TRANSACTIONS OF THE MALAYSIAN SOCIETY OF PLANT PHYSIOLOGY VOL. 30

INNOVATION, CHALLENGES, AND PERSPECTIVES IN PLANT PHYSIOLOGY

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Plant Growth and Development

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Changes in Stand Structure and Species Composition Over a Second Growth Forest: A Study at Lesong Forest Reserve, Pahang

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Introduction

The forest in Peninsular Malaysia is managed through sustainable forest management practice. This practice required a careful selective logging system that is advocated as a significant mean to provide timber, protect biodiversity, and reduce carbon emissions from tropical forest. It is believed that natural forests are able to recover and grow resiliently in any disturbances. However, the recovery and stand conditions of the second growth forests are much dependent on the management system applied on the ground (Wan Mohd Shukri et al., 2004). The knowledge on any stand structures are useful to initiate proper stands management plans. The productive forest is mainly composed of dipterocarps and is a major source of valuable timbers, which can be used to satisfy increasing market demands. The use of Selective Management System (SMS) has been applied since 1978 as a prescription for timber harvesting in Peninsular Malaysia. Under this management system, the trees are initially selected based on pre-felling inventory data and to determine the cutting limits (Thang, 1987). It will ensure a continuous supply of timber whereby harvesting is also regulated by area control and estimation of volume are prescribed accordingly in the management plans recommended by each state (Noraida et al., 2018). According to Otani et al. (2012), the original timber stocks in a production forest are deemed to regain back to its original state within 25 to 30 years after the first logging. However, Samsudin et al. (2010) and Saiful and Latiff (2014) stated that these second growth forests may be highly variable in terms of structure, species composition and productivity. It was further stated that these forest stands may not fully recover or reach the optimum sizes as the initial primary forest where large trees ($\geq$50 cm dbh) remained lower even after 41 years of logging (Okuda et al., 2003). With this information, various approaches have been carried out to overcome these impacts. Hence, the objective of this study was to investigate any stand structure and species composition changes using two logging cutting limits: 45 cm and 60 cm diameter-at-breast-height (dbh) over second growth forest.

Materials and Methods

Study area

This study was conducted in Compartment 181, Lesong Forest Reserve, Rompin, Pahang. It is located at the southeast of Peninsular Malaysia and adjacent to the state of Johor (Figure 1). The total area for this compartment accounted for about 85-hectares (ha) with elevation ranges between 150 to 300 metres above sea level (asl). According to a previous record, this is a logged-over lowland dipterocarp forest since 1978 and mainly dominated by *Dryobalanops aromatica* (Kapur) and *Elasteriospermum tapos* (Perah) stands. Prior to the logging operations, the study area was divided and demarcated equally into two blocks with about 42.5-ha per block (Figure 1). There were two logging cutting limits: 45 cm and 60 cm dbh and labelled as CL45 and CL60, respectively.

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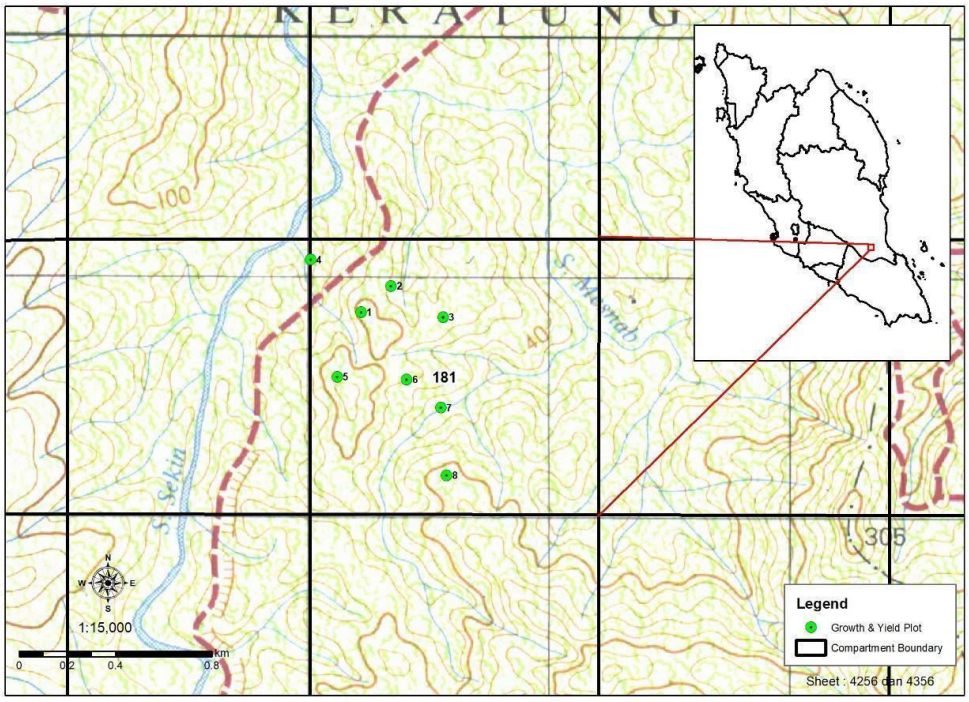

Figure 1: Location of study area.

Sampling and data collection

Four 100 × 100 m growth and yield plots were established within each block and with different topography conditions i.e., ridge, mid slope and valley bottom. The trees were measured and tagged since 1979 and these include trees with dbh ≥ 10 cm within the plots. These measurements were taken periodically between 2019-2012 and regarded as the second growth forest type. All tree identification was made on the groundworks till species level. Any unidentified trees were tagged and specimens were brought back to FRIM's Herbarium Laboratory. For identification purposes, Tree Flora of Malaya was used as a reference.

Data analysis

Any past changes in stand structures were calculated and analysed based on cutting limits, the basal area, tree density and tree volume.

a) Basal area for tree density is based on the area of a given section of land that is occupied by the cross-section of a tree:

$$BA = \pi r^2$$
$$= 3.142 \times (dbh/200)^2$$

Where, BA is basal area (m^2), r is radius of tree (cm) and dbh is diameter-at-breast-height (cm).

b) Tree density is the number of trees in a given area. This information is significant for specific species indication so as to facilitate efforts in management and conservation purposes:

Tree density = total number of tree/area in m^2

c) Tree volume is used for assessment of growing stock, timber valuation, selection of forest areas for harvests and for growth and yield studies, $V = \frac{\pi \times dbh^2 \times L \times f}{4 \times 10{,}000}$

Where, V is tree volume in m^3, π is equal to 3.142, dbh is diameter-at-breast-height, f is form factor with 0.65 and L is merchantable height/length. According to the SMS manual, the length of the timber is calculated based on the standard diameter class as shown in Table 1.

Table 1: Calculation of diameter class, length and equivalent merchantable heights.

Diameter class	Number of logs 5 m	Equivalent merchantable heights (m)
+ 30-60 cm	2	10
+ 60-75 cm	3	15
+ 75 cm	4	20

The analysis includes logging treatments and species groups; Dipterocarp (D) and Non-dipterocarp (ND). A One-way Analysis of Variance (ANOVA) was then performed to test differences between means of large tree volume. While species composition refers to an assemblage of tree species for particular forest areas. All data were analysed using R-statistical software, including the 'RStudio' package.

Results and Discussion

Stand structure

Both tree density and volume give large trees with dbh ≥ 55 cm as these trees played crucial roles for sustainable timber stocks production. Figure 2 demonstrates there are changes in the mean total basal area in the cutting limits by 13-14% from 1979 and 2012. The BA for CL45 increases from 30.28 m^2/ha to 34.40 m^2/ha while for CL60 it increases from 31.78 m^2/ha to 35.80 m^2/ha. This marginal increment further demonstrates that the forests are regenerating to normal growth conditions.

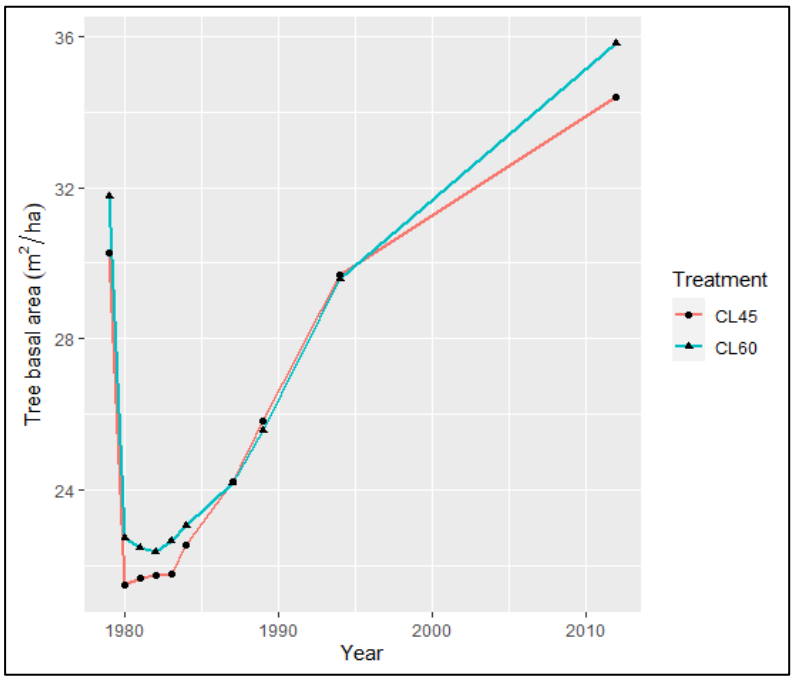

Figure 2: Temporal changes for mean total BA in CL45 and CL60.

Other changes in the mean density for larger trees (dbh ≥ 55 cm) by cutting limits from 1979 to 2012 were shown as in Figure 3. These larger trees exhibited higher density at early stages but decreased

thereafter. The decrease of large tree density in both cutting limits may be due to high mortality rate resulting from logging damages. Despite the increment in the 33-year period, the large tree density in 2012 has not recovered to at least similar as recorded on the first census. The number of large trees depreciated around 12-14% as compared to the density on the first census for both treatments. This indicates the both cutting limits were affecting the trend of large tree density within the stated period.

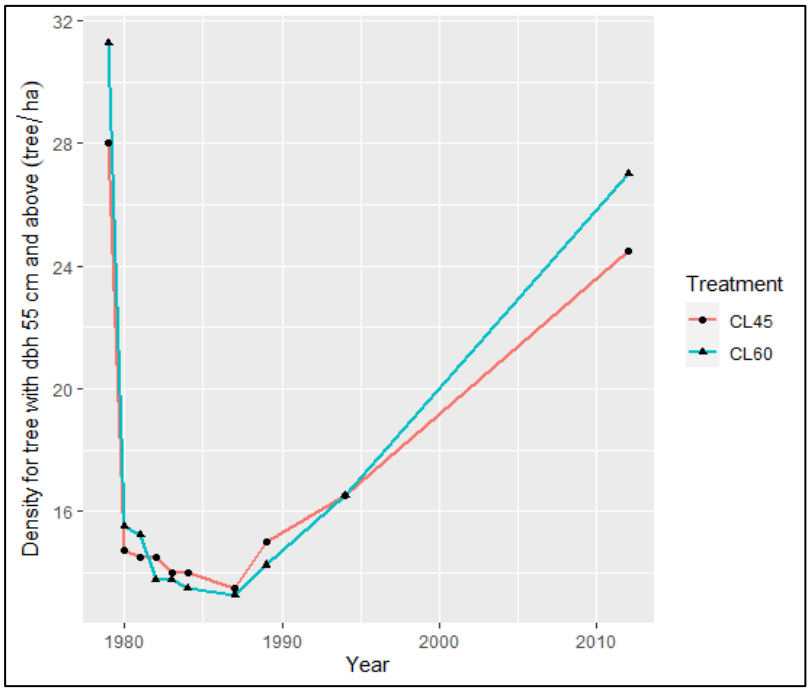

Figure 3: Temporal changes in mean density for trees with dbh ≥ 55 cm.

The analysis on tree volume for larger trees by cutting limits and species groups is useful to examine the status of forest productivity throughout 33 years after logging. Thus, the temporal changes in the tree volume for large trees (dbh ≥ 55 cm) by cutting limits and species groups were displayed as in Figure 4. According to analysis, this study found that the volume of dipterocarp trees exceeded the volume of non-dipterocarp trees in the first census for CL45 and CL60. All species groups show decreasing trends in 1980 except CL45-ND. The volume for non-dipterocarp trees was gradually increasing from 48.03 m^3/ha in 1979 to 71.48 m^3/ha in 2012 for CL45. Results from ANOVA showed that there is a significant difference in the means of volume between logging treatments and species groups ($p < 0.05$). Multiple comparison tests (Tukey test) were then performed to compare the means of volume between CL45-D, CL45-ND, CL60-D and CL60-ND in 2012. The mean of tree volume for the CL45-D is significantly different from CL45-ND ($p=0.021$). This indicates that the implementation of cutting limits, 45 cm and below may promote the growth of non-dipterocarp trees and possibly suppress the productivity of valuable timbers especially from dipterocarp species within production forests.

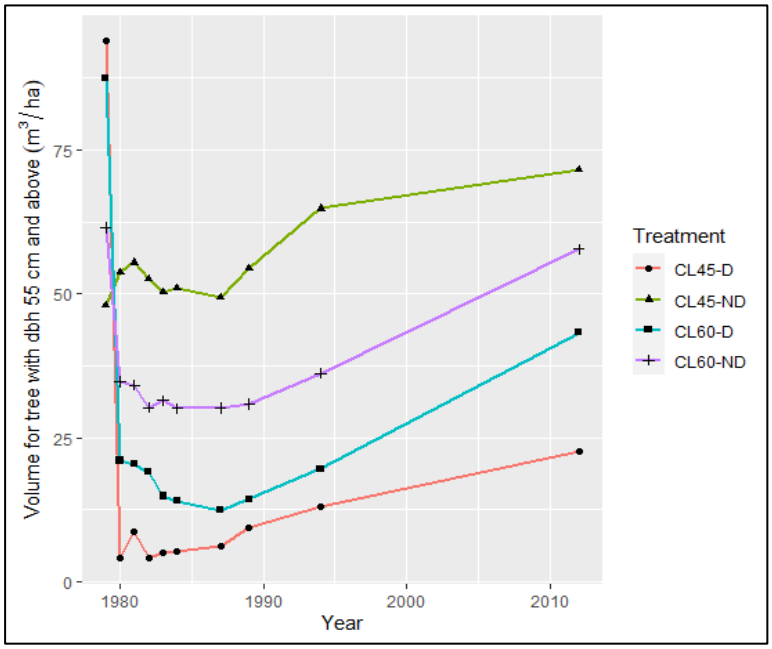

Figure 4: Temporal changes in the mean tree volume for large trees at dbh ≥ 55 cm and species groups.

Species composition

Overall, there are 602 species and 71 families recorded within the growth and yield plots. The ten common species with highest mean total basal, and tree density within 1979 and 2012 for treatments CL45 and CL60 are as shown in Table 2. This indicates that there is a species basal area one year after logging for *Dryobalanops aromatica* and *Elasteriospermum tapos* in both treatments. However, census data in 2012 later showed that the species composition in the study area had changed substantially with *E. tapos* having higher BA at 14.47% and 12.38% in both treatments. This demonstrated that logging activities have growth effects on *D. aromatica* and other dipterocarp trees in treatment CL45.

Any forest openings through logging activity showed that a pioneer species such as *Macaranga conifera* was introduced and shown to achieve higher BA. Furthermore, there was presence of *M. conifera* with greater density in CL45 than in CL60 with 27.50 tree/ha and 9.50 tree/ha, respectively. According to Otani et al. (2012), any succession by a pioneer species, for example *Macaranga* spp, can grow to reach their maximum number several years after logging. As this pioneer tree is light demanding species, any disturbance within a forest area may encourage them to grow and subsequently function as to provide optimum conditions for other shade-tolerance tree species to grow. Hence, it further suggests that any selection of logging treatments especially on lower cutting limits can significantly affect species composition for a production forest and to sustain logging activities.

Table 2: Comparisons between ten common species with highest mean total BA and tree density based on CL45 and CL60 cm.

Year: 1979 Treatment: CL45				Year: 2012 Treatment: CL45			
Species	BA		Density	Species	BA		Density
	(m^2/ha)	(%)	(tree/ha)		(m^2/ha)	(%)	(tree/ha)
Dryobalanops aromatica	7.17	23.69	22.25	*Elateriospermum tapos*	4.98	14.47	37.75
Elateriospermum tapos	4.07	13.45	40.75	*Dryobalanops aromatica*	2.71	7.87	76.75
Syzygium sp.	1.27	4.20	20.00	*Macaranga conifera*	1.05	3.05	27.50
Santiria sp.	1.24	4.10	17.00	*Shorea macroptera*	0.96	2.78	9.00
Litsea sp.	0.82	2.72	15.00	*Endospermum diadenum*	0.90	2.63	19.75
Shorea macroptera	0.64	2.11	8.75	*Syzygium anisosepalum*	0.60	1.75	3.50
Shorea acuminata	0.45	1.47	5.50	*Shorea acuminata*	0.59	1.72	4.00
Dipterocarpus concavus	0.38	1.26	3.75	*Shorea multiflora*	0.52	1.51	8.00
Gluta elegans	0.35	1.15	5.50	*Santiria rubiginosa*	0.45	1.31	4.00
Shorea multiflora	0.35	1.14	5.00	*Santiria laevigata*	0.44	1.28	2.50

Year: 1979 Treatment: CL60				Year: 2012 Treatment: CL60			
Species	BA		Density	Species	BA		Density
	(m^2/ha)	(%)	(tree/ha)		(m^2/ha)	(%)	(tree/ha)
Dryobalanops aromatica	6.36	20.00	25.75	*Elateriospermum tapos*	4.43	12.38	38.75
Elateriospermum tapos	3.32	10.43	36.00	*Dryobalanops aromatica*	3.75	10.47	53.50
Syzygium sp.	1.36	4.27	22.25	*Endospermum diadenum*	0.96	2.68	25.00
Santiria sp.	0.80	2.52	11.50	*Teijsmanniodendron simplicifolium*	0.79	2.21	14.50
Hopea nervosa	0.65	2.05	11.00	*Shorea acuminata*	0.63	1.76	6.25
Teijsmanniodendron simplicifolium	0.61	1.92	13.25	*Streblus taxoides*	0.54	1.51	22.50
Streblus taxoides	0.53	1.66	21.25	*Scaphium macropodum*	0.52	1.45	4.75
Dipterocarpus concavus	0.52	1.64	2.25	*Hopea nervosa*	0.50	1.40	9.75
Litsea sp.	0.52	1.62	10.50	*Macaranga conifera*	0.48	1.33	9.50
Koompassia malaccensis	0.50	1.58	2.25	*Palaquium hexandrum*	0.44	1.24	2.00

Conclusion

The changes in stand structure and species composition by logging treatments of two cutting limits, i.e., 45 cm and 60 cm dbh over second growth forest of Lesong Forest Reserve, Pahang were investigated in this paper. Over a 33-year after logging, the basal area of the second growth forest in 2012 was recovered and even greater as compared to the first census in both treatments. Besides that, the large tree density ($\geq$ 55 cm dbh) was also increased but not attained as in the initial census within these long-term monitoring plots. There is a significant difference in the means of tree volume between logging treatments and species groups. The implementation of a cutting limit of 45 cm, promoted the growth of non-dipterocarp trees and suppressed the productivity of dipterocarp trees.

The species composition has changed over 33-year after logging. *Elasteriospermum tapos* and co-dominated with *Dryobalanops aromatica* in terms of basal area in 2012. *Macaranga conifera* were introduced and exhibited higher tree density in CL45 than CL60 in 2012. Overall, this study shows that the CL45 provided long-term impacts toward the structure of large trees especially from dipterocarp trees and species composition over CL60. Findings from this paper may provide insight towards the establishment of proper management plans for production forests within Peninsular Malaysia.

Acknowledgement

We gratefully acknowledge the Malaysian Government for financial support granted through Rancangan Malaysia Ke-11 Fund (RMK-11). Special thanks to Pejabat Hutan Daerah Rompin and Jabatan Perhutanan Negeri Pahang for allowing us with permission to enter Compartment 181, Lesong Forest Reserve, Pahang. Thanks also to all our staff from the Forest Management Branch and Climate Change and Forestry Programme for their assistance in data collections. Last but not least, we wish to express our thanks to Ms. Rodziah Hashim from Geoinformation Programme, FRIM for providing us with the map of the study area.

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Landscape Plant Diversity Index at Taman Awam Bukit Lagi, Perlis

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Introduction

Tree diversity in an urban green area is crucial for providing environmental services in terms of social and psychological well-beings. Any sustainable green urban park allocated are required to have proper management and reliability practices so to maximise usage. The planted trees information could provide us with a better understanding of the available stands and species diversity as these could further lead to improving resilience and flexibility in urban parks sustainability. The trees diversity within an urban green space increases as species richness and uniformity increases. Furthermore, by increasing tree diversity will create high resistance, tolerance, and adaptability to the trees as caused by the biotic or abiotic factors. Any mitigations action on specific pests or diseases in the urban landscape can be controlled by first understanding the trees species present. As such, it is crucial to carry out preliminary tree inventory in order to monitor its main composition and health conditions.

