20 min) and 20% A (20-25 min). Sample injection volume was set at 10 µL with detection at the absorbance of 226 nm.

Results and Discussion

The linear calibration curves for both MG and 7-OH are shown in Figure 1. Both the calibration curves were linear with concentration for MG ranging from 1-500 µg/mL (equation y = 39972x - 148640 and R^2 = 1.0), while the concentration range for 7-OH was 0.1-25 µg/mL (equation y = 77113x - 14582 and R^2 = 1.0). The quantification limit was satisfactory.

a)



b)

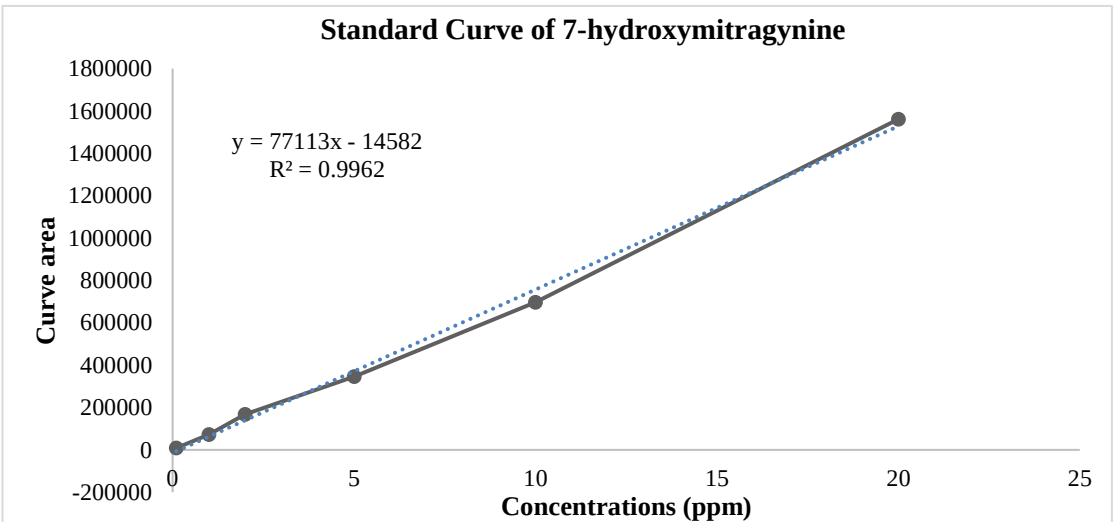


Figure 1: Calibration curve of (a) mitragynine and (b) 7-hydroxymitragynine.

The HPLC chromatogram profiles for the reference standards and sample were observed at a maximum UV absorption detection of 226 nm. The mean retention time for MG was 16.23 min while 7-OH at 12.16 min (Figure 2). The sample profile peak has a close retention time as the reference standard profile of MG, which was 16.15 min with 1.4 absorption units (AU) at 226 nm. The minimal amount of 7-OH in the sample caused difficulty to visualize the peak based on retention time. Considering that 7-OH is a minor alkaloid in *M. speciosa*, baseline separation was tough (Mudge and Brown, 2017). Therefore, the peak was processed by Empower Software System. The UV absorption spectra of the sample and the reference standard were compared at retention time near 12 min. Likewise, in the sample profile, 7-OH retention time was similar to the reference standard profile, 12.18 min at 226 nm.