Species richness describes the number of species in a community or ecosystem, while the evenness index compares the number of individuals between species to the relative abundance of each species. Sreetheran et al. (2011) found that the Species Diversity Index (SDI) for road trees in Kuala Lumpur showed a respectably low compared with the SDIs for various urban cities worldwide as measured by Sun (1992). Jim et al. (2000) have developed several guidelines for urban parks in Hong Kong to improve the quantity and quality within parks based on a complete tree survey of ten major urban parks. This valuable information to local authorities will also enable us to execute an excellent practice in conserving and maintaining tree diversity in Malaysia. As such, this study was conducted to examine the species diversity and composition at Taman Awam Bukit Lagi (TBL), Perlis. Information available will assists researchers, management park personnels, and local authorities in making crucial decisions for management purposes of green parks.

Materials and Methods

Study site

A survey was carried out in a public park, Taman Awam Bukit Lagi (TBL), Kangar, Perlis (6°24'58.37"N; 100°11'30.77"E) (Figure 1). The study site is one of the popular parks used by the local community for leisure. It has approximately 75.07 ha of green spaces and majestic limestone hill surrounding the area.

Figure 1: Map of the study site at Taman Awam Bukit Lagi, Kangar, Perlis.

Data collection

The trees species and the number of individuals for each species were identified in order to determine the SDI. The data was subsequently analysed using the Ecometh ver 7.4 statistical software package to determine species heterogeneity and evenness measures.

Data analysis

Descriptive analysis and the non-parametric approach measures of heterogeneity were adopted. A widely used of heterogeneity diversity index is Simpson's diversity and Smith and Wilson's index (Krebs, 1989).

Species diversity

The Simpson's Index (D) with

$$D = \frac{\sum n\ (\mathrm{n}-1)}{\mathrm{N}-(\mathrm{N}-1)}$$

Where:
n = number of individuals of each species
N = total number of individuals of all species

The reciprocal Simpson's Index of Diversity (1-D) measured the probability that two individuals randomly sampled belong to different species based on simple population calculation. The SDI index quantifies biodiversity by taking into account species richness and evenness, and it can be used to compare communities to identify intrinsic qualities. Pielou (1969) advocated and demonstrated that the precise estimation for a finite population sample is:

$$D = 1 - \sum \quad [\frac{\mathrm{n}\,(\mathrm{n}-1)}{\mathrm{N}-(\mathrm{N}-1)}]$$

Where:
n = number of individuals of each species
N = total number of individuals of all species

Value 1, indicate infinite diversity, and 0 absolute no diversity.

Species evenness

The diversity species evenness index calculates the number of individuals in genus or species in a habitat community based on the circumstances of the organism's population.

Simpson's Index of Diversity (1/D) of evenness

$$E_{1/D} = \frac{1/\hat{D}}{S}$$

where:

$E_{1/\hat{D}}$ = Simpson's measures of evenness
$\hat{D}$ = Simpson's index
S = Number of species

This index ranges from 0 to 1 and is relatively unaffected by the rare species in the sample.
Smith and Wilson's index of evenness

$$E_{var} = 1 - \left(\frac{2}{\pi}\right)\left[arctan\left\{\frac{\sum_{i=0}^{s}(log_e(n_i) - \sum_{j=1}^{s}(log_e(n_j)/s}{s}\right\}\right]$$

Where:
E_{var} = *Smith and Wilson's index of evenness*
n_i = Number of individuals in species *i* in sample (*i= 1,2,3,4…s*)
n_j = Number of individuals in species *j* in sample (*i= 1,2,3,4…s*)
s = Number of species in entire sample

Value 0, absolute no evenness.

Results and Discussion

Species diversity compositions

Results obtained showed that there are 281 individuals, 18 families, 27 genera, and 30 species of landscape trees including palms (Table 1; Figure 2). The most diverse family was Myrtaceae, consisting of four species and three genera namely *Melaleuca cajuputi*, *Syzygium polyanthum*, *Callistemon citrinus*, and *Syzygium myrtifolium.* The Myrtaceae (58 individuals), and Combretaceae (56 individuals) represent almost 40.6 % of the total individuals in the park. The highest taxa richness and individuals from the Myrtaceae family were *M. cajuputi* (30), *S. polyanthum* (13), *C. citrinus* (12), and *S. myrtifolium* (3). The highest planted tree species in the park is *Terminalia mantaly* 17.8% (variegated and non-variegated species), followed by *Cyrtophyllum fragrans* (11.0%), *M. cajuputi* (10.6%), *Mimusops elengi* (10.6%) and others species which are below 10% of the total population (Table 1). Instantly, *Calophyllum soulattri, Cananga odorata* and *Tamarindus indica* are the only landscape tree representing one individual for each species. About 66.7% of the planted landscape tree is native, and 33.3% are exotic; both landscape tree classes demonstrate significant growth within the park and play a major role as an aesthetic value and shading the area. The species diversity at TBL contributes to various stand characteristics such as tree form, leaf pattern, and fruit and leaves colour. These characteristics also increases the values and serves as part of ecological services that benefit the community.

Figure 2: Trees of *T. mantaly* variegated leaf (a), *M. cajuputi* (b) and *C. fragrans* (c).

Table 1: List of landscape trees including palms at Taman Awam Bukit Lagi, Kangar, Perlis.

No.	Botanical name	Local name/common name	Family	Origin	Number of trees
1.	*Terminalia mantaly*	Pokok doa	Combretaceae	exotic	50
2.	*Crytophyllum fragrans*	Tembusu	Gentiaceae	native	31
3.	*Melaleuca cajuputi*	Gelam	Myrtaceae	native	30
4.	*Mimusops elengi*	Bunga tanjung	Sapotacae	native	30
5.	*Syzygium polyanthum*	Kelat salam	Combretaceae	native	13
6.	*Dypsis leptocheilos*	Redneck palm	Myrtaceae	exotic	13
7.	*Callistemon citrinus*	Crimson bottlebrush	Myrtaceae	exotic	12
8.	*Lagerstroemia speciosa*	Bungor	Lythraceae	native	12
9.	*Saribus rotundifolius*	Serdang	arecaceae	native	11
10.	*Barringtonia racemosa*	Putat	Lecythidaceae	native	11
11.	*Hibiscus tiliaceus*	Bebaru	Malvaceae	native	7
12.	*Alstonia angustiloba*	Pulai	Apocynaceae	native	6
13.	*Terminalia catappa*	Ketapang	Combretaceae	native	6
14.	*Barringtonia acutangula*	Bungor	Lecythidaceae	native	6
15.	*Milettia pinnata*	Mempari	Fabaceae	native	5
16.	*Lagerstroemia floribunda*	Bungor	Lythraceae	native	5
17.	*Cocos nucifera*	Kelapa	Arecaceae	native	4
18.	*Mesua ferrea*	Penaga lilin	Hypericaceae	native	4
19.	*Cratoxylum cochinchinense*	Derum	Hypericaceae	native	3
20.	*Peltophorum pterocarpum*	Jemerlang	Fabaceae	exotic	3
21.	*Lophanthera lactescens*	Golden chain	Polygonaceae	exotic	3
22.	*Filicum decipiens*	Kiara payung	Sapindaceae	exotic	3
23.	*Diospyros* sp.	Kayu arang	Ebenacae	Unknown	2
24.	*Ficus* sp.	Ara	Moraceae	Unknown	2
25.	*Syzygium myrtifolium*	Kelat paya	Myrtaceae	native	3
26.	*Coccoloba uvifera*	Sea grape	Malpighiaceae	exotic	2
27.	*Cananga odorata*	Kenanga	Annonaceae	native	1
28.	*Calophyllum soulattri*	Bintangor	Calophyllaceae	exotic	1
29.	*Tamarindus indica*	Asam jawa	Fabaceae	exotic	1
30.	*Gardenia carinata*	Cempaka hutan	Rubiaceae	exotic	1
				Total	281

Species diversity index

The Simpson's Diversity Index (1-D) showed that the high diversity index value, which is closest to 1 (0.925), indicates there is a high probability of 92.5% chance that any two randomly selected individuals from the community are of different species. Reciprocal of Simpson's 1/D value can be used and interpreted as the number of equally common species required to generate the observed heterogeneity of the sample, which means a different community with 13 equally-common species has an equivalent diversity with TBL. In comparisons, the evenness index for Simpson's (0.438) and Smith and Wilson's (0.468) are relatively low (Table 2). This suggests that by improving or increasing the evenness among species at TBL would enhance the diversity index value. Thus, the total number of species or individual distribution among species should be considered when planning and designing an urban park. The local authority and park management should implement solid arboriculture practices to maintain and minimise long-term concerns in the landscape area. Wang et al. (2022) indicate that densely planted trees face even more harsh circumstances, necessitating more consideration and upkeep in the long run. These conditions would decrease the tree's performance in its ecological capacities. According to Hasan et al. (2017), in the early 1990s, local authorities used popular species for street planting because they provided immediate shade, produced distinctive flowers and leaves and added aesthetic value to the area. However, after 17 years, these trees became more fragile and prone to hazardous conditions whereby any branches or stems breakage could lead to serious consequences such as properties and even life.

Table 2: Diversity indices at Taman Awam Bukit Lagi, Kangar, Perlis.

	Diversity Indices	Value
Species diversity	Simpson (1-D)	0.928
	Simpson's Reciprocal 1/D (diversity equivalents for comparisons)	13.336
Species evenness	Simpson's E 1/D	0.438
	Smith and Wilson E*var*	0.468

Conclusion

Stands richness and evenness are the two main factors influencing species diversity whereby the diversity index increases when the richness and evenness of the community increases. The trees community is considered as highly diverse but low in species dominance and diversity. It was however, concluded that this diverse tree species population has low evenness in species distribution at the site.

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Photosynthetic Efficiency of 18 Selected Timber Species in the Nursery

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Introduction

About 47 million ha of primary forests were depleted between 2000 and 2020 globally (FAO, 2022). These are mainly associated with climatic and biodiversity changes, plant diseases, health related and long-term food security reasons. From 2001-2021, it was reported that about 8.67 million hectares of trees cover were depleted, resulting in 29% reduction of tree cover, 4.99 Gt of CO_2e emissions and 93% deforestation rates (GFW, 2022). Malaysia is committed to the 1992 Rio Earth Summit Agreement to maintain at least 50% of its land mass under forest cover at the Rio Earth Summit in 1992 as forest loss is occurring at an alarming rate and requires immediate attention. Nevertheless, neither halting deforestation nor restoring degraded ecosystems can further improve the ecosystem services provided such as biodiversity conservation, climate regulations and enhancing economic growth. It was reported by the Forestry Department Peninsular Malaysia that under the national planting programme, about 5,235,570 trees have been planted in the Peninsular Malaysia. These include various programmes such as Mangrove Tree Planting and Suitable Species Project in the National Coastal Area, Central Forest Spine Project (CFS) and Restoration, Reclamation and Rehabilitation Project of Degraded Forest Area in Peninsular Malaysia (FDPM, 2020). In order to ensure the success of these rehabilitation programmes, various factors must be taken into considerations and these include species-site matching, site conditions and other silvicultural treatments particularly during early growth and adequate funding. However, selection of appropriate species is of great importance to ensure survival, growth as well as ability to support socio-economic and/or ecological purposes.

Rehabilitation and restoration is defined separately by Lamb and Gilmour (2003) with each having its own unique characteristics, applied according to the objectives and end results expected. In either category, selection of appropriate species is of great importance to ensure survival, growth as well as ability to support socio-economic and/or ecological purposes. An example is the tree species from Dipterocarpaceae family which consists of dominant tree species found in the rainforests of South-East Asia (Widiyatno et al., 2014; Hamdan et al., 2015). Some Dipterocarps are known for its valuable tropical timber and resin. *Shorea pauciflora* and *S. macrophylla* were reported to be used in enrichment programmes in Peninsular Malaysia due to considerable growth rates (Appanah and Weinland, 1993) while *S. leprosula, S. parvifolia, S. macrophylla* and *S. johorensis* were among the 23 Dipterocarps found to be suitable for rehabilitation on degraded tropical rainforest due to higher growth and survival rates (Ang and Maruyama, 1995; Widiyatno et al., 2014). Other factors affecting plant growth such as light intensity, soil fertility, water availability and air temperature have also been reported (Abdu et al., 2008). In response to inevitable deforestation and forest degradation, concerted efforts to rehabilitate and restore these areas have been carried out as one of the most viable and environmentally sustainable solution. Species-site matching selection is an important criteria as some of these plants are reported to survive and grow in poor sites and under harsh growing environmental conditions. Hence, this study was conducted to screen 18 indigenous timber species to understand their physiological performance in nursery conditions and to identify potential species for out-planting in rehabilitation and restoration purposes.

Materials and Methods

A total of 18 species were selected from families comprises of Dipterocarpaceae, Fabaceae and Lauraceae. These seedlings were raised in FRIM's main nursery. Three seedlings from each species were selected and placed under shade for height and other physiological measurements. The latter measurements were taken from two first fully matured leaves from three seedlings of each species.

The seedlings were dark-adated for 30 minutes using leaf clips prior to each measurement for leaf chlorophyll fluorescence (ChlF) using Pocket Plant Efficiency Analyser (Hansatech Instruments, King's Lynn, UK) and with a single strong light pulse of 3,500 µmol m^{-2} s^{-1} for one second. The chlorophyll content was measured using a SPAD-502 Plus Chlorophyll Meter (Konica-Minolta, Osaka, Japan). Data presented are mean ± standard deviation. Statistical analysis was carried out using analysis of variance (ANOVA) followed by the Tukey's HSD test (p = 0.05) by IBM SPSS Statistics Version 20.

Results and Discussion

Table 1 presents a list of 18 species selected from FRIM nursery for this study and based on their conservation status. A majority of the selected species are classified as being threatened according to the International Union for Conservation of Nature (IUCN) Red List. Among these, *Dipterocarpus semivestitus* or keruing padi is recognised as critically threatened or endangered. *Hopea helferi* recorded the best height (129.0±56.6 cm), while *Dipterocarpus* seedlings had the lowest height ranging from 13 to 25 cm. Similarly, leaf chlorophyll content was also found to be highest in *D. semivestitus* but lowest in *S. macrophylla* (Table 1).

Table 1: Conservation status, mean height and leaf chlorophyll content of 18 selected timber tree species grown in FRIM.

No.	Species	IUCN	Others	Height (cm)	Chlorophyll
1	*Dipterocarpus baudii* (DB)	VU (2017)	LC (MPRL, 2021)	19.0±5.6	20.6±4.6
2	*Dipterocarpus cornutus* (DC)	CR (1998)	LC (MPRL, 2021)	21.7±2.9	42.2±6.3
3	*Dryobalanops oblongifolia* (DO)	LC (2018)	LC (MPRL, 2021)	57.2±10.6	23.6±4.8
4	*Dipterocarpus semivestitus* (DS)	CR (1998)	CR (MPRL, 2021)	15.7±1.5	48.9±3.0
5	*Eusideroxylon zwageri* (EZ)	VU (1998)	NE (MPRL, 2010)	64.7±16.7	23.6±4.8
6	*Hopea helferi* (HH)	EN (2017)	VU (MPRL, 2021)	129.0±56.6	45.0±5.4
7	*Hopea odorata* (HO)	VU (2017)	VU (MPRL, 2021)	36.0±6.6	40.0±3.7
8	*Intsia palembanica* (IP)	NT (2021)	NE (PMPRL, 2010)	25.0±10.4	36.3±4.8
9	*Neobalanocarpus heimii* (NH)	EN (2017)	NT (MPRL, 2021)	49.7±14.6	37.9±5.4
10	*Shorea bracteolate* (SB)	EN (2017)	LC (MPRL, 2021)	52.3±5.5	40.0±3.6
11	*Shorea hemsleyana* (SH)	VU (2020)	CR (MPRL, 2021)	27.3±2.3	21.8±3.7
12	*Shorea leprosula* (SL)	NT (2017)	LC (MPRL, 2021)	49.3±19.9	32.5±3.9
13	*Shorea macrophylla* (SM)	LC (2019)	VU (SPRL, 2014)	56.0±2.0	17.8±4.5
14	*Shorea roxburghii* (SR)	VU (2017)	NT (MPRL, 2021)	45.3±4.5	24.2±3.4
15	*Shorea singkawang* (SS)	VU (2017)	EN (MPRL, 2021)	27.3±7.1	35.5±3.8
16	*Shorea maxwelliana* (SW)	EN (1998)	LC (MPRL, 2021)	60.3±9.1	31.6±4.0
17	*Shorea mecistopteryx* (SX)	VU (2019)	LC (SPRL, 2014)	27.7±5.1	22.5±4.4
18	*Vatica umbonata* (VU)	LC (2017)	LC (MPRL, 2021)	57.7±9.0	33.0±3.7

Note: IUCN – International Union for Conservation of Nature, MPRL – Malaysia Plant Red List (Yong et al., 2021), PMPRL – Peninsular Malaysia Plant Red List (Chua et al., 2010), SPRL – Sarawak Plant Red List, CR – Critically Endangered, EN – Endangered, LC – Least Concern, NE – Not Evaluated, NT – Not Threatened, VU – Vulnerable.

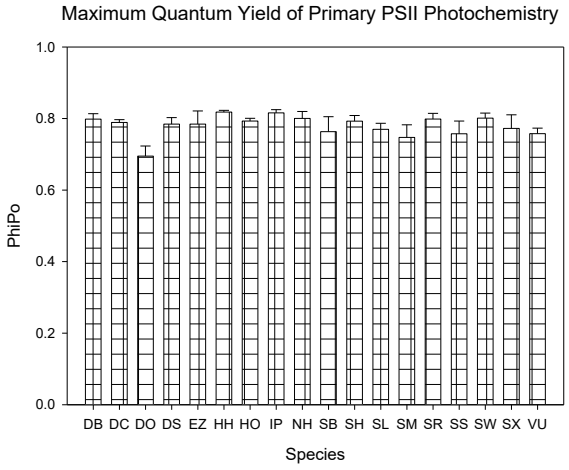

Figure 1: Maximum quantum yield of primary PSII photochemistry (φP_o).

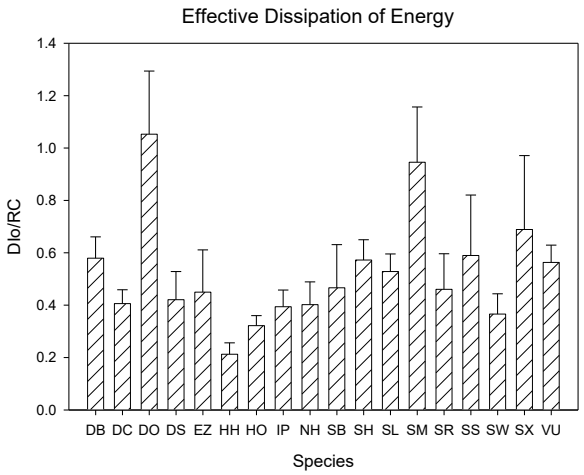

Figure 2: Effective dissipation of energy in active reaction center (DI_o/RC).

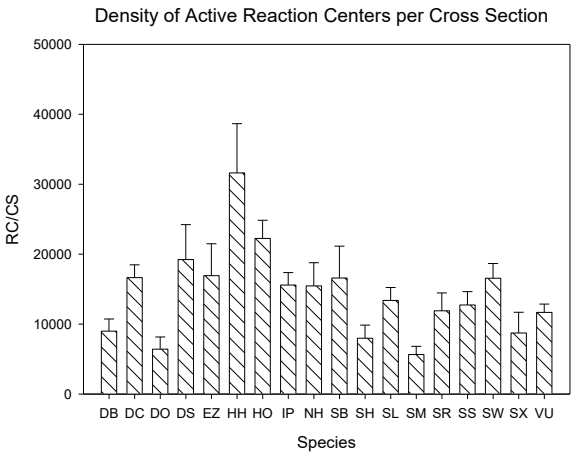

Figure 3: Density of reaction centres per cross section (RC/CS).

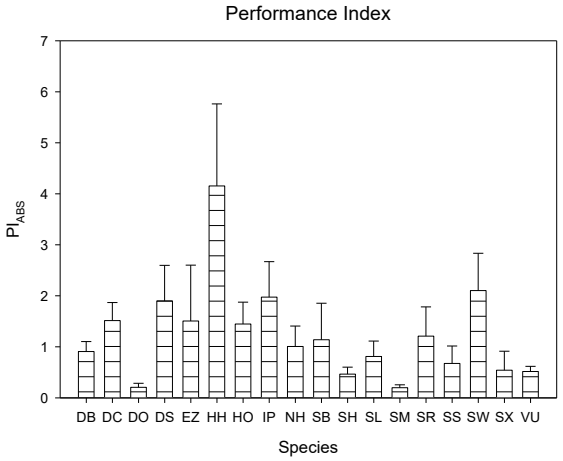

Figure 4: Performance index on absorption basis (PI_{ABS}).

This study showed that maximum quantum yield of primary PSII photochemistry (φP_o) is not a very sensitive indicator for selecting high performance among the species (Figure 1). The insensitivity of φP_o to nutrient deficiency, water availability and drought stress have discussed in some other studies by Bukhov and Carpentier, 2004; van Heerden et al., 2007; Živčák et al., 2014. Interestingly, we observed that DI_o/RC which represented the dissipation of energy from reaction center (RC) provided a more pronounced pattern that differentiates the effectiveness of each species (Figure 2). Similar results have also been shown by Jiang et al. (2008) who found that an increase in DI_o/RC and a decrease in Ψ_{Eo} can be used to identify photoinhibition rather than F_V/F_M or (φP_o). This highest average of DI_o/RC was obtained in *D. oblongifolia* and *S. macrophylla*. Furthermore, *Dryobalanops oblongifolia* and *S. macrophylla* also showed exceptionally higher absorption flux per RC (ABS/RC) (data not shown). This parameter characterises the ratio of energy fux absorbed (ABS) by all PSIIs to the number of active PSII reaction centers (RC) (Khan et al., 2021). Increased in ABS/RC is usually associated to an increase in antenna size or partial inactivation of PSII RC. The latter can be confirmed by lower active RC per excitation cross-section (RC/CS) of those two species above in comparison to significantly higher average value in *H. helferi* (Figure 3). According to Stefanov et al. (2011) and Lepeduš et al. (2011), the inactivation of RC can be linked to a photo-protective mechanism which then transforms part of the RC into 'heat sink' which dissipates excess excitation energy to prevent

excessive excitation of PSII. The increase in dissipated energy is evidently shown in the increase of DI_o/RC in both *D. oblongifolia* and *S. macrophylla*.

In terms of performance index (PI), it consolidates three individual JIP parameters (Stirbet et al., 2018): (i) the apparent antenna size of an active PSII (ABS/RC); (ii) the likelihood that an absorbed photon can be trapped by PSII RCs (Fv/Fm =φP_o); and (iii) the efficiency with which electrons are transferred beyond Q_A in the electron transport chain (ψP_o). Thus, this is regarded as a sensitive indicator in antenna properties, trapping efficiency or electron transport beyond Q_A (Kalaji et al., 2016). Figure 4 shows the performance index on absorption basis (PI_{ABS}) as it was a more sensitive parameter as compared to φP_o. There is a more pronounced PI_{ABS} ($p < 0.05$) in *H. helferi* but lowest in *D. oblongifolia* and *S. macrophylla,* thus again indicating that these two species have overall lower photosynthetic performance associated with decrease of leaf electron transport capacity.

Conclusion

The quality of seedlings is critical to the success of forest restoration programmes. Therefore, rapid assessment of seedling physiological and growth performances will contribute to the establishment of high quality seedlings following out-planting. In this nursery-stage study, it was found that *H. helferi* performed significantly better compared to other selected species. However, further studies are recommended to examine the performance of other dominant or emergent species in response to stress conditions and as a reliable indicator for plants with higher photosynthetic efficiency.

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Development of Propagation Methods for *Xylocarpus rumphii* (Nyireh Pasir)

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Introduction

Xylocarpus Koenig or locally known as Nyireh is a small genus of plants in the mahogany family (Meliaceae). It includes two true mangrove species namely *Xylocarpus granatum* J. Koenig (Nyireh bunga) and *X. moluccensis* (Lam.) M. Roem. (Nyireh batu), however *X. rumphii* (Kostel) Mabb. (Nyireh pasir) is a non-mangrove species that usually grows above the limit of tidal reach in cliffs, rocks and sandy upland areas (Duke, 2006). *X. rumphii* is less common in distribution, abundance and in research compared with the two other genus. *X. rumphii* is found along the coasts of East Africa and Madagascar towards India, Burma (Myanmar), Indochina, the Ryukyu Island, Thailand, the Malesia region, tropical Australia and the Pacific, east to Tonga and Fiji. In Peninsula Malaysia, it has been recorded in Kedah, Penang, Perak, Negeri Sembilan, Johor, Pahang, and Terengganu (https://www.mybis.gov.my/sp/46251). *X. rumphii* is listed as LC (Least Concerned) in Malaysia Plant Red List Peninsular Malaysia version 3.1, 2021. But in Thailand, Koh Mak Tambon Administrative Organisation is fighting to save a single *X. rumphii* on a small uninhabited Koh Khai Hua Roh islet in Trat province from selfie-crazy tourists who have been blamed for its deteriorating health (https://www.scmp.com/week-asia/lifestyle-culture/article/3181924/thailand-wants-selfie-taking tourists-stop-abusing). *X. rumphii* is a small tree that can grow from 4 to 12 metres tall and is utilized for its timber, tannins, and medicinal properties. In 2018, a project proposal was submitted in the interest of saving and conserving the only *X. rumphii* found in Penang National Park. The study was divided into two parts propagation study on macro propagation and tissue culture. Unfortunately, none of the studies produced rooted planting materials.

Figure 1: *Xylocarpus rumphii* at Penang National Park, George Town, Penang.

Efforts were taken to search for areas that have *X. rumphii* populations that can give more options to experiment. These areas include Pulau Singa Besar and Pulau Ayer Hangat in Pulau Langkawi; and Telok Dalam and Telok Segadas in Pulau Pangkor.

The objective of this study is to develop propagation techniques of *X. rumphii* which include seed germination, stem cuttings and marcotting for conservation and planting. Plant propagation is the process of increasing the number of plants of a specific species or cultivar. It involves sexual reproduction which produces viable seeds. Seed germination is a complex process that occurs when a viable seed with proper internal conditions is exposed to favourable environmental conditions. Internal conditions that will affect seed germination include seed coat properties and dormancy. Environmental factors that influence seed germination includes moisture, temperature, air (oxygen) and light in

certain species. Ideal germination conditions vary by species (https://extension.missouri.edu/publications/mg3).

Vegetative propagation using stem cuttings and marcotting techniques provides an opportunity to harness and exploit genetic variation directly (Zobel and Talbert, 1984). Stem cutting involves rooting a severed piece of stem containing at least one node of the parent plant (Nor Hasnida et al., 2019) whereas marcotting is a method of propagation now known as air layering, whereas a stem is inducted to create roots while being present within the parent plant. The stem is then partly cut and rooting hormones are applied to enhance rooting. It is then wrapped with moist peat moss and sphagnum and tied with a plastic film (https://homework.study.com/explanation/what-is-marcotting.html). The formation of roots on the marcots requires continuous moisture, adequate aeration, and moderate temperatures (Puran Bridgemohan et al., 2016). Stem cuttings and marcotting are the major methods of vegetative propagation for mangrove species as these are low-cost, less time-consuming and yet simple technologies (Clough, 1993; De Silva and Amarasinghe, 2010; Wetlands International, n.d.). Indetermined the success of rooting, several factors affect the ability of rooting, has been reported widely, as the effectiveness of hormone in promoting root development (Leakey et al., 1982; Hartmann et al., 1990; Tchoundjeu and Leakey, 1996; Aminah et al., 2006).

A previous study on the propagation of *X. rumphii* in Sri Lanka by Palihakkara et al. (2013), used seeds soaked in seawater for 12 hours which gives a high percentage of germination while peeled seed shows the lowest. As for stem cutting and marcotting fails to root after 4 months. The rooting hormone used is commercial hormone Secto (NAA+fungicide) and control with no hormone. However, not much detail was available regarding the germination percentage rate.

Materials and Methods

Seed collection

The location of the tree was identified in December 2020 at Pulau Singa Besar, Langkawi, Kedah and was fruiting (Figure 2). The fruit type is a capsule with corky testa seed (Figure 3). The seeds float just below the seawater surface and are being dispersed by ocean currents. The single tree was facing the open sea at the sandy beach. Reachable fruits were wrapped using a net to prevent dropping on the beach and drifting away (Figure 4). Fruits in the net were collected on the tree and seeds dispersed from fruits were collected near the tree trap under the dead leaves and twigs during the second visit to the site in April 2021. Seeds were separated from the fruit on site.

Figure 2: Bonsai shape of *X. rumphii* at Pulau Singa Besar, Langkawi, Kedah.

Figure 3: Young fruit of *X. rumphii*.

Figure 4: Fruits are wrap with net to avoid seeds drop on beach being drifted away by ocean currents.

Figure 5: Seeds were collected during the second visit.

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Coir is separate from the testa (Figure 6) and was nicked using secateurs to allow moisture and gases in (Figure 7). Seeds are then sown in the germination bed in lines and are arranged at an appropriate distance (Figure 8). A thin layer of sieved river sand is applied on top of the seeds. Watering with fresh water is done twice daily using an automatic sprinkler system. Observation for seed germination was done from week 2 till week 12.

Figure 6: Coir is separated from testa using secateur.

Figure 7: Scarification to enhance germination.

Figure 8: Seeds were arranged in lines, at an appropriate distance, pressed down to the surface of the sand bed. A thin layer applied on top to avoid exposing the seeds.

Stem cutting

Cutting sources are taken from two sites, two trees at Teluk Dalam (NPTD 1 and 3) (Figure 9) and one tree (NPTS 1) at Teluk Segadas, Pulau Pangkor (Figure 10). Cuttings were selected and taken from young coppices or orthotropic shoots. The length of cutting is 40–50 cm to facilitate delivery to the nursery. Cuttings are harvested and kept moist in a black polyethylene bag size 22” x 42”. A layer of hydrogel is put in the base of the bag to avoid the base cutting dried out.

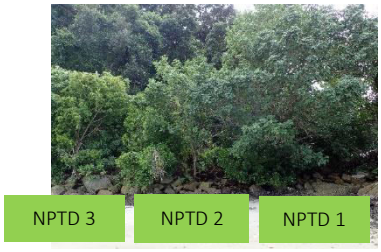

Figure 9: NPTD 1 to 3 in row (from right to left) in Teluk Dalam, Pulau Pangkor.

Figure 10: NPTS 1 with sprouting shoots in Teluk Segadas, Pulau Pangkor.

Stem cutting between 10 cm to 12 cm with two to three nodes was used in the experiment. Cuttings are isolated from each source. Each source was divided into softwood, semihard and hardwood. Cutting segments were treated with Seradix no 1–0.1 % IBA (Indole butyric acid) and no 3–0.8% IBA. Cuttings were planted in a rooting planting bed of semi-coarse river sand (Figure 11). Cuttings are kept moist by using an automatic mist system. The rooting bed was enclosed using a translucent plastic sheet and covered with black netting with 80% shade (Figure 12).

Figure 11: Cuttings were planted in rooting beds.

Figure 12: Rooting bed were enclosed with a layer of translucent plastic sheet and covered with black netting with 80% shade.

At week 7, dead cuttings were removed and the remaining cuttings were cut back at the base and reapplied with gel rooting hormone (0.3% IBA). Cutting experiments were closed after 16 weeks (4 months).

Marcotting

Five number of marcot were done on *X. rumphii* (NPSB 1) at Pulau Singa Besar, Langkawi in December 2022 (Figure 13). The media used for the marcotting technique is sieved soil and fine cocopeat fibres at a ratio of 1:1. Rooting hormone used is seradix no 3 (0.8% IBA). A sheet of polyethylene film is used to wrap the marcot media. Two ends of the sheet are secured tight to ensure media are not dried up after a certain period. Observation was done after 3–4 months to check for rooting.

Figure 13: Marcotting were done to the selective orthotropic shoots.

Results and Discussion

Seed propagation technique

The seed germination rate is 91% and started in week 3 and ended in week 11 (Figure 14 and 15).

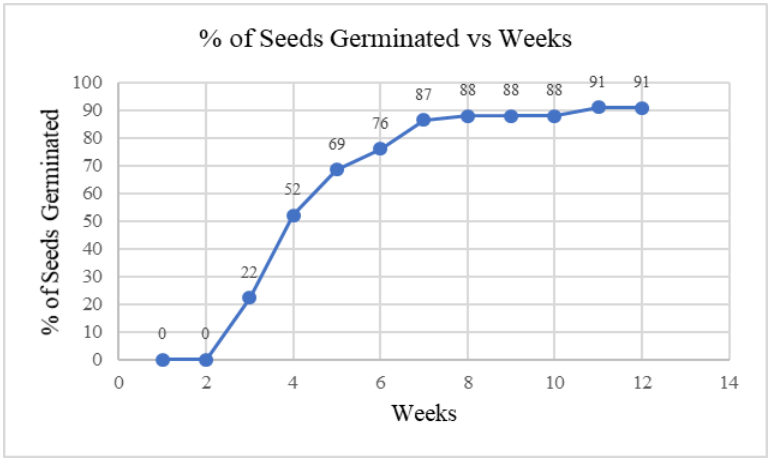

Figure 14: Percentages of seeds germinated.

Figure 15: Close-up picture of seed germination (left) and *X. rumphii* in germination bed ready for potting in polyethylene bags.

Scarification done to seeds testa has shown a very promising result for germination of *X. rumphii*. It is a pre-treatment of seeds that aims to break seed dormancy and accelerate the occurrence of uniform

seed germination. It is a way to provide a permeable condition of seeds through puncturing, burning, breaking, filing, and scratching with knives, needles, sandpaper, and other tools (Ardiarini et al., 2021). Sukardjo (1998) stated that seed viability for *X. granatum* decreased rapidly upon storage and this factor was considered during the germination test for *X. rumphii*. Seeds from *X. granatum* have about 70% germination in 1–2 ½ months which is shorter than *X. rumphii.*

Macro/vegetative propagation

A. Stem cutting technique

A total of 166 numbers of stem cuttings were tested (Figure 17) showing that new shoots were observed in 2nd week. The leaf stalk started to drop and the base of the cuttings started to rot in 3rd week. The cutting experiment was terminated due to 78% mortality at week 7. Observation at week 12, only 5 cuttings survive from the 36 cuttings from the previous terminated trial (7 weeks old cutting) (Figure 16), and in week 16, one of the surviving cuttings produce rooted cutting (Table 1). The cutting was identified from a source taken from NPTS 1 Pulau Pangkor.

Table 1: Type of hormone, number of cuttings and type of cuttings used in the experiment.

Rooting hormone	Type of cuttings	No. of cutting	Observation
Seradix No 1 (0.1 % IBA)	Softwood Semi-hardwood Hardwood	31 21 28	No rooting at week 7. Experiment terminated
	Total	80	
Seradix No 2 (0.3 % IBA)	Softwood Semi-hardwood Hardwood	33 22 31	No rooting at week 7. Experiment terminated
	Total	86	

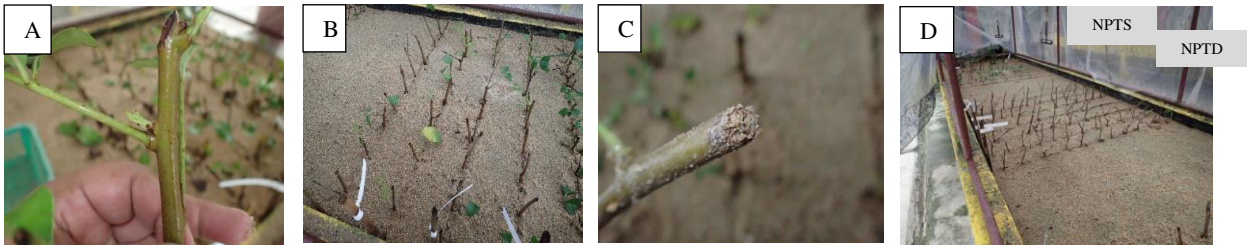

Figure 16: A – New shoots started to grow in week 2. B – Petioles started to drop in NPTD 1 and 3. C – base of cuttings starts to rot. D – In Week 4, 25% of NPTS 1 cuttings observed with leaves attaches but all the leaves dropped for NPTD 1 and 3.

Figure 17: Rooting occurs on the swollen stem, but the base of the cutting seems to rot.

Hartmann and Kester (1983) stated that IBA could be used in a wide range of concentrations without giving a toxic effect on cuttings. The tendency to perform roots is controlled not by the age of the cutting, but by the age of the tree from which cutting is taken (Thimann et al., 1939).

B. Marcotting/air layering technique

From the study, 40% (2 out of 5) have produced rooting (Figure 18). Constant moisture is one of the essential conditions for successful air layering (Nautiyal 2002, Kadami and Dabral, 1954) as the climate in December 2022 is wet with 321 mm of rainfall over 17 days (https://www.whereandwhen.net/when/southeast-asia/malaysia/langkawi-island/december/). The physiological basis of regeneration by marcotting and cuttings is similar, marcotting is usually more successful (Ruviv and Reuven, 1984). In marcotting, there is also a possibility of maintaining as many leaves as possible, thus maintaining photosynthesis. The continued manufacture of photosynthate increases the capacity of the layer to produce adventitious roots (Haissig 1984, 1986). Auxin is very effective for many difficult-to-root species (Davis, 1988) and it is recommended for general use because of its non-toxic nature to plants over a wide range of concentrations compared with NAA or IAA (Hartmann et al., 2010).

Figure 18: A two-rooted marcot from NPSB 1 at Pulau Singa Besar, Langkawi (left). The successful marcot brought back to nursery at FRIM Mata Ayer Research Stations, Perlis (right).

Conclusions

The seed propagation technique shows that it is the best method to produce *X. rumphii*. Fruits need to be wrapped with a net to avoid seeds' dispersions onto the beach and drifting out into the sea. The advantages of using this technique because seeds are easily transported from sites to a nursery, need simple equipment and fewer facilities, have low possibility of transmitting diseases from the mother plant to the new plant and plant breeders are able to develop new varieties. As for stem cutting, although it produces less rooting and takes 16 weeks to produce rooting there are possibilities to use juvenile planting materials from seedlings or from cutting back of tree branches to promote coppicing as a source of cutting materials. On the other hand, the marcotting technique shows the possibilities of methods that can be used to produce *X. rumphii*. It is best done in the wet season when growth is active and using the rooting hormone 0.8% IBA. It is an alternative way of propagation for difficult-to-root plants.

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Growth Performance and Survival of Selected Dipterocarp Species in the Intensive Forest Management Area of Besul Forest Reserve, Terengganu

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Introduction

Forests are crucial for human well-being by contributing numerous ecosystem services, including carbon storage, clean water, source of non-wood products and habitats for biodiversity. Forests also play a significant role in Malaysia's economy by providing source of timber supplies. These resources must be managed properly and sustainably to ensure the perpetuity of forest products for the next generation. In Peninsular Malaysia, timber extraction within inland dipterocarp forests is managed using the Selective Management System (SMS).

The SMS involves the selection of management (felling) regime based on inventory data, instead of arbitrary prescription, which is equitable to both logger and forest owner as well as to ensure ecological balance and environmental stability and quality. Recent findings on the development of the residual stands in logged over forest provided adequate stocking for subsequent cycles. Previous study by Samsudin et al. (2010) stated that analysis over post felling inventories on current logged over forests found that desired species composition in the residual stands are not sufficient for the next cycles.

The intensive forest management (IFM) has been introduced through an operational study in Peninsular Malaysia. The concept of IFM recognises the importance and needs for biodiversity conservation and forest services. This study is currently executed in Besul Forest Reserve, Dungun, Terengganu. In addition, the IFM is focussing on clear cutting about 30% out of total production area for timber extraction by employing good practices and intensive management, while, another 70% area is conserved for biodiversity, carbon storage and other ecosystem services.

The 30% clear cutting area is planted with selected local commercial tree species. Wood production within production forests can be tailored according to sustainable and timely supply of adequate volume of specific timber species to the targeted timber based on industrial mills. All planted trees were managed intensively within the first five years and to be abandoned to grow naturally afterwards in the study area. From the current records, the IFM study area was planted with various species including commercial trees from dipterocarp.

Dipterocarp trees could be broadly categorised as shade-tolerant, while the seedlings require an increase in light for satisfactory establishment and growth (Ashton, 1998). Previous study by Hai et al. (1996) also showed that shade did not influence the performance of the dipterocarp trees, whereas Mun et al. (2022) suggested that *Shorea leprosula* can be planted in areas with partial shade or in gaps. However, this situation depends on the species where the growth of some planted seedlings from genus such as *Shorea* was found stunted in open areas (Ishak et al., 2020).

Seedling survival is site specific, according to biotic, microclimatic, edaphic characteristics and wildlife interruptions. Intensive management on the planted trees may reduce the risk of mortality and increase survivability as well as growth of tree stands in the study area. Therefore, this paper highlights the growth performance and survival of selected dipterocarp species from the genus *Shorea, Dipterocarpus, Dryobalanops, Hopea,* and *Vatica* planted in the IFM study area of Besul Forest Reserve, Terengganu.