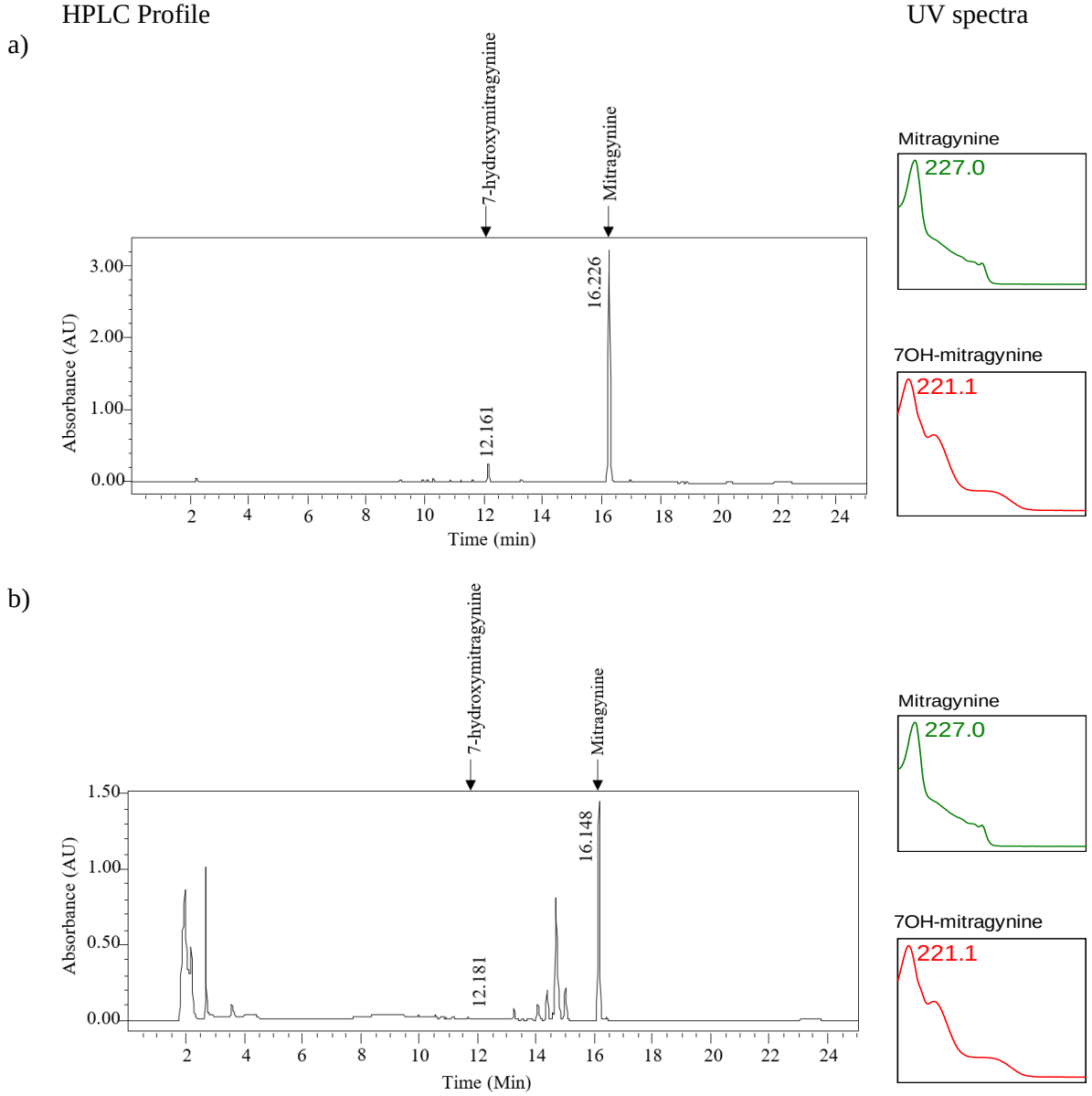


Figure 2: HPLC chromatogram profiles for (a) 7-hydroxymitragynine and mitragynine reference standards and (b) a representative *Mitragyna speciosa* sample at the wavelength of 226 nm.

Conclusion

The method for MG and 7-OH determination in *M. speciosa* has been optimized for a better profile resolution. The method will be further validated for future work.

Acknowledgement

The research funding from the Ministry of Education Malaysia is gratefully acknowledged (FRGS/1/2019/STG05/NRE/2).

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