Materials and Methods

Study area

This study was carried out in Compartment 17, Besul Forest Reserve in Dungun, Terengganu (4˚ 39.00'N, 103˚ 9.37'E). This study area is located northeast of Peninsular Malaysia. Compartment 17 is a second growth production forest and covers an area of about 235 hectares (ha). Following the IFM concept, 30% out of this area which equal to 70.5 ha was determined to be felled (Figure 1). Physically, the area still consists of striped forest stand zones in every 20 metre (m) of felling area with purpose for ecological services. After forest operation, this 30% area was then divided into four blocks for tree planting purposes. The topography for the study site varied including hilly, undulating, and flat areas. These four blocks were planted with selected commercial tree seedlings by phases (e.g., phase 1 is for block 1, etc.).

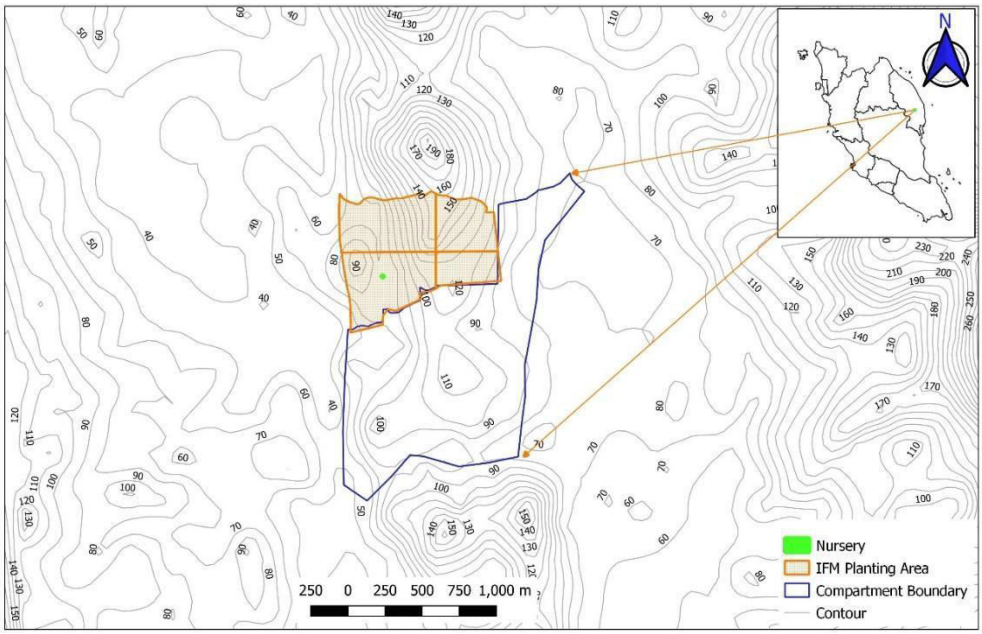

Figure 1: Location of the study area.

Sampling and data collection

Tree planting activity was set in all blocks of the study area. The selection of seedlings was made from local commercial tree species of dipterocarp and non-dipterocarp groups. All seedlings were planted and managed intensively in an open area starting from August 2019 by stages in all four blocks. In this case, intensive activities in the study area are as follows; within the first five years, all trees are provided with adequate fertilizer, weeding, and climber cutting as per schedule and the tree growth are recorded annually. All these activities will be dropped, and trees will be abandoned to grow naturally after five years from the date of planting. To monitor growth performances and tree survival, 10% out of total planted trees for each species were sampled and tagged. The initial basal diameter (mm) and height of the trees (m) were measured and recorded immediately after completion of planting. The first quarter of 2022, the growth performance and survival of the planted trees were recorded by measuring the basal diameter and height using digital calliper and measuring stick, respectively.

Growth performance and survival analysis

In terms of survival, if sampled trees found damage such as missing or dead in the study area, the information on the cause of the damage was taken, for example, wildlife disturbance, erosion, etc. The

growth performance data, i.e., basal diameter and height as well as tree survival were analysed using R-statistical software.

Results and Discussion

Table 1 summarizes the result of the mean annual basal diameter increment, annual height increment and survival rate for all dipterocarp trees planted in the study area. This table shows that *Shorea sumatrana* was found highest in annual basal diameter increment (13.41 mm/year) and followed by *Dryobalanops oblongifolia* and *S. roxburghii* (10.92 mm/year and 10.00 mm/year, respectively). Three species with the least basal diameter increment as affected from intensive management were *S. multiflora, Hopea odorata* and *S. ovalis.*

In terms of height, *S. glauca* was recorded as the species with the highest height increment among other species planted in the IFM area (0.75 m/year). Three species of *Shorea* emerged with a high rate of annual height increment as compared to the other tree species, i.e., *S. glauca*, *Shorea* sp. (Balau) and *S. leprosula*. Apart from that, *Vatica* sp. and *D. aromatica* were also among tree species with high annual height increment. The smaller height increment was observed in *S. multiflora* (0.10 m/year).

Meanwhile, *Vatica* sp. has the highest survival rate (100.00%) followed by *S. multiflora* (90.00%) and *S. leprosula* (88.07%). The tree species with the lowest survival rate was *H. odorata* (62.50%). Basically, all dipterocarp trees survived more than 62% for about 1.5 years after planting in this study area. This condition shows that all dipterocarp trees planted in this area were robust as they were able to withstand under the open environment.

Table 1: Mean annual basal diameter increment, annual height increment and survival of selected dipterocarp trees in Besul Forest Reserve, Terengganu.

Species	Number of samples	Annual basal diameter increment (mm/year)	Annual height increment (m/year)	Survival (%)
Shorea sumatrana	63	13.41	0.53	82.54
Dryobalanops oblongifolia	4	10.92	0.14	75.00
Shorea roxburghii	580	10.00	0.57	81.60
Shorea glauca	58	9.22	0.75	65.52
Dryobalanops aromatica	67	7.97	0.60	68.19
Vatica sp.	11	7.16	0.62	100.00
Hopea sp. (Giam)	60	6.60	0.28	78.33
Shorea curtisii	23	6.56	0.45	73.91
Shorea leprosula	243	6.45	0.65	88.07
Shorea sp. (Balau)	120	6.30	0.72	85.00
Shorea acuminata	957	5.45	0.54	64.13
Shorea lepidota	33	5.36	0.30	78.79
Hopea nervosa	10	4.90	0.19	70.00
Dipterocarpus sp.	333	4.78	0.36	66.15
Shorea multiflora	10	3.28	0.10	90.00
Hopea odorata	192	3.08	0.32	62.50
Shorea ovalis	10	1.63	0.29	80.00

Basal diameter and height increments relationship for selected dipterocarp trees; *D. oblongifolia, S. curtisii, S. glauca, S. roxburghii, S. sumatrana* and *Vatica* sp. were shown in Figure 2. This analysis was done to discover the effect of intensive management towards the trends of basal diameter increment vs. height increment for selected tree species in the study area. This figure shows that the basal diameter increment was directly proportional with the height increments for all selected six species. This early analysis on trees planted in IFM area demonstrated that as the diameter increases, the height of trees also increases by various rates for these six species. Moreover, most trees display height increment below than 2.0 m/year except *S. roxburghii* where some trees exhibited higher than 2.0 m/year. In addition, *S. curtisii* and *Vatica* sp. showed height increments lower than 1.0 m/year for

all trees. Some *S. roxburghii* trees have higher basal diameter increment over the other species in the study area by reaching an increment of more than 30 mm/year.

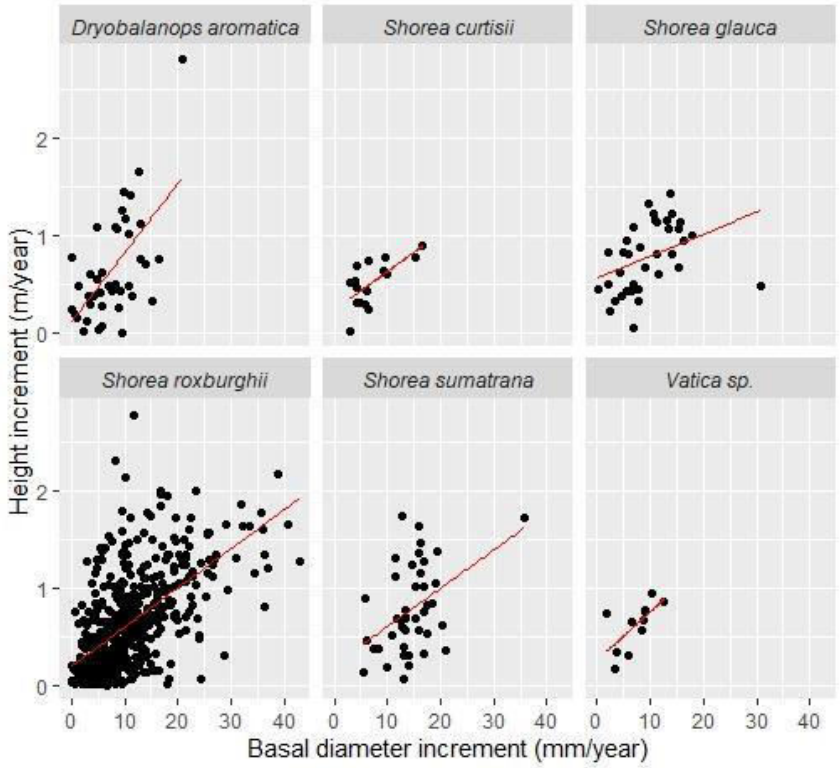

Figure 2: Basal diameter and height increments relationship for six species of selected dipterocarp trees.

All trees were planted in open areas regardless of the topography and planting site conditions. These conditions may affect the growth performance and survival of some of the planted trees in the study area. The survival of planted trees was also influenced by the disturbances from natural and anthropogenic sources, such as soil erosion, wildlife interruption, falling of dead branches from remaining trees and human activities. A study showed that some *Shorea* species were slow growing and shade tolerant where the seedlings were severely damaged due to prolonged exposure of sunlight (Ishak et al., 2020). While this study found that several *Shorea* species were quite sturdy by surviving in intensive management of open area of Besul Forest Reserve. For example, the highest diameter increment recorded in this current study was 13.41 mm/year which belonged to *S. sumatrana*. Another previous finding indicated that dipterocarp trees growing on the lower slope gave better growth and survival than trees growing on the upper hill slope and the shade had no significant effect on the growth of the dipterocarp trees (Hai et al., 1996).

Apart from that, *D. aromatica* is categorised as a fast-growing dipterocarp which can produce marketable size timbers and can have potential yield compared to other dipterocarp trees (Ahmad-Zuhaidi et al., 2003). The basal diameter and height increments for *D. aromatica* for current study also appeared to be similar with the figures stated in the previous study, i.e., 0.7-0.8 cm/year and 0.6-0.7 m/year for diameter increment and height increment, respectively. Besides that, the regular usage of fertiliser in the study area may increase the tree growth. A study showed that the usage of nitrogen, phosphorus and potassium fertiliser or commonly known as N-P-K fertiliser provided a positive effect on 1-year-old and 6-year-old plantations of *Tectona grandis* (Fernández-Moya et al., 2017). Trees require adequate nutrients to grow, thus, use of fertiliser may provide growth responses on the planted tree, especially in this intensive management.

Conclusions

The growth performance and survival of dipterocarp were assessed in the IFM study area of Besul Forest Reserve, Terengganu. Through intensive management, all dipterocarp trees planted in the study area showed incremental trends in the growth performance (diameter and height) by various rates based on species. The survival rate for all dipterocarp trees in the study was more than 62%. Based on the analysis, leading tree species with better growth performance and survival rate from best to least order are *S. roxburghii, S. sumatrana, S. glauca* and *D. aromatica*. Therefore, further assessments are needed in the study area to monitor the growth performance and survival on every species as impact from this intensive management. Long-term studies and effective management strategies are essential to attain sustainable timber stock for the industry in the future.

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Rainfall Effects on Field Seeding of *Mucuna bracteata*

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Introduction

Many oil palm and rubber planting sites in Malaysia have leguminous cover crop (LCC) for soil moisture conservation, erosion control, reduced weeding and improved crop nutrition through the symbiosis between the legume and rhizobia (Samedani et al., 2012; Nasir Ahmad et al., 2020; Rao et al., 2021; Yao et al., 2021). *Pueraria javanica*, *Pueraria phaseoloides*, *Centrosema pubescens*, *Calopogonium mucunoides* and *Calopogonium caeruleum* are LCC adopted by the planters for more than half a century. Later, *Mucuna bracteata* was introduced and gained popularity because of its thicker ground coverage and ability to smother weeds more effectively than the conventional LCC. It is also more efficient in fixing atmospheric nitrogen and produces a threefold biomass, thus it is more beneficial in enhancing nutrition and organic matter content in the cropping fields (Mendis et al., 2022).

Conventional LCC is mostly introduced to planting areas through seeding in the field. However, *M. bracteata* is germinated in the nursery in plug trays or polybags followed by transplanting of the seedlings to the field. The different handling techniques for these LCCs are attributed to the prices of the seeds. The seeds of conventional LCC are produced locally and cheaper enabling seeding directly to the field at rates ranging from 4 kg/ha to as high as 9 kg/ha. Conversely, the seeds of *M. bracteata* are imported and much more expensive. Hence, transfer of the seedlings saves the quantity of seeds needed, being approximately 200 g/ha. Moreover, *M. bracteata* faces the risk of death when encountered with harsh condition in the field during its slower initial growth stage after germination. While labour shortage is widespread in the above-mentioned economic cropping, field seeding of *M. bracteata* could be feasible and even cost effective in certain areas. Rainfall has always been a concern for the success of rain fed plants (Metz et al., 2018). Thus, this study reports the germination and growth of *M. bracteata* seeds sown directly in the oil palm field in Sungai Buloh, Selangor. Seeding of this LCC was studied at two different months in 2017. Simultaneously, the experiments were also aimed to determine the effect of seeding densities on faster ground cover potential. For promoting the growth of this LCC, controlled release fertilizer (CRF) incorporated during seeding procedure was studied as a labour saving strategy.

Materials and Methods

Study location

A total of two experiments on field seeding of *M. bracteata* were carried out in a rain fed oil palm estate in Sungai Buloh, Selangor (3.22 °N, 101.50 °E). The study sites were clay loam, sandy clay loam and sandy loam in mixture according to soil particle size analysis.

Field preparation and seeding procedure

The experimental sites within the estate were cleared for oil palm replanting prior to field seeding of *M. bracteata*. Lining followed by planting hole preparation for oil palms were carried out before seeding of this LCC.

Scarified *M. bracteata* seeds (RediMb®) having 85% germinability according to a laboratory test were applied in the field seeding experiments. Seeding densities of 400, 600 and 800 points/ha were studied for early ground coverage potential. The seeding densities were respectively fulfilled through seeding points made at 1.5 m, 2.3 m and 3 m apart between rows of oil palms. For each seeding point, the seeds were sown at a depth of approximately 2 cm. Holing was made using a hoe handle with a sharpened end. This seeding hole preparation tool is locally known as *tugal*. It enables fast holing and efficient seeding procedure, especially practical and advantageous during sunny time in the field. A total of three seeds were placed in each seeding point. This brought about seed requirements of 200 g/ha, 300 g/ha and 400 g/ha. Then, the seeding points were covered with the soil.

Fertilizer application

Sowing density was studied concurrently with the fertilizer treatments of controlled release fertilizer (CRF) and nitrophoska fertilizer (NPK) in contrast to control with no fertilization. Application of CRF was carried out simultaneously during seeding procedure as single pocket application. CFR was applied at a distance of 5-10 cm from the seeding point. Holing for CRF was also carried out using *tugal*. CRF was also covered with soil after application. On the other hand, NPK was broadcast around the seedlings at one month after seeding.

Experiment 1

In Experiment 1, field seeding was carried out in the middle of April in 2017 according to a completely randomized design. Seeding densities of 400, 600 and 800 points/ha were combined with control (no fertilization), CRF 17:8:9:3 at 10 g and NPK 15:15:15 at 15 g per seeding point in this experiment. Each treatment was replicated four times with five seeding points per replicate.

Experiment 2

Field seeding for Experiment 2 was performed later in the middle of August in 2017. Seeding densities of 400, 600 and 800 points/ha were also studied but combined with two different CRF treatments in this subsequent work. Thus, the fertilizations included control (no fertilization), CRF 17:8:9:3 at 20 g, CRF 10:26:10:3 MgO+TE at 20 g and NPK 15:15:15 at 20 g per seeding point. CRF 10:26:10:3 MgO+TE offered lower nitrogen supply and was cheaper than CRF 17:8:9:3. The experiment was also based on a completely randomized design with four replicates. Each replicate also consisted of five seeding points.

Data collection

Data gathered in these experiments were in-house monthly rainfall and number of rainy days, seeding survival rate and growth of vines. Monthly rain data from April to November 2017 were obtained with the aid of a manual in-house rain gauge in the estate. Rain availability was discussed with regards to its importance in field seeding success of *M. bracteata*.

To elucidate the seed germination of this LCC in each experiment, the monthly seeding survival rate was calculated as follows.

$$Rate = \left(\frac{n}{N}\right)$$

where,
n = number of seeding points with at least a seedling on the monthly assessment day
N = total number of seeding points

On the other hand, the length of the longest main vine from each seeding point was measured monthly to indicate the initial growth of this cover crop. The length of vine was measured using a measuring

tape. Both the seeding survival rate and growth of vines in each experiment were monitored for three months after seeding procedures.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 28. A plot of rainfall and number of rainy days against the studied months was prepared for each experiment. For the LCC, the survival rates and length of vines as impacted by seeding density and fertilization in each experiment were respectively subjected to Kruskal-Wallis test. Means were compared using Mann-Whitney U test at 5% level of significance where applicable.

Results and Discussion

Under rainfed condition, the rain availability, especially in terms of the number of rainy days, had a substantial impact on the field seeding success of *M. bracteata*. In the seeding trial initiated in the middle of April in 2017 and monitored for the following three months until the middle of July, there was 640.50 mm rain and 42 rainy days in total (Figure 1). Sufficient rain in the month of May after seeding procedure resulted in a high germination rate of seeds. There was an above 86% survival rate by seeding points regardless of the seeding densities studied (Table 1; Figure 2). Nonetheless, the seeding survival was significantly reduced to 50% to 78% in the subsequent dryer month of June which recorded only 5 rainy days, although an acceptable amount of rain, i.e. 98 mm rain, was received in this month (Figure 2). The increased mortality of the seedlings could be associated with the seeding pattern, i.e. seeding at points of at least 1.5 m apart. Such a seeding method was attempted as a seed saving strategy in view of the price of the seeds; 400 seeding points/ha, 600 seeding points/ha and 800 seeding points/ha only require approximately 200 g/ha, 300 g/ha and 400 g/ha seeds, respectively. With seeding carried out in points, the erect seedlings of only approximately 10 cm at one month after seeding faced intense water stress as the exposed soil around them dried with delayed rain, leading to potential death of these tender plants (Figure 3). It was in opposed to broadcast seeding of conventional LCC in rows at much higher rates which ranged from 5 kg to 9 kg/ha. Such a higher seeding rate in conventional LCC cultivation was advantageous and reduced mortality despite temporary drought as the seedlings at vicinity could hinder drastic soil moisture loss through an increased ground cover and shading effect (Yue et al., 2023). The subsequent month of July experienced more than 10 rainy days retaining the seeding survival rate as 44% to 73%.

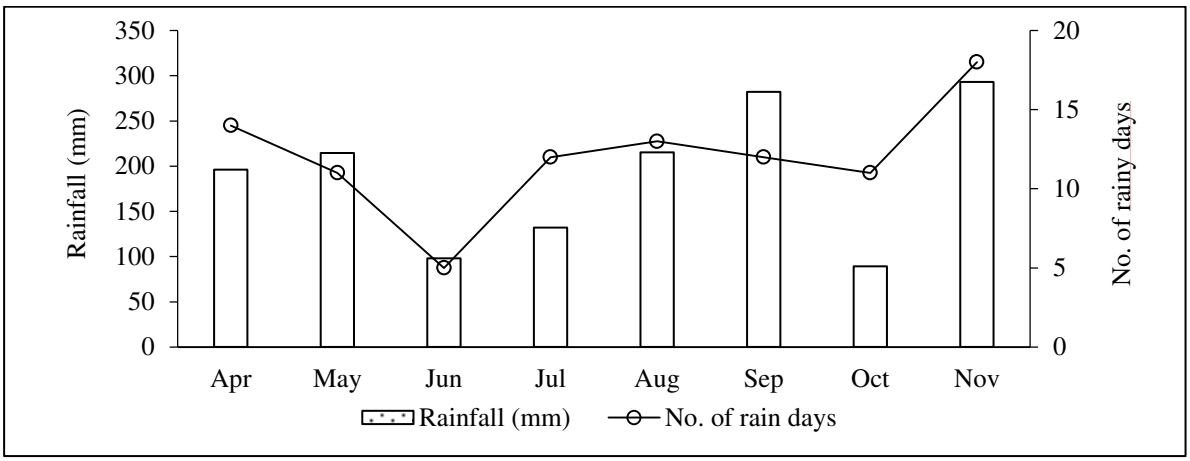

Figure 1: Rainfall and number of rainy days during experimentations from April to November 2017.

Table 1: Significance (P-value) for survival rate and length of vine as affected by seeding density and fertilization from middle of April to middle of July 2017.

Factor	Survival rate by period (months)			Length of vine by period (months)		
	1	2	3	1	2	3
Seeding density	>0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Fertilization	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

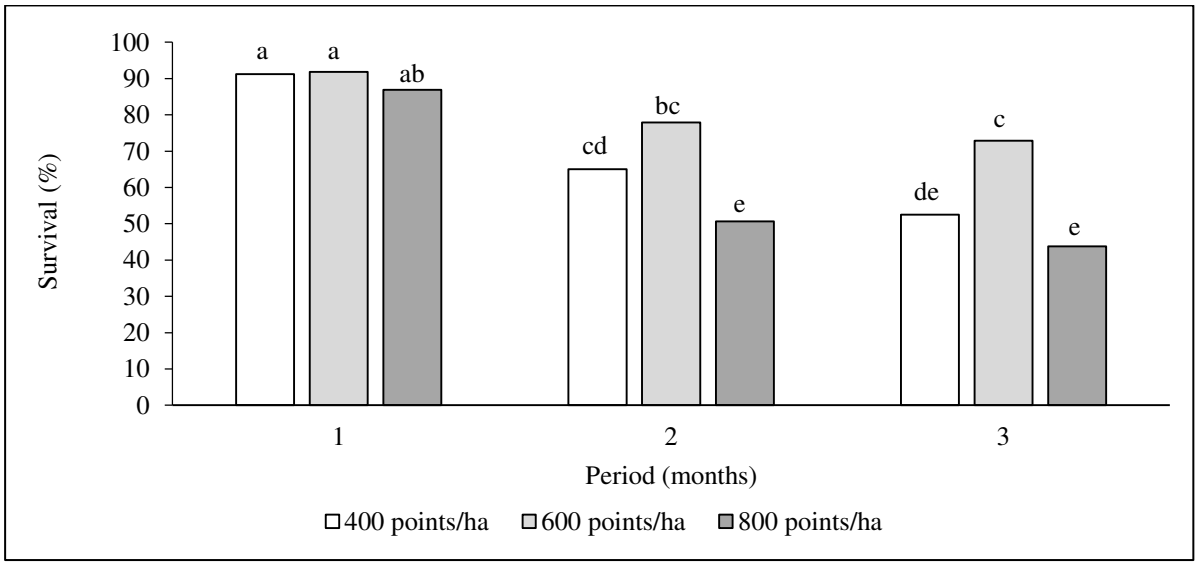

Figure 2: Seeding survival rate according to seeding density in the experiment carried out from middle of April to middle of July 2017; means having similar letter are not significantly different at 5% level of significance.

The rain availability, with the number of rainy days being critical, affected the effectiveness of fertilization for this cover crop (Grabau et al., 2011; Yue et al., 2023). In this experiment, both the CRF and NPK fertilizations were not significantly different from the control for vine elongation (Table 1). While the seedlings had a height of only approximately 10 cm after one month, the limited number of rainy days in the following month of June had a negative impact on the growth of the vines at 400 seeding points/ha, probably because the seedlings were separated farther from one another (Figure 3). The plants could have spent energy to conserve water, instead of utilizing the nutrients for extending their vines. During this relatively dryer month, many apical shoots of the vines also died. Thus, there was generally not much vine elongation observed in the following month of July by three months after seeding.

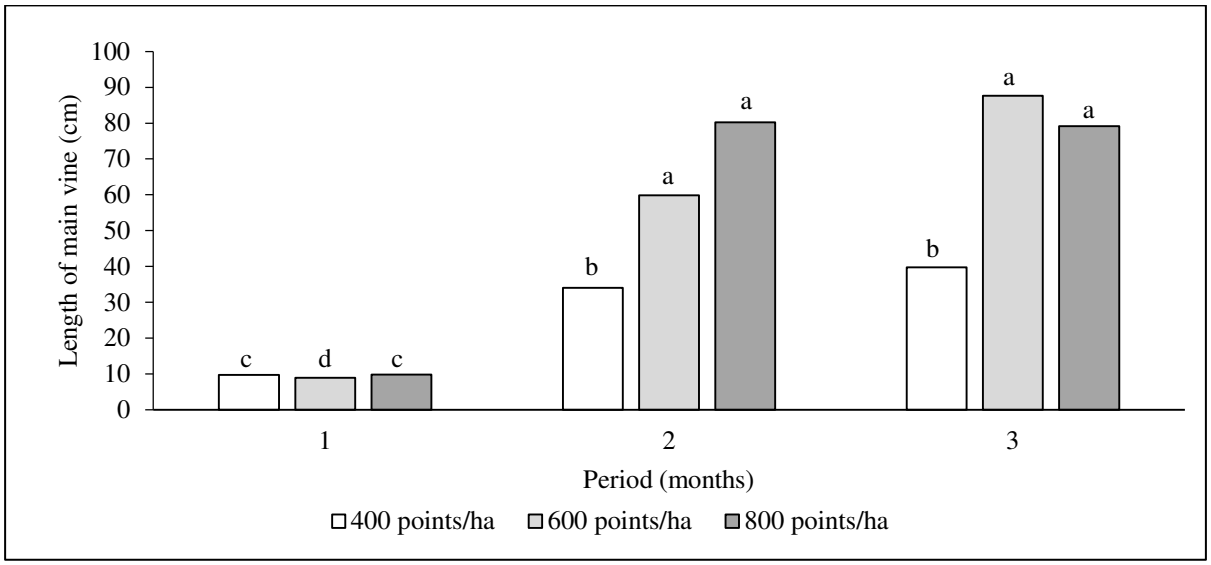

Figure 3: Length of main vine according to seeding density in the experiment carried out from middle of April to middle of July 2017.
Means having similar letter are not significantly different at 5% level of significance.

In the next experiment when seeding was started in the middle of August in the same year, there was also lower rainfall in October at two months after seeding (Figure 1). However, there were more than 10 rainy days in this month. In total, the months spanning from August to November in 2017 had 897 mm rainfall and 54 rainy days (Figure 1). Thus, the survival rate of the seeding points was generally retained above 76% over this period of three months, except for that with 400 seeding points/ha (table 2; Figure 4). Its survival rate was reduced to 61% at three months after seeding. It implied that the number of rainy days was critical for field seeding success. The germinating seeds and the newly developed seedlings needed a constant water supply during their early growth stage since they have yet developed deeper root systems to source for water further down in the ground.

Table 2: Significance (P-value) for survival rate and length of vine as affected by seeding density and fertilization from middle of August to middle of November 2017.

Factor	Survival rate by period (months)			Length of vine by period (months)		
	1	2	3	1	2	3
Seeding density	>0.05	>0.05	<0.05	<0.05	<0.05	>0.05
Fertilization	>0.05	>0.05	>0.05	<0.05	<0.05	<0.05
Interaction	>0.05	>0.05	>0.05	<0.05	<0.05	<0.05

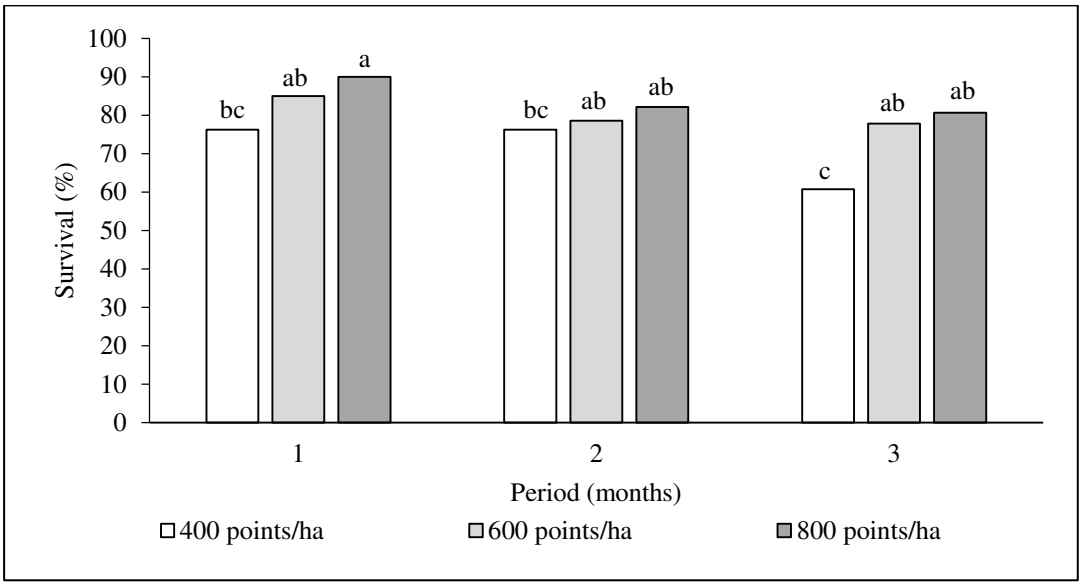

Figure 4: Seeding survival rate according to seeding density in the experiment carried out from middle of August to middle of November 2017.
Means having similar letter are not significantly different at 5% level of significance.

With above 10 rainy days monthly, the seedlings in Experiment 2 responded positively to fertilizer application while the soil variety could have confounded the seeding density effect on their initial growth (Table 2). Likewise, the seedlings also had a height of approximately 10 cm after one month, regardless of the seeding density or fertilization (Table 3). In the subsequent months, the seedlings applied with CRF or NPK generally had better growth performance in terms of their length of main vines. It was encouraging to note that CRF 10:26:10:3 with lower nitrogen content and price was appropriate for cost saving. CFR also reduced labour usage since it could be applied concurrently during seeding procedure.

Table 3: Length of main vine (cm) as affected by the interaction between seeding density and fertilization in the experiment carried out from middle of August to middle of November 2017.

Seedling density (points/ha)	Fertilization	Period (months)		
		1	2	3
400	Control	11.36^{ab}	12.80^{c}	22.80^{bc}
	CRF 17:8:9:3	10.19^{bc}	53.35^{ab}	139.59^{a}
	CRF 10:26:10:3	12.39^{a}	47.59^{ab}	86.93^{ab}
	NPK	9.72^{bc}	30.57^{b}	188.02^{a}
600	Control	7.10^{d}	9.45^{c}	10.00^{c}
	CRF 17:8:9:3	8.14^{cd}	62.46^{a}	124.70^{a}
	CRF 10:26:10:3	8.80^{bcd}	57.93^{ab}	127.55^{a}
	NPK	8.67^{bcd}	53.34^{ab}	116.49^{a}
800	Control	7.50^{d}	11.78^{c}	20.65^{bc}
	CRF 17:8:9:3	7.36^{d}	13.58^{c}	115.62^{a}
	CRF 10:26:10:3	9.95^{bc}	51.11^{ab}	116.14^{a}
	NPK	7.69^{d}	14.20^{c}	99.91^{a}

Means having similar letter within column (period) are not significantly different at 5% level of significance.

Conclusion

Field seeding of *M. bracteata* was recommended as a fast, easy and cost-effective approach during rainy season or in areas with regular rainy days. A total of 3 seeds could be sown at a distance of 1.5 to 3 m for this purpose, resulting in seed requirements of 200 g/ha to 400 g/ha. Fertilizer application, especially the labour saving and single application of low-cost CRF, enhanced the initial growth of the seedlings for early ground coverage potential, despite containing lower nitrogen.

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Preliminary Findings on the Survival and Growth Performance of Belimbing Buluh, Asam Gelugor and Petai Grown in Two Agroforestry Models

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Introduction

Agroforestry is defined as the practice of growing trees and crops in interacting combinations (Nair et al., 2010). It has gained the attention of the scientific community due to its potential to provide environmental benefits such as enhanced nutrient cycling, soil conservation and biodiversity conservation (Leakey, 2017), and its potential for carbon sequestration (Nair et al., 2010). Currently, a wide range of agroforestry systems are being practiced in many different parts of the world, in industrialised regions such as Europe, Australia and New Zealand (Nair, 2013), and in developing regions such as sub-Saharan Africa (Nair, 2014). Agroforestry play very different roles in different countries – its main purpose in industrialized nations is providing ecosystem services and providing a method of sustainable land use, whereas in developing countries agroforestry is practiced to alleviate poverty, maintain nutritional security, and arresting land degradation (Nair et al., 2008).

According to Leakey (2017), trees in agroforestry systems able to improve the soil through four ways. Firstly, they increase nutrient inputs by capturing nutrients from atmospheric deposition, biological nitrogen fixation, and from deep in the subsoil, and storing them in their biomass. Secondly, trees enhance internal cycling by increasing the availability of nutrients in the soil through the conversion of nutrients to more labile forms of soil organic matter. Thirdly, trees in agroforestry systems decrease nutrient losses from the soil. Finally, trees prevent soil loss through runoff and erosion by protecting soil surface via two canopies: the litter layer and the leaf canopy, which can also maintain or improve soil physical properties.

One of the models of agroforestry that is widely practiced is known as hedgerow intercropping (Nair, 2014). The hedgerow intercropping involves growing one/two rows of perennials trees or shrubs (preferably leguminous) at a close spacing in the form of hedges, and crops are grown simultaneously in between the hedgerows (Kaushal et al., 2021). In some cases, the woody species in the field is pruned to reduce the shading of crops, and the pruning can be used as animal fodder, or applied as mulch to the alleys as a source of organic matter and nutrients (Nair, 2014). Sometimes, intercropping can be planted in rows, with alternating species of crops with each row (Nair, 2014).

In Malaysia, agroforestry is mainly practiced by rural communities, and some of them are found to be not aware that they are involved in the agroforestry sector (Musa et al., 2019). In the consideration of its various benefits, agroforestry has been made as one of the strategic action plans of the Third National Agriculture Policy (NAP3) to maximize utilization and returns on the same piece of land (Ahmad, 2001). It has been promoted among farmers, and multiple research projects are done on agroforestry. Some of the most researched agroforestry systems in Malaysia are the inter-row integration, block planting, perimeter or border planting, and hedge planting system (Ahmad, 2001). However, much of the focus is placed on the viability of plantation crops such as rubber, oil palm, and certain timber species (Ahmad, 2001). There is a need to study how food crops interact with each other in an agroforestry model. In this study, three different types of crops are planted in those agroforestry models for the purpose of studying the agroforestry system's viability. The plants studied were the Belimbing buluh (*Averrhoa bilimbi*), Asam gelugor (*Garcinia atroviridis*), and Petai (*Parkia speciosa*).

Belimbing buluh can grow up to 15 m, and has sparse branches. The fruit juice is sour and extremely acidic, and has been widely used for making sambal hitam in Malay delicacy. Beside this, it is used in the traditional medicine, and is therefore of great interest in the field of phytochemistry and pharmacology (Alhassan and Ahmed, 2016). Asam gelugor can grow up to 27 m height and 70 cm girth with a deep monopodial crown of dense, slender and drooping branches (Shahid et al., 2022). The trees are able to provide food, nutrition as well as generate income to the farmer. The fruit is valued for its distinctive sour taste and has been traditionally used in food preparation and cooking (Shahid et al., 2022). Asam gelugor or Kandis is a medium hardwood timber species which can be used as posts and fence posts. This heavy duty species is suitable for temporary use in construction for bridges or railways.

Petai can grow up to 15–40 m in height with a 50–100 cm stem diameter (Lim, 2012). The seeds are valued because they can be eaten raw or roasted or cooked as vegetable (Lim, 2012). It is light hardwood timber species and widely used for plywood, boxes and crates. They are suitable for interior work for partitions, stair railings, floors and walls skirtings. Although the fruits of these plants are widely used in Malaysian cuisine (Lim, 2012; Alhassan and Ahmed, 2016), their viability in agroforestry models has yet to be studied. As such, this study aims to investigate the viability Belimbing buluh, Asam gelugor, and Petai in two agroforestry models – namely the hedge-row model and the inter-row model – by studying their survival and growth rates after six months of planting.

Materials and Methods

Experimental design

The study site was established at Forest Research Institute Malaysia (FRIM) Research Station (SPF) in Maran, Pahang. In this study, the three crops Belimbing buluh, Asam gelugor, and Petai are planted in two agroforestry models, namely the hedge-row model and the inter-row model in different combinations. The agroforestry models and the combination of crops are the treatments as listed below:

Treatment H1 : Combination of Belimbing buluh and Asam gelugor in a hedge-row model.
Treatment H2 : Combination of Belimbing buluh and Petai in a hedge-row model.
Treatment I1 : Combination of Belimbing buluh and Asam gelugor in an inter-row model.
Treatment I2 : Combination of Belimbing buluh and Petai in an inter-row model.

The crops were planted in October 2021. The crops were given fertilizers every three months for the first year, in which each plant receives 100 g of commercial fertilizer (with a N:P:K ratio of 15:15:15).

Agroforestry models

The arrangement of the plants in the hedge-row and the inter-row systems are shown in Figure 1 and Figure 2, respectively. In the hedge-row system, the Asam gelugor or Petai were planted 6 meters apart from each other into a “hedge”. The Belimbing buluh were planted in 5 rows, with each plant spaced 3 meters apart within the “hedge”. Triplicates of the model for each treatment (H1 and H2) are constructed.

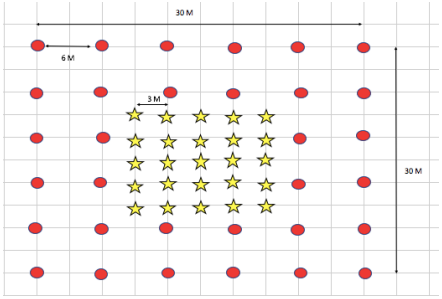

Figure 1: Schematic representation of plants in the hedgerow model, with yellow stars representing the Belimbing buluh plant, whereas the red circles represent either Asam gelugor (in treatment H1) or Petai (in treatment H2).

In the inter-row model, the Asam gelugor or Petai was also planted 6 meters apart from each other into 6 rows. In between each row of the aforementioned crops, the Belimbing buluh was planted 3 m apart. Triplicates of the model for each treatment (I1 and I2) are constructed.

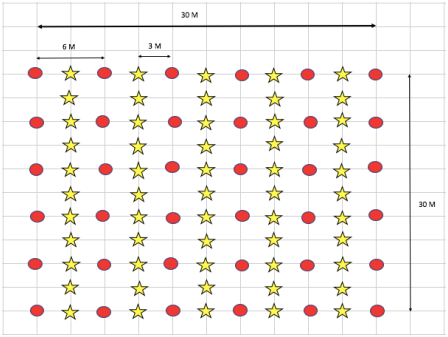

Figure 2: Schematic representation of plants in the inter-row model, with yellow stars representing the Belimbing buluh plant, whereas the red circles represent either Asam gelugor (in treatment I1) or Petai (in treatment I2).

Measured variables

The height of the crops was measured at three and six months after planting (in January 2022 and April 2022, respectively), during which the crops were given fertilizers. The height of crops and their height increase after a period of three months and six months are used as a measure of growth performance. The survival rate of the plants was also recorded as a percentage of the number surviving crops after a period of three months and six months separately.

Statistical analysis

The data obtained are subject to statistical analysis using the SPSS software (IBM SPSS Statistics Version 26; IBM Corporation, Armonk, NY). The data obtained is analysed using the analysis of variance (ANOVA) which is performed on the survival rate of Belimbing buluh, Asam gelugor, and Petai in the two different agroforestry models. ANOVA is also performed on the plants' height and their height increase. The purpose of ANOVA is to test the significance of the effect of planting these crops in Agroforestry models on their survival rates and growth performance. If a significant difference is detected, Tukey's Honestly Significant Difference (Tukey's HSD) test will be conducted on the data groups as the post-hoc test of choice. The post-hoc test indicates if there is significant difference among the data sets yield from different agroforestry models. The probability of statistical significance between the means of the results is observed at $p \leq 0.05$.

Results and Discussion

The results on the survival rates of the plants in the agroforestry models are depicted in Table 1. In the hedgerow system, the Belimbing buluh, Asam gelugor, and Petai had survival rate of 100.00%, 97.92% and 96.30%, respectively, which was higher than the plants in the inter-row system. The Belimbing buluh plants' in the inter-row models recorded the survival rate of 98.20% and 98.18% when paired with Asam gelugor and Petai, respectively. The Asam gelugor and Petai has a survival rate of 96.30% and 85.98%, respectively in the inter-row model. These preliminary results suggest that the hedge-row system provides an environment that facilitates plant survival better than the inter-row model. Another point of interest is that in both models, Asam gelugor displayed a higher survival rate compared to Petai. Asam gelugor recorded a survival rate of 97.92% and 96.30% in the hedge-row and the intercrop model, respectively; whereas the Petai has a survival rate of 89.52% (hedge-row) and 85.98% (inter-row model). Petai had a significantly lower survival rate in both agroforestry models. These early findings suggest that Belimbing buluh is more suitable to be planted together with Asam gelugor in an agroforestry model, compared to Petai.

Table 1: Survival rate of plants in the agroforestry models.

Agroforestry model		Plant species	Survival rate of the crop (%)	
			3 months after planting	6 months after planting
Hedge-row	H1	Belimbing buluh (*Averrhoa bilimbi*)	100.00^{a}	100.00^{a}
		Asam gelugor (*Garcinia atroviridis*)	100.00^{a}	97.92^{a}
	H2	Belimbing buluh (*Averrhoa bilimbi*)	100.00^{a}	100.00^{a}
		Petai (*Parkia speciosa*)	98.96^{a}	89.58^{bc}
Inter-row	I1	Belimbing buluh (*Averrhoa bilimbi*)	100.00^{a}	98.20^{a}
		Asam gelugor (*Garcinia atroviridis*)	99.07^{a}	96.30^{ab}
	I2	Belimbing buluh (*Averrhoa bilimbi*)	100.00^{a}	98.18^{a}
		Petai (*Parkia speciosa*)	93.46^{b}	85.98^{c}

**Means in the same column with the same letters are not significantly different at $p < 0.05$ (using Tukey's HSD Test).*

The height of the plants at three and six months after planting are depicted in Table 2. The results shown that after six months, both Asam gelugor and Petai recorded a higher growth rate in the hedge-row model compared to the inter-row model. Petai in the hedge-row model grew 30.29 cm on average after six months, whereas in the inter-row model measured 27.19 cm in the same duration. Asam gelugor in the inter-row model grew 12.87 cm after six months, whereas in the inter-row model, it recorded 10.83 cm in height. These results may indicate that the hedge-row model facilitates better plant growth.

Table 2: Height of the plants in the agroforestry models after 6 months of planting.

Agroforestry model		Plant species	Average Height of plant (cm)			Height increase of plant (cm)	
			Initial height	3 months after planting	6 months after planting	3 months after planting	6 months after planting
Hedge-row	H1	Belimbing buluh	64.55^{c}	71.52^{cd}	91.95^{cd}	6.97^{bc}	27.40^{bcd}
		Asam gelugor	40.17^{a}	41.27^{a}	53.04^{ab}	1.10^{a}	12.87^{ae}
	H2	Belimbing buluh	68.36^{c}	77.00^{de}	92.88^{cd}	8.64^{c}	24.52^{cd}
		Petai	23.73^{b}	28.03^{b}	54.02^{b}	4.30^{abc}	30.29^{b}
Inter-row	I1	Belimbing buluh	69.62^{c}	78.32^{e}	99.24^{d}	8.70^{c}	29.62^{bcd}
		Asam gelugor	37.86^{a}	39.26^{a}	48.69^{a}	1.40^{ab}	10.83^{a}
	I2	Belimbing buluh	64.89^{c}	69.75^{c}	85.18^{c}	4.86^{abc}	20.29^{de}
		Petai	24.43^{b}	25.22^{b}	51.62^{ab}	0.79^{ab}	27.19^{bc}

**Means in the same column with the same letters are not significantly different at $p < 0.05$ (using Tukey's HSD Test).*

Belimbing buluh planted together with Asam gelugor has shown a higher growth rate in the span of six months. The Belimbing buluh grew by 27.40 cm and 29.62 cm on average in the hedge-row model and inter-row model, respectively; whereas the Belimbing buluh planted with Petai have grew by

24.52 cm and 20.29 cm on average in the hedge-row model and inter-row model, respectively. This reinforces the previous suggestion that Belimbing buluh is more suited to be planted together with Asam gelugor, compared to Petai.

Conclusion

The preliminary results show that the hedge-row agroforestry model is more favourable for plant survival and growth compared to the inter-row model for both Petai and Asam gelugor. Both of these plants performed better in terms of survival rate and growth rates in the hedge-row model. It was also found that the survival rate and growth rates of Belimbing buluh was higher when it was planted together with Asam gelugor in both agroforestry models, compared to Petai. Thus, it can be said that Belimbing buluh planted together with Asam gelugor in a hedge-row agroforestry model has shown the best performance in terms of crop survival rates and growth rate.

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Continuous Oil Palms Breeding Yield Improvement in FGV: Yangambi Materials

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Introduction

The origin of the genus Elaeis, which is a part of the Cocoideae subfamily of the Palmaceae, is either in America or Africa. Prehistoric drift between the American and African continents is thought to have caused the two oil palm species to divide (Zeven, 1965). Two less popular Elaeis species exist is *E. odora* (South America) and *E. madascariensis* (Africa). *Elaeis guineensis* Jacq., also known as the "oil palm of commerce," is endemic to tropical Africa and can be found from Senegal (16 °N) in the north through Angola (15 °S), which borders Namibia, to Zanzibar and the island of Madagascar in the east (Hartley, 1988).

Generally, the wild grove's yield is lower than 0.5 tonne ha^{-1} $year^{-1}$ of oil. However, oil yield at commercial plantations' could increase up to 8 tonne ha^{-1} $year^{-1}$ through breeding and selection (Hartley, 1967). Although it is an out-pollinating monoecious crop, maternal and paternal lineages are independently developed to produce seeds based on the presence or absence of a shell ring in the fruit. *Pisifera* palms typically is female sterility, thick-shelled *dura* palms generate less oil than *tenera* heterozygotes. Thin-shelled *tenera* derives from the crosses of *dura* × *pisifera,* have full fertility restored, and producing 30% more oil than their *dura* parents.

The Institut National pour l'Etude Agronomique du Congo (INEAC) was focusing on the Yangambi population. One open-pollinated *tenera* bunch from the Djongo (best) palm at the Eala Botanical Garden and nine bunches from palm groves in Yawenda served as the foundation for its breeding programme, which began in Yangambi, Zaire, in 1922 (Hardon, 1976). In 1924 and 1927, other illegitimate *teneras* from N'gazi and Isangi were planted at Yangambi. FGV acquired Yangambi materials in the early 1970s as a consequence of an exchange programme with Institut Recherche Huiles Oleagineux (IRHO). In the 2000s, FGV produced D × P Yangambi as its main product after conducting an extensive breeding program. To maintain its quality, FGV has developed into the third generation of Yangambi. This trial is crucial for assessing the performance of the progeny as improvements are made. Potential palms identified in progeny will next be tested to confirm their performance.

Materials and Methods

The trial consisted of six progenies derived from Yangambi-NPM, Virescens × Yangambi, Dumpy-AVROS × Yangambi, Yangambi × MPOB AVROS and Yangambi × Yangambi. The trial was planted as T261 at FGV AS Jengka 24, Pahang, in December 2011 with a single common standard cross. On inland soil (3º42'7.909"N, 102º25'35.961"E), the materials were planted in a randomised complete block design (RCBD), with two replications and 16 palms per plot. The descendants are described in Table 1.

Table 1: List of progenies and their origin.

Origin	Cross-Type	Progenies	Pedigree
Yangambi-NPM Virescens × Yangambi	T×P	TK4045	EB3/10 × EB3/20
Yangambi-NPM Virescens × Yangambi	T×P	TK4311	B36/35 × EB3/20
Dumpy-AVROS × Yangambi	T×P	TK4033	HLK 5 × FC2933.10.5/2
Yangambi × MPOB AVROS	T×P	TK3933	FK6/61 × 0.174/480
Yangambi × Yangambi	T×P	TK3964	FK6/61 × FC2933.10.5/1
Yangambi × Yangambi	T×P	TK4031	FK6/61 × FC2933.10.5/2
Banting × Yangambi	D×P	SC13	FOP 19 × ML 161

SAS 9.4 software was used to analyse the fresh fruit bunch and bunch analysis data. To examine the differences between the progenies and parental groups of planting materials, an analysis of variance (ANOVA) was used, followed by a comparison of means using Duncan's Multiple Range Test (DNMRT).

Results and Discussion

Table 2 shows the performance of bunch yield and its components (fresh fruit bunch (FFB), bunch number (BNO), and average bunch weight (ABW)) over six years. FFB, BNO, and ABW trial means were 132.95 kg palm^{-1} year^{-1} (18.08 tonne ha^{-1} year^{-1}), 13.70 bunches palm^{-1} year^{-1}, and 9.87 kg palm^{-1} year^{-1}, respectively. Progeny TK3964 (FK6/61 × FC2933.10.5/1) generated the highest FFB yield at 161.26 kg palm^{-1} year^{-1} (21.93 tonne ha^{-1} year^{-1}), with BNO and ABW at 16.52 bunches palm^{-1} year^{-1}and 10.10 kg palm^{-1} year^{-1}, respectively. There were no significant differences in FFB between TK4045 and SC13, BNO between TK4045 and SC13, and ABW between TK3933, TK4031, TK4033, and TK4311. Progeny TK3933 (FK6/61 × 0.174/480) had the lowest FFB, BNO, and ABW at 117.55 kg palm^{-1} year^{-1} (15.97 tonne ha^{-1} year^{-1}), 11.59 bunches palm^{-1} year^{-1}, and 9.91 kg palm^{-1} year^{-1}. This demonstrates that the FK6/61 combines poorly with MPOB AVROS *pisifera* but does well with Yangambi *pisifera*. However, no significant differences were found in FFB for TK4031, TK4033, TK4045, TK4311, and SC13, BNO for TK4031, TK4033, and TK4311 BNO, and ABW for TK3964, TK4031, TK4045, TK4311, and SC13.

Table 2: Progeny mean for bunch yield components.

			Mean 6 yrs (2015-2020)		
No	Progeny	Rep	FFB	BNO	ABW
			(kg palm^{-1} year^{-1})	(bunches palm^{-1} year^{-1})	(kg palm^{-1} year^{-1})
1	TK3933	2	117.55^{b}	11.95^{c}	9.91^{ab}
2	TK3964	2	161.26^{a}	16.52^{a}	10.10^{ab}
3	TK4031	2	128.81^{b}	13.43^{bc}	9.82^{ab}
4	TK4033	2	129.87^{b}	12.91^{c}	10.33^{a}
5	TK4045	2	141.71^{ab}	16.04^{ab}	8.92^{b}
6	TK4311	2	130.80^{b}	13.32^{bc}	9.97^{ab}
7	SC13	2	141.40^{ab}	16.67^{a}	8.53^{b}
Mean (1-6)			132.95	13.77	9.87
P ≤ 0.05			22.01	2.61	1.11

**FFB = Fresh Fruit Bunch; BNO = Bunch Number; ABW = Average Bunch Weight*
**Means with the same letter (s) in the same column are not significantly different at P ≤ 0.05 with Duncan New Multiple Range Test (DNMRT).*

The overall mean for mesocarp to fruit (M/F), oil to dry mesocarp (O/DM), and oil to bunch (O/B) was 84.56%, 78.08%, and 30.22%, respectively. The progeny TK3964 (FK6/61 × FC2933.10.5/2) had the highest O/B at 31.67%, which is 1.45% higher than the mean (Table 3). Additionally, it produced the highest oil yield (OY), and total economic product (TEP) each at 7.08 tonne ha^{-1} and 7.60 tonne ha^{-1}, respectively. This result is in line with progeny testing by Chin et al. (2005a) which highlighted the unique qualities of *pisifera* Yangambi ML161 as the best male parental for the development of high oil-yielding planting materials. In addition, in an earlier study by Chin et al. (2005b), Yangambi ML161 was the most stable *pisifera* when compared to the others for FFB, BNO, OY, and TEP.

Nevertheless, no significant differences were found in O/B for TK4031, TK4033, TK4045, and SC13, as well as OY and TEP for TK4045.The progeny TK4311 (B36/35 × EB3/20) had the lowest O/B at 28.13%, which was 2.09% lower than the trial mean. For O/B for TK3933, there is no significant difference, whereas for OY and TEP, there are no significant difference between TK4031, TK4033, SC13, and TK3933.

Table 3: Progeny means for bunch quality components.

No	Progeny	M/F (%)	O/DM (%)	O/B (%)	OY (tonne ha^{-1})	TEP (tonne ha^{-1})
1	TK3933	85.84^{ab}	76.24^{b}	28.60^{bc}	4.63^{d}	5.00^{c}
2	TK3964	87.73^{a}	79.59^{a}	31.67^{a}	7.08^{a}	7.60^{a}
3	TK4031	82.83^{bc}	79.84^{a}	31.01^{ab}	5.84^{bc}	6.42^{b}
4	TK4033	83.13^{bc}	78.81^{a}	30.70^{ab}	5.72^{bc}	6.33^{b}
5	TK4045	86.29^{ab}	76.33^{b}	31.21^{a}	6.29^{ab}	6.75^{ab}
6	TK4311	81.17^{c}	77.09^{b}	28.13^{c}	5.13^{cd}	5.67^{bc}
7	SC13	86.53^{ab}	75.87^{b}	29.94^{ab}	5.77^{bc}	6.19^{b}
Mean (1-6)		84.56	78.08	30.22	5.75	6.26
P ≤ 0.05		3.51	1.55	2.33	0.97	1.03

**M/F = Mesocarp to Fruit; O/DM = Oil to Dry Mesocarp; O/B = Oil to Bunch; OY = Oil Yield; TEP = Total Economic Product. *Means with the same letter (s) in the same column are not significantly different at P ≤ 0.05 with Duncan New Multiple Range Test (DNMRT).*

Conclusion

The result reveals that the Yangambi × Yangambi gave an excellent performance by out-yielding the SC13 (an elite D×P Yangambi). This shows that the Yangambi line has significantly improved for the bunch quality components across the generation. For the planting materials to continue to meet the high standard required, these characteristics must be kept and improved upon for the following generation. Although Progeny TK4045 (Yangambi-NPM Virescens × Yangambi) demonstrated acceptable yielding, ongoing development in this material is required for even better results than Yangambi × Yangambi.

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Comparison of Variability Between Derived Progenies for Selfed and Cloned Oil Palm Germplasm Line

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Introduction

Elaeis guineensis or oil palm, the world's main vegetable oil crop is characterized by high oil productivity and has a long lifespan of more than 25 years. Present with an outcrossing species and a generation cycle of about 8-10 years, any genetic improvement through conventional breeding such as using phenotypic selection could take up to 19 years/cycle. Commercial oil palm hybrid progenies are known to be comparatively heterogeneous genetically (arising from non-fully inbred parents) whereby individual palms can yield >30% of the mean. Successful cloning of a selected number of highly productive oil palms leads to the possibility of establishing uniform tree stands (clones) and hence the potential to achieve higher productivity. Cloning from less advanced populations, in early/recombinant phases of hybrid breeding, or early exploitation of new genetic material from introgression programs (wide intra or inter-specific crosses) were seemed to be more advantageous, as it is more efficient due to broader genetic variability and higher heritability for better yield and other desirable traits (Soh, 2012). Hence, the objectives of this preliminary study were to perform a comparison of yield variability between (i) germplasm line BM530 parent population, (ii) selfed BM530/21 population and (iii) BM530/21 derived ramet population.

Materials and Methods

BM530 was part of the Sabah Exchange Programme, for which the pedigree was listed as LC(T)2 self, originating from Pamol, Cameroons (Congo basin), while LC(T) lines were associated with Unilevers, Cameroons. BM530, were planted in complete block design with other DxD, TxT, TxD, DxT, and TxP (D=*Dura*, T= *Tenera*, P=*Pisifera*) crosses in August 1966. Teneras from this trial were selected and crossed as part of a *pisifera* fruit improvement program for continued breeding, whereby BM530/21 was selfed and subsequently selected as an ortet for tissue culture and clones propagated materials. Progenies and ramets were planted in Randomized Complete Block Design (RCBD) since March 1995 and October 2002 respectively. There were two-four replicates used and 12-16 palms each repeated in each progeny per replicate per trial with spaced triangularly pattern. Planting, upkeep and maintenance were carried out according to standard estate practice. Yield recording and bunch analysis were performed and as shown in Table 1 and according to standard practice as carried out by Rao et al., 1983.

Statistical analysis was performed using Minitab and the data were based on individual palm data for fresh fruit bunch (FFB) and oil-to-bunch ratio (O/B). Standard deviation and variance values indirectly determine the stability of yield (risk) and degree which yield change over time. Yield distributions of clonal palms are expected to narrow significantly compared to culled DxP and DxP populations (Mutert and Fairhurst (1999). Findings may contribute to further cloning, and re-cloning selection for future breeding programmes.

Table 1: Summary at oil palm germplasm and progenies used in the study.

Trial/ Treatment/Year	Type	Fruit type	No palms	Soil type	Density	Yield record/ Bunch analysis
PTA/T9/1966	BM530 [LC(T)2 self]	Tenera	42	Briah clay	148/ha	1970-1974
PTB/T2/1995	BM530/21 x BM530/21	Tenera	29	Selangor	138/ha	1997-2002 1999-2005
PTB/T3/1995	D x P control	Tenera	62	Selangor	138/ha	1997-2002 1999-2005
PTB/T4/1995	D x P control	Tenera	62	Selangor	138/ha	1997-2002 1999-2005
PTC/T7/2002	BM530/21 ramets	Tenera	33	Jawa/Tongkang	148/ha	2005-2010 2010-2013
PTC/T5/2002	D x P control	Tenera	36	Jawa/Tongkang	148/ha	2005-2010 2010-2013
PTC/T6/2002	D x P control	Tenera	32	Jawa/Tongkang	148/ha	2005-2010 2010-2013

Results and Discussion

Basic descriptive statistics and histograms with normal curves based on FFB and O/B for BM530, BM530/21 selfed and BM530 ramets are as shown in Table 2, Table 3, and Figure 1 respectively. For FFB and O/B, equal variance was detected between T2 (selfed) and T7 (ramets) versus 2 sets of controls namely T3, T4; T5, T6 and T9 (Figure 2).

Table 2: Descriptive statistics of the different progenies of an oil palm germplasm line BM530 based on FFB (kg/p/yr).

Variable	Treatment	N	Mean	SE Mean	StDev	Variance	CoefVar
FFB (kg/p/yr)	T2	29	75.13	4.30	23.17	537.02	30.84
	T3	62	146.08	3.31	26.09	680.60	17.86
	T4	62	146.58	3.15	24.78	613.87	16.90
	T5	36	132.97	4.05	24.33	591.81	18.29
	T6	32	144.13	2.94	16.63	276.61	11.54
	T7	33	124.80	3.54	20.33	413.13	16.29
	T9	42	121.33	6.59	42.70	1823.00	35.19

SE=standard error; StDev=standard deviation; CoefVar=coefficient of variance.

Table 3: Descriptive statistics of the different progenies of an oil palm germplasm line BM530 based on O/B value.

Variable	Treatment	N	Mean	SE Mean	StDev	Variance	CoefVar
O/B	T2	29	23.90	0.66	3.53	12.46	14.77
	T3	62	24.05	0.33	2.63	6.92	10.93
	T4	62	25.86	0.33	2.57	6.62	9.95
	T5	28	28.44	0.76	4.04	16.33	14.21
	T6	20	26.64	0.92	4.13	17.01	15.49
	T7	22	25.88	1.01	4.73	22.37	18.28
	T9	25	20.19	0.67	3.37	11.35	16.68

SE=standard error; StDev=standard deviation; CoefVar=coefficient of variance.

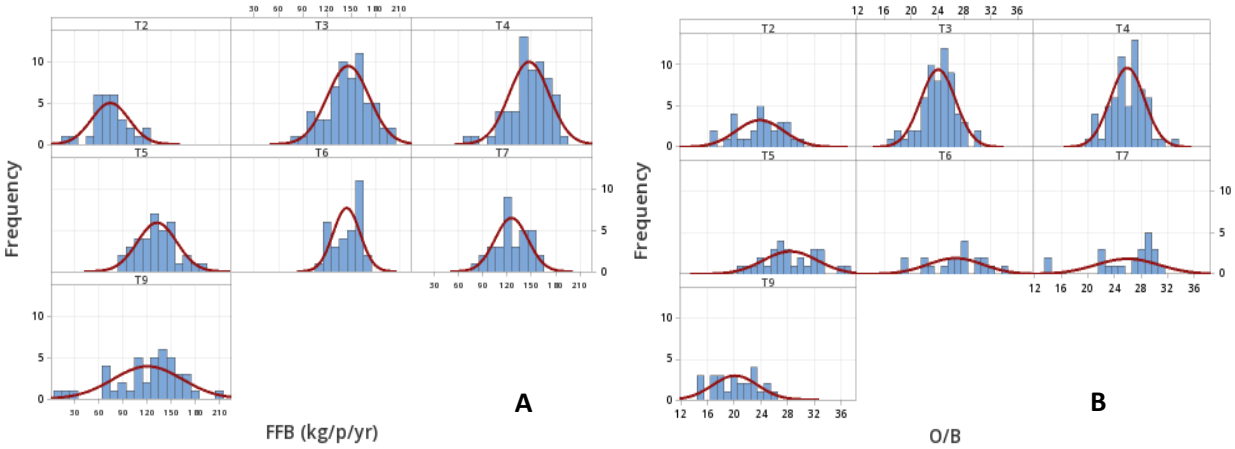

Figure 1: (A) Histogram with normal curve of FFB kg/p/yr) by treatment and (B) Histogram with normal curve of O/B by treatment.

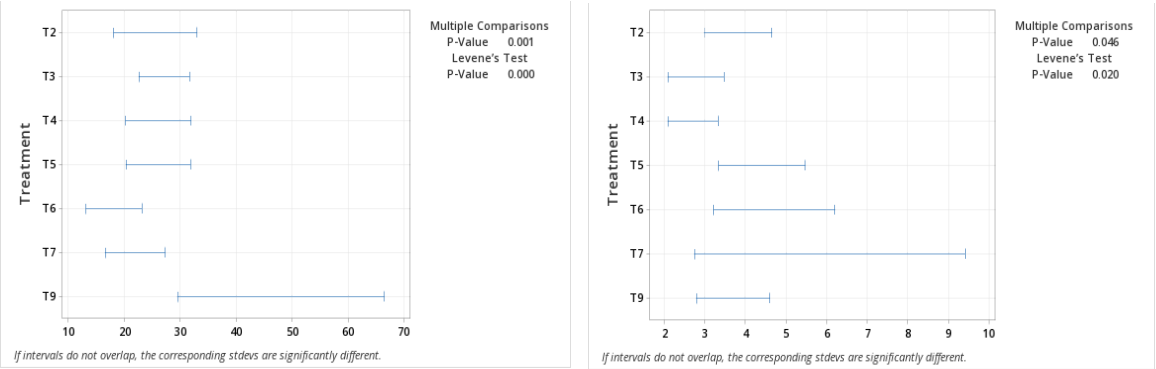

Figure 2: Results of test for equal variances showing multiple comparison intervals based on FFB (kg/p/yr) (left) and O/B values (right) versus treatment, for the standard deviation α = 0.05.

One way ANOVA test showed there was significant difference for FFB of T2 which was 48.68% lower than T3, T4 (controls). Significant difference was also detected between T7 and T6 control, but not T5 control, with FFB difference of 9.57% (Figure 3). In terms of O/B value, there were similarity in T2 and T3, but T4, control was significantly different from both T2 and T3. On average, O/B value of T2 was 4.4% lower than T3, T4. No significant difference in O/B was detected between T7 and T5 and T6 controls; with the former being 5.82% lower (Figure 3). From collected yield records, the ortet (BM530/21) averaged FFB of 155kg/p/yr and O/B value of 23.6. Average values for T9 (BM530 progeny population) were FFB of 121.33kg/p/yr and O/B of 20.19. Hence, in terms of FFB, the yield gap between T7 (ramets)-controls was much reduced compared to T2 (selfed).

The average FFB of T7 (ramets) was 124.80 kg/p/yr) and this was closer to T9 (BM530) with 121.33 kg/p/yr) but was less than the selected ortet in BM530/21 with 155 kg/p/yr). However, the T2 (selfed) recorded a lower FFB at 75.13 kg/p/yr. This significant reduction in FFB could be due to possible inbreeding depression (as a result of multiple generations of selfing) (Faizah et al., 2016; Simiqueli et al., 2018). Other abnormalities such as early stage bunch rotting and stunted palms were observed for eight palms (out of 80 field planted palms) and this warrants further studies. In terms of O/B value, T7 (25.88) was similar to the ortet (23.60) and higher than T9 (20.19), with T2 having similar value to ortet (23.90). Soh (2012) reported that selection based on O/B was more efficient than on FFB yield, as ascribed to higher broad-sense heritability (h2B) in palms from families of more out-bred parents (0.26). Further, the selection using ortet (individual palm) was also reported to have performed well

for early exploitation of new genetic materials because of higher genetic variability and yield traits heritability (Potier et al., 2006; Soh et al., 2011).

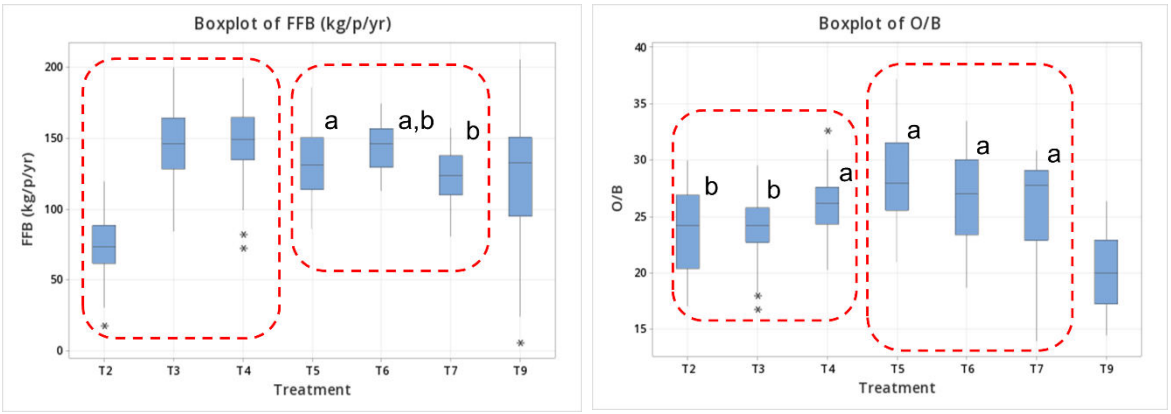

Figure 3: Boxplots depicting one way ANOVA results for (left) FFB (kg/p/yr) versus treatment and (right) and O/B versus treatment (α = 0.05). T2=BM530/21 selfed, T3, T4, T5, T6=control, T7=BM530/21 clone, T9=BM530 family. Means with the same letter are not significantly different.

Conclusion

Results showed that oil palm germplasm line can be gained in clones as compared to control by narrowing restricting variability in FFB. The closed yield gap between ortet and clone versus selfed progenies (although this trend not clear for O/B), showed that uniformity and homogeneity for clonal planting material can be achieved. However, more studies are recommended for re-cloning and hybrids on elected clones that may affects costs, somaclonal variations, pests and disease resistance, and environmental stress vulnerabilities.

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FGV Oil Palm Progenies Performances of Advance × Germplasm Material

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Introduction

The oil palm (*Elaeis guineensis* Jacq.) is a tropical palm that originated from West and Central Africa and could produce three to eight times more oil from mesocarp and palm-kernel oil from the endosperm compared to any other tropical or temperate oil crops (Sheil et al., 2009). Palm oil is one of the world's major commodities and has significantly contributed to many countries' economic growth since it has been involved in a wide range of consumer products like cosmetics, surfactants, soaps, herbicides, and cooking oil (Constantin et al., 2017). From a renewable energy perspective, the concern on global warming issues and the prediction of the depletion of fossil fuel resources have boosted the demand for biofuel. This gave a high impact on palm oil as one of the main ingredients to make biodiesel which is mainly produced in the Southeast Asia region (Kurnia et al., 2016). Thus palm oil demand is increasing tremendously.

Taking a wild and naturally reproduced plant into mass planting raises many questions about planting, seed treatment, diseases, and other related issues (Corley and Tinker, 2016). The selection of these wild oil palm materials needs to go through a strict selection process because improper selection can lead to fewer heritability traits. In oil palm breeding, heritability traits are identified through the *dura* × *pisifera* or *dura* × *tenera* progeny test because it led to significant yield improvement in oil palm (Swaray et al., 2020). Both cross-pollination produced the majority of the *tenera* progeny whose fruit shell was thinner than *dura* palm. One or more traits from the parents shall be inherited by the majority of their progenies and this could be indicated that the traits have high heritability (Constantin et al., 2017). Once the high heritability trait has been determined, the parents could be further exploited to become potential commercial parents by using the self-pollination approaches. Further self-pollination could enhance the heritability of the traits. However, repeated procedures in oil palm breeding will harm the genetic information of the breed, which will cause genetic depression. The inbreeding depression arises due to crossing between relatives or self-pollinated (Simiqueli et al., 2018). According to Corley and Tinker (2015), Deli *dura* populations already had narrow genetic variations due to the self-pollinated for several generations and most of the wild variation traits have disappeared after several selections and only certain interest traits remain such as large bunch sizes.

This study aims to investigate the fresh fruit bunch (FFB) yield of germplasm and its potential to be incorporated background as future oil palm varieties for commercialization. Therefore, the intensive exploitation of germplasm material is crucial to unravel other abilities such as materials that are tolerant to *Ganoderma* or nutrient efficiency materials that could reduce dependency on high fertilizer application in the future. Finally, the exploitation of germplasm material could broaden the genetic pool in oil palm research.

Materials and Methods

The trial consisted of six progenies and was planted using Randomised Complete Block Design (RCBD) in 2012 at FGV Agri Services Sdn. Bhd. (FASSB) plantation, Jengka 24. The trial also consists one standard cross (SC) progeny. All the progenies and their linage are listed in Table 1.

Table 1: List of progenies and their pedigree on the trial.

Progeny code	Parentage		Cross-type	Lineage
	Female	Male		
NK35	C2721D	FJ646P	D×P	Clonal Deli × NPM-Yangambi
NK40	C2720D	FJ646P	D×P	Clonal Deli × NPM-Yangambi
NK45	C2720D	FJ646P	D×P	Clonal Deli × NPM-Yangambi
NL75	C2721D	IBB19T	D×T	Clonal Deli × ZPM-AVROS
NL78	C2721D	IBB19T	D×T	Clonal Deli × ZPM-AVROS
NL84	C2721D	IBB19T	D×T	Clonal Deli × ZPM-AVROS
SC10	FGV-Deli	FGV-Yangambi	D×P	Deli × Yangambi

A total of 80 palms of each progeny have been recorded in terms of bunch weight (BWT) and bunch number (BNO) for three times a year. The effect of planting materials was analysed using analysis of variance (ANOVA) by SAS® 9.4 TS1M7 software. Subsequently, the significant test between treatments was determined by Duncan new multiple range test (DNMRT) at 95% confidence level.

Results and Discussion

The performance of bunch yield and its components consisting of FFB, BNO and average bunch weight (ABW) for five years recordings are presented in Table 2. The overall FFB mean of all crosses comprising clonal Deli × ZPM-AVROS, clonal Deli × NPM-Yangambi, and Deli × Yangambi was 100.61 kg palm^{-1} yr^{-1}. While, the overall BNO mean was 12.73 bunches palm^{-1} yr^{-1} and the ABW was 7.91 kg bunch^{-1}. A large difference in FFB yield was observed between these two groups of elite-germplasm at 29.47 kg palm^{-1} year^{-1} may indicates that the NPM-Yangambi has a better combination than ZPM-AVROS in this trial. This result might also be due to the NPM line at FGV was undergo an outstanding palm selection process by MPOB (Nor Azwani et al., 2020).

Further analysis has been conducted to determine the performance among the progenies and the results showed standard cross (SC) producing the highest FFB yield at 123.29 kg palm^{-1} year^{-1}, which was slightly higher and not significantly different from progeny NK35 (NPM-Yangambi) which is also the second highest FFB at 116.71 kg palm^{-1} year^{-1} (Table 2). This NK35 progeny also constitutes the highest BNO at 14.75 bunches palm^{-1}year^{-1} which overcomes the SC at 14.63 bunches palm^{-1}year^{-1}. The outstanding combination of the elite Deli × Yangambi is on the BWT was well known and it shows significantly higher compared to other progeny at 8.44 kg bunch^{-1}. Besides FGV, an extensive study on germplasm materials was also conducted by Malaysian Palm Oil Board (MPOB). Then, they have introduced their elite-germplasm introgression materials known as the PORIM Series (PS) (Wahid et al., 2005).

Table 2: Mean performance of progenies for bunch yield (2016-2020).

Progeny	Lineage	Rep	Bunch yield (2016-2020)		
			FFB (kg palm^{-1} yr^{-1})	BNO (bunches palm^{-1} yr^{-1})	ABW (kg bunch^{-1})
NK35	Deli clone x NPM-Yangambi	4	116.71^{ab}	14.75^{a}	7.91^{bc}
NK40	Deli clone x NPM-Yangambi	4	107.28^{cd}	14.01^{abc}	7.67^{cd}
NK45	Deli clone x NPM-Yangambi	4	110.69^{bc}	14.11^{ab}	7.85^{c}
Group Mean			111.56	14.29	7.81
NL84	Deli clone x ZPM-AVROS	4	79.49^{cd}	9.91^{cd}	8.07^{bcde}
NL75	Deli clone x ZPM-AVROS	4	79.73^{cd}	10.63^{c}	7.54^{g}
NL78	Deli clone x ZPM-AVROS	4	87.04^{bc}	11.04^{bc}	7.91^{def}
Group Mean			82.09	10.53	7.84
SC	Deli x Yangambi	4	123.29^{a}	14.63^{a}	8.44^{a}
Overall Mean			100.61	12.73	7.91

**FFB = fresh fruit bunch; BNO = bunch number; ABW = average bunch weight; Means with the same letter(s) in the same column are not significantly different at P < 0.05 with Duncan New Multiple Range Test (DNMRT).*

Conclusions

In conclusion, the NPM-Yangambi line has compatible performance compared to the elite cross, which is Deli-Yangambi progeny as represented as SC in the trial. The study also provides evidence that the FFB performance of the germplasms can be increased further by introgression with elite palm, especially from the Yangambi line and the special trait from this germplasm could conserve for the next generation.

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Fertilization and Sucker Pruning Effects on Sago Palm Planted on Alluvial Soil: Growth Performance at the Sixth Year

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Introduction

Sago palm (*Metroxylon sagu* Rottb.) grows vigorously in the coastal region and flood plains of rivers in the swampy area of Southeast Asia and New Guinea Island (Flach, 1997; Ehara, 2015). In Sarawak, sago palms are found growing extensively in the low-lying mineral or alluvial soils and swampy forest of Mukah and Betong Division, along the lower stretches of the Balingian, Mukah, Oya, Igan, Lupar, Saribas, and Rimbas Rivers (Hassan, 2002). This palm belongs to the Arecaceae family, genera *Metroxylon* and species *sagu* which is both hapaxanthic (once-flowering) and soboliferous (suckering) (Flach, 2005; Ehara, 2015). Depending on the soil type, sago palm has an average life cycle of 10 to 15 years (Hassan, 2002), while Flach (1997) reported that sago palm can reach harvestable stage in 8 to 10 years on mineral soil. Although Sarawak is not the world's largest sago producer, however, it is the sole exporter of world sago starch (PELITA, 2013). World demand on sago starch, especially for food industries, has increased tremendously but the supply of sago logs is insufficient.

Limited information on the proper agronomic and cultural practices for sago palm planted on different soil types has resulted in sago palms left to grow without any proper and systematic palm maintenance. This has caused low and inconsistent sago log productivity with longer harvesting intervals (Ipor et al., 2005). Observations on normal sago palm growth have found that the ability to produce suckers has an adverse effect on the palm growth performance (Howell et al., 2017). Without any system for sucker regulation, it may eventually result in a dense cluster where palms of different growth stages compete for nutrients, light, and growing space, limiting optimum palm growth (Irawan et al., 2015; Howell, 2017).

Previous studies by CRAUN on agronomic and cultural practices for sago palm were mostly conducted on deep peat area and the results obtained were not very satisfactory from the commercial point of view. Sim et al. (2017) reported that a lower degree of humification in woody materials in the underlying peat may have led to inconsistent sago palm growth, possibly affecting sago palm study results on deep peat. Fariza et al. (2018) reported that fertilizer application did improve sago palm's growth performance on deep peat area but the result was not too impressive, which might be due to the chemical and physical properties of peat soil. Peat soil is composed primarily of humic substances which are complicated with heterogenous compounds resulting from the process of decomposition or humification. Low degree of humification, low pH and high leaching incident contributing to poor uptake of major and trace nutrient elements, hence, make deep peat soil less fertile and not favourable for plant growth. Alluvial soil in contrast, is one of the most fertile soils as it is rich in minerals, light, porous and tillable, which is ideal for crop growth. It has a loamy texture and is rich in humus. Therefore, it has good water retention and absorbing capacity. The objective of this study was to determine the suitable fertilizer and sucker pruning combination to be included in the standard agronomic and cultural practices for optimum sago palm growth performance and starch yield on alluvial soil.

Materials and Methods

Palm materials and experimental location

The study was conducted on two hectares sago smallholder plot under the LCDA SSSED extension project in Senau, Oya, Dalat District. The plot was established in November 2015 and planted using sago clonal planting materials supplied by CRAUN Research. The soil chemical properties before planting are illustrated in Table 1.

Table 1: Study plot soil chemical properties.

Properties	Horizon depth (cm)	
	0 – 10	30 – 40
Total N (%)	0.76	0.15
Total P (mg kg^{-1})	122.4	180.4
Available P (mg kg^{-1})	52.4	24.0
Acid-Extractable K (mg kg^{-1})	396.2	1589.0
CEC (cmol kg^{-1})	48.2	8.87

Treatments and experimental design

There were nine treatment combinations arranged using Split Plot Design in three replicates in this study. The treatment combinations consisted of three different fertilizer applications (F0, F1 and F2) as the main plot treatments and sucker pruning times (P0, P1 and P2) as the sub plot treatments as shown in Table 2. The standard fertilizer rates for this study are shown in Table 3.

Table 2: Description for fertilization (F) and sucker pruning (P) treatment combinations.

Treatment	Description
F0P0	No fertilization + no sucker pruning
F0P1	No fertilization + sucker pruning with one succession palm retained every 12 months.
F0P2	No fertilization + sucker pruning with one succession palm retained every 18 months.
F1P0	Fertilization at vegetative stage + no sucker pruning
F1P1	Fertilization at vegetative stage + sucker pruning with one succession palm retained every 12 months.
F1P2	Fertilization at vegetative stage + sucker pruning with one succession palm retained every 18 months.
F2P0	Fertilization until harvesting stage + no sucker pruning
F2P1	Fertilization until harvesting stage + sucker pruning with one succession palm retained every 12 months.
F2P2	Fertilization until harvesting stage + sucker pruning with one succession palm retained every 18 months.

Sucker pruning treatment started after 3 years from field planting.

Table 3: Fertilization programme.

	Rate (kg $cluster^{-1}$)						
Type of fertilizer	During planting	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6
LSD	2.500	0	0	0	0	0	0
RP	0.200	0	0	0	0	0	0
Compound 45 + TE	0	0.6	1.2	1.5	3.0	4.5	6.0

Nutrient content of fertilizer: Limestone dust (LSD): 55% CaO; Rock phosphate (RP): 34% P_2O_5; Compound 45 + TE: 12% N - 12% P_2O_5 - 17% K_2O - 2% MgO + TE.

Growth performance-related parameters

Five growth performance-related parameters for the six-year-old sago clusters were recorded. Number of trunking palms was recorded through physical observation whereas trunk height, diameter and base girth size were measured using conventional measuring tape.

Statistical analysis

Analysis of variance (ANOVA) using Statistic Analysis System software version 9.4 (SAS 9.4) was used to analyse the significance of different fertilization and sucker pruning treatment combinations for trunk height, diameter and base girth size. Duncan's New Multiple Range Test (DNMRT) at $P = 0.05$ was used to compare the means for all growth performance parameters.

Results and Discussion

Percentage of trunking following different treatment combinations for mother and succession sago palms is shown in Table 4 while trunking performance for different succession palm levels is shown in Figure 1. Data for mother palm trunk height, diameter and base girth size following different treatment combinations are presented in Table 5.

Table 4: Percentage of trunking in mother and succession sago palms following different treatment combinations at 6 years after field planting.

Treatment combination	Trunking of mother palms (%)	Trunking of succession palms (%)
F0P0	100	11.1
F0P1	100	16.7
F0P2	100	50.0
F1P0	100	41.7
F1P1	100	33.3
F1P2	100	39.3
F2P0	100	36.1
F2P1	100	57.4
F2P2	100	39.3

The result showed that all mother sago palms reached trunking stage at six years after planting regardless of the different treatment combinations. High trunking percentage for mother sago palms might be due to high fertility in alluvial soil. The soil contained adequate nutrients for trunk development. Succession palms, on the other hand, also achieved trunking stage but at a lower percentage. Sago clusters that underwent both fertilizer application and sucker pruning or with only either one of the applications demonstrated higher trunking for the succession palms. F2P1 palms showed the highest trunking percentage of 57.4% as compared to sago clusters receiving no fertilizer and sucker pruning (P0F0) that showed only 11.1% reaching trunking stage. Moreover, sago clusters that underwent both the fertilizer application and sucker pruning or with only either one of the applications showed more succession sago palms reaching trunking stages. These sago clusters achieved three or more succession palm levels (S1-S5). Succession palm levels were numbered according to the sequence of retaining suckers based on the sucker pruning treatment. The numbered succession palms S1, S2, S3, S4, S5 refer to the first, second, third, fourth and fifth retained succession palms. Sago clusters without any fertilizer and sucker pruning showed only the first (S1) and second (S2) retained succession palm reaching trunking stage (Figure 1). These results suggested that both fertilization and sucker pruning significantly improved sago palm trunking performance as previously reported by Howell et al. (2017) and Fariza et al. (2018). Availability of nutrients and minimum degree of competition between individual sago palms within the cluster had enhanced its growth performance.

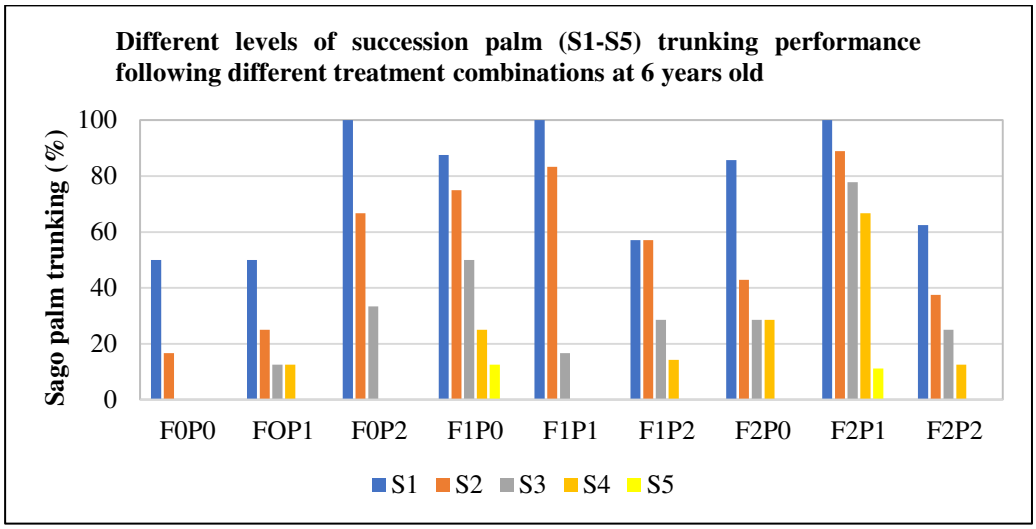

Figure 1: Sago palm trunking percentage and different levels of retained succession palm sucker in response to different fertilizer and sucker pruning treatments at 6 years old.

Table 5: Mean palm trunk height, diameter and basal girth size of mother sago palms following different treatment combinations at 6 years after field planting.

Treatment combination	Trunk height (m)	Trunk diameter (cm)	Basal girth size (cm)
F0P0	3.3±0.4^{b}	61.0±4.2^{a}	224.7±17.5^{b}
F0P1	3.8±0.2^{ab}	69.5±1.8^{a}	259.3±6.3^{a}
F0P2	3.3±0.2^{b}	67.7±2.8^{a}	246.7±11.4^{ab}
F1P0	3.8±0.3^{ab}	66.8±6.3^{a}	242.5±7.5^{ab}
F1P1	4.3±0.3^{ab}	67.3±0.3^{a}	245.0±3.0^{ab}
F1P2	4.2±0.3^{ab}	65.3±2.2^{a}	254.3±5.8^{ab}
F2P0	4.5±0.1^{a}	61.8±1.8^{a}	231.5±5.5^{ab}
F2P1	3.7±0.2^{ab}	69.3±0.7^{a}	260.3±4.7^{a}
F2P2	3.7±0.2^{ab}	65.1±2.6^{a}	246.7±3.8^{ab}

Mean value ± standard error. Values followed by the same alphabetical letter by column indicate the absence of a significant difference by ANOVA DNMRT ($p < 0.05$).

The above recorded mean data for mother sago palm's trunk height, trunk diameter and trunk base girth size did suggest that both fertilizer application and sucker pruning can improve sago trunk quality as compared to sago trunk from cluster without fertilizer and sucker pruning. These results were best explained by the minimum competition for nutrient, sunlight and growing spaces created when only certain numbers of sago palms were allowed to develop at certain period (Howell, 2017). Nevertheless, sago palm clusters following different treatment combinations did not show much difference by visual observations (Figure 2).


F0P0


F0P1


F0P2


F1P0


F1P1


F1P2


F2P0


F2P1


F2P2

Figure 2: Sago palms following different treatment combinations at six years after field planting.

Conclusions

At the sixth year, growth performance data indicated that the combination of fertilization and sucker pruning significantly improved the trunking performance of both mother and succession sago palms. Combination of fertilizer application and sucker pruning resulted in 57.4% trunking for succession palms compared to 11.1% for sago clusters without any application. Mother sago palm's trunk was much taller and broader with fertilization or sucker pruning or both. This study indicated that the combination of fertilizer application and sucker pruning had the potential to enhance trunking development for sago palms. Nevertheless, this study will be continued until sago palm reaches maturity stage for more concrete conclusion.

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FGV 3 Way Oil Palm Variety Inflorescence Sex Ratio

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Introduction

Oil palm, or *Elaeis guineensis* Jacq, is one of the major oil-producing crops. The palm is a member of Arecaceae family, a perennial tree that needs three to four years after planting to bear fruit (Adam et al., 2005). The fruits are composed of hard-shelled nuts covered with mesocarp, which produces the opulent oil. Additionally, the nut can be used to make kernel cake, which is commercially used as animal feed. Oil palm is a monoecious tree, producing both male and female flowers. There are also reports that oil palm is 'temporally dioecious' because it produces functional unisexual male and female flowers alternately (Adam et al., 2011). As with other palm species, the oil palm is a single-stemmed, long-lived tree that generates new leaf primordium approximately every two weeks (Corley and Gray, 1976). Occasionally, mixed-sex inflorescences are recorded in oil palms. Unlike the male inflorescence, which has individual staminate flowers, the female inflorescence generates floral triads composed of a pistillate bloom flanked by two staminate flowers (Adam et al., 2011).

Oil palm is widely farmed in Africa, South America, Central America, and Southeast Asia (Corley, 2015), primary economic contributor being Ghana, accounting for more than 75% of total export revenue (Sapey et al., 2017). To date, oil palm has been the most spectacular crop for oil production because it used only 7% of land for oils but produced the higher percentage of vegetable oils (38%) Furthermore, global demand for vegetable oil is expected to exceed 240 million tonnes by 2050 (Corley and Thinker, 2015). Although *E. guineensis* is indigenous to West Africa, Malaysia and Indonesia account for 84% of oil palm production. Because oil palm used the least land area per oil production, scientists and botanists from many countries across the world are collaborating to boost oil palm yield.

Oil palm cannot fully self-pollinate or cross-pollinate without the assistance of a pollination agent. Wind pollination alone is insufficient to support fruit set formation. *E. kamerunicus* was introduced into the Malaysian population in 1981 to increase the effectiveness of oil palm pollination (Norman et al., 2018). Numerous reasons contributed to the inconsistent and decreasing FFB provided by oil palm, one of them is declining in oil palm fruit formation in Sierra Leone, Colombia, Malaysia, and Indonesia. Given the fact that oil palm produces unisexual males and females alternately, reducing the likelihood of self-pollination (Norman et al., 2018; Kushairi et al., 2019). The inflorescence sex ratio (ISR) is the proportion of female blooms to the total number of flowers in a single tree. The sex ratio is highly influenced by variation of biotic and abiotic factors. For instance, a substandard irrigation system may affect the female flower formation per palm (Corley and Hong, 1982). Additionally, intense competition for sunlight as a result of dense planting may reduce the oil palm's dry matter production. As the dry matter production is reduced, oil palm intends to produce more male inflorescence than female (Corley and Hew, 1976). On the contrary, stress and defoliation are causing the oil palm to produce more male flowers, which is critical for pollen production (Corley and Thinker, 2015).

One of the popular oil palm varieties in Malaysia is FGV 3 Way, developed by FGV Holdings, which yielded up to 8.5 tonnes per hectare of oil. FGV 3 Way is a highly sorted variety by oil palm farmers, especially smallholders. This variety can produce high bunch numbers with moderate bunch weight despite being planted at high density. The name '3 Way' is derived from the three different cities of origin of the planting materials that is from Deli, Nigeria, and Yangambi (Congo). This small trial was conducted to determine the inflorescence sex ratio range of FGV 3 Way variety by counting male flower (Figure 1) and female flower and thus, to design the most accurate and practical method for determining inflorescence sex ratio in FGV trial plot.

Figure 1: Male flower (1) and female flower (2) of Yangambi 3 Way progenies in Kampung Kota Gelanggi 5.

Materials and Methods

Materials

Various FGV 3 Way crosses, the PN 165, PN 91, PN 84, and PN 167 were selected for this study. The male pollen comes from Yangambi, Zaire, while the female flower was an outcross of Nigerian Prospected Material (NPM) and Deli Dura (Indonesia). Standard Cross 8 (SC8), a progeny that came from Deli x Yangambi cross is used as a control for this trial. The trial was arranged in a randomized complete block design (RCBD) with 4 replications. SC8 is one of the progenies of the commercial FGV's D x P Yangambi. Around 1600 palms are involved in this project.

Field location

The progenies were planted in Ladang KG 5, Kampung Kota Gelanggi, Pahang (GPS coordinate: N 3°57'42.32", E 102° 35'36.87"). The area is flat and a little/slightly undulating in certain areas. While the soil series is Bukit Tuku and the area also receive a good amount of rainfall (2000-2400 mm) annually.

Data collection and identification of ISRs

Data were collected from January to December 2022, by performing a gender ratio census that was conducted once every quarter (Q1-Q4). This procedure identifies the female flower (F_f, the male flower (F_m), and abortive bunches. The census began by identifying the youngest frond that was completely opened, followed by marking it with red paint as an indicator of the first frond. The palms' anthesis times were carefully established by observing the inflorescence position at the anthesis. Finally, the number of flowers counted was summed after completing the 4^{th} census during the study period. The ratio of the male flowers to female flowers was calculated using the following formula:

$$\frac{F_{f1} + F_{f2} + F_{f3} + F_{f4}}{\sum F_{m1-4} + \sum F_{f1-4}} \text{ X } 100 = \text{Inflorescence Sex Ratio}$$

$\sum F_{1\text{-}4}$: sum of male/female flowers throughout four round of census
$F_{m(n)}$: number of male flowers on the period of the census
$F_{f(n)}$: number of female flowers on the period of the census

Statistical analysis

The data collected were analyzed using SPSS for analysis of variance was performed using the normal linear model. The P-value was set below 0.01 ($P < 0.01$). The means were compared using the Least Significant Difference (LSD) at the 5% level of probability.

Results and Discussion

Until June 2022, two rounds of flower census had been completed. The analysis was conducted based on data from the two rounds of flower census (Table 1). An analysis of variance (ANOVA) was performed to test the p-value of 3 Way variety inflorescence Sex Ratio. As expected, the sex ratio for all of the progenies was significantly different ($p < 0.01$). The SC8, obtained the highest sex ratio compared to the other 3 Way varieties. Based on two rounds of sex ratio census, the sex ratio was SC8 at 88% followed by PN165 at 85% and PN84 at 81%. While the two lowest sex ratio progenies were PN167 and PN91, at 74% and 70%, respectively.

Table 1: Mean inflorescence sex ratio for Yangambi 3 Way progenies and SC8.

Progeny	Mean (%)
PN165	85[a]
PN167	74[b]
PN91	70[c]
PN84	81[d]
SC8	88[e]

**Mean in the column with the different letters indicates significant differences at $p \leq 0.05$ level according to LSD.*

Trial yield data from 2018-2020 were used to complement the inflorescence sex ratio. The yield and bunch data were obtained from Trial T276, at Kota Gelanggi 5, Pahang (Ahmad et al., 2020). In 2020, SC8 led in total yield compared to 3-Way progenies. SC8 produced 129.2 kg of fresh fruit bunch (FFB), per palm followed by PN165 at 127.5 kg while, the yield for PN84, PN167, and PN 91 were 123.4 kg, 113.7 kg, and 99.7 kg, respectively. For bunch number, SC8 led for 3 years compared to other progenies at 22.8 followed by 20.9 for PN165. Meanwhile, for PN84, PN167, and PN91, the mean bunch number was 20.2, 19.3, and 17.7, respectively. Based on the results of yield, bunch number, and inflorescence sex ratio, the performance of SC8 was better than the other FGV 3 Way varieties (PN 165, PN167, PN84, and PN91).

Table 2: Mean yield, bunch number and bunch weight of FGV 3 Way varieties and SC8.

Progenies	2020			Mean 3 yr (2018-2020)		
	FFB (kg)	Bunch No	Bunch Wt(kg)	FFB (kg)	Bunch No	Bunch Wt(kg)
PN167	109.14[d]	14.70	7.57	113.45[d]	19.23	6.18
PN84	118.52[c]	14.30	8.41	123.38[c]	20.19	6.47
PN91	93.90[b]	12.70	7.46	99.70[b]	17.65	5.89
SC8	146.03[a]	19.81	7.43	129.24[a]	22.78	5.82

**Mean in each column with the different letters within the column indicates significant differences at $p \leq 0.05$ level according to LSD.*

One of the ways in evaluating the early performance of oil palm progenies is to determine the sex ratio because it is directly related to the bunch formation of oil palm (Breure et al., 1990). Thus, in order to maintain the performance of an oil palm good management of elite planting materials need to be done in oil palm plantations. There are a lot of challenges in managing palm plantations and one of them is the uncertainties of climate (Rival, 2017). This will slightly affect water availability for oil palm. Climate change has been one of the important topics discussed internationally and the expectation of oil palm yield decline is inevitable. To overcome this situation, efforts of selecting and breeding high-performance oil palms are being done in the countries that commercially plant these trees (Swaray et. al, 2021).

Since the inflorescence sex ratio is largely influenced by environmental factors, a big challenge for breeders to produce an oil palm planting material that can withstand climate uncertainties in upcoming years. Adaptations of new agricultural practices need to be implemented for the sustainability of the agriculture industry with the harsh environmental changes. Oil palm bears both male and female flowers in the same tree making the sex ratio very important to form a good fruit set. Domination of male inflorescence in an oil palm tree could lead to poor performance of the palm. In order for a palm to produce a good and nice bunch, the pollination of male and female flowers needs to occur efficiently. Oil palm flowers bloom mostly in an alternate sex cycle, but sometimes hermaphrodite flowers appeared affecting the bunch yield. The development of the male flowers could take up to a month before being aborted. These can be induced by stressing the palm and by defoliating it and removing the leaves (Corley and Tinker, 2015). Usually these methods are used to develop and utilize *pisifera* plants for oil palm breeding.

In general, high-performance oil palms have a range of inflorescence sex ratios around 70-80%, thus contributing to a high bunch yield (Rival, 2017). Based on the result of the inflorescence sex ratio, SC8 showed a higher potential yield than FGV 3 Way progenies (PN165, PN167, PN91, and PN84). Supposedly FGV 3 Way progenies produced more bunches or female flowers than FGV D x P Yangambi's (Chin et al., 2008). The result for ISR in this trial might not be good for FGV 3 Way since the 2nd round of census results does not represent the actual sex ratio performance of the progeny in this trial. Furthermore, this result might be hugely influenced by environmental factors, the final conclusion cannot be explained since the full round of sex ratio census is not completed yet.

Conclusion

Up to 2nd round of sex ratio census in 2022, the sex ratio percentage for all progenies in this trial is quite impressive between 70-90%. But, the sex ratio of standard control progeny exceeds all the FGV 3 Way varieties. One of the biggest factors contributing to the inflorescence sex ratio is nutrient availability. Meanwhile, poor irrigation management and bad soil fertility availability could increase the number of male flowers.

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Growth and Rooting Ability of *Moringa oleifera* Cutting as Affected by Juvenility and Growing Media

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Introduction

Moringa (*Moringa oleifera* L.) is a perennial crop and a type of local herb that has been widely cultivated in most South Asian countries and has been globally neutralized (Gandji et al., 2018). Moringa belongs to the family of Moringaceae. This tree is also known as Kelor, Merunggai, Mulangay, Drumstick tree and it has earned its name as 'the miracle tree' due to its remarkable healing abilities for a variety of ailments, including some chronic diseases (Abdull Razis et al., 2014). Because of the high nutritional value and essential phytochemicals found in its leaves, pods, and seeds, this crop has gained popularity in the food and medical industries (Saini et al., 2016).

Moringa can be grown in any tropical or subtropical region with temperatures ranging from 25 °C to 35 °C. This tree thrives in sandy and loamy soils with pH levels ranging from slightly acidic to alkaline (Chukwuebuka, 2015). Due to the lack of scientific knowledge, little is known about cultural practices like pruning, cutting, and fertilization that have not been scientifically applied (Isah et al., 2014). In terms of propagation technique, most growers prefer to propagate by seed rather than stem cutting due to its high germination rates and the availability of seeds throughout the year. However, the moringa plant is a naturally and highly cross-pollinated plant that produced seeds that have segregation and variation in terms of quality, growth, and yield (Bhattacharya and Mandal, 2004).

Vegetative propagation by stem cuttings produces plants that are genetically identical to the parent plant and regenerate large quantities of planting materials. Selection of cutting is also a crucial part since every plant part, age and size of the cuttings will contribute to the successful cutting percentage. Juvenility is related to a plant's capability to be vegetatively propagated, yet most plants have no clear transition from the juvenile to the mature phase (Bonga, 1982). Goldschmidt and Samach (2004) defined juvenility as the incapacity to stimulate flower development, fruit bearing while displaying a high predisposition for rapid, vegetative growth. This stage may last even several years and is characterized by the plant. Juvenility or age of the mother plant of a cutting material also plays an important role in cutting to success and grew vigorously.

Other factors that should also be considered are accurately supplying the proper media, temperature, moisture, light, and air for successful growth, rooting formation and results on cuttings to succeed. Vegetative propagation such as cutting for moringa has been reported previously but information on cutting technique, particularly for cuttings with less than 3 cm in diameter is still lacking. It has also been reported that moringa cuttings are difficult to produce roots even with the use of a high concentration of rooting hormone (Rufai et al., 2016). Therefore, this study was conducted to evaluate the growth and rooting ability of cuttings of *Moringa oleifera* L. taken from a different ages of mother plants; young (<6 months) and old (>2 years) cultivated using different growing media.

Materials and Methods

Plant materials

This research was conducted at the MARDI Headquarters Herbs Nursery in Serdang, Selangor. The moringa cuttings were taken from a different age of mother plants; young (<6 months) and old (>2 years) at two different locations which are MARDI Headquarters and Global Moringa Plantation at

Kampung Parit Jelutong, Parit Raja, Johor in March 2022. To prevent fungal infections, clean-cut cuttings ranging from 16 cm to 20 cm in length and 1.6 mm to 2.5 mm in diameter (Figure 1) were quickly dipped into a fungicide solution (active ingredient: thiram) (Figure 2).

Figure 1: Moringa cuttings length.

Figure 2: Moringa cuttings were soaked with fungicides (a.i: Thiram).

Treatment and experimental design

The present experiment was conducted as two factorials which were different ages of cuttings as the first factor and different growing media as the second factor. The moringa cuttings taken from a different age of mother plants were grown in three different growing media; soil, sand, and soil and sand mixture (1:1). The experiment was set up as a split-plot design with four replications, each containing 60 cuttings. Therefore, a total of 240 cuttings were prepared and planted in a planting box (Figure 3) with no hormone application.

Data collection

Data on successful cutting percentage (%) and rooting performance (number of roots, root diameter, root length and root dry weight) of moringa plant were collected at 60 days after planting (DAP).

Figure 3: Experimental plot with a split plot design.

Successful cutting category (%)

The percentage of successful cutting was evaluated and calculated. All 240 cuttings from different juvenility of the mother plants; young (<6 months) and old (>2 years) was counted using the formulation below:

$$\frac{Number\ of\ successful\ cutting\ (shoot\ emergence, cm)}{Total\ number\ of\ cutting} \times 100 = Successful\ cutting\ (\%)$$

Successful cutting percentage for both juvenilities of cutting age were evaluated and grouped based on their morphological difference (shoot emergence length (cm)). The cuttings were ranked into five

categories i.e., A) unsuccessful cutting; B) shoot emergence, 0–10 cm; C) emergence shoot, 10–20 cm; D) emergence shoot, 20–40 cm and E) emergence shoot, >40 cm) (Figure 4).

Rooting performance

The cuttings were checked for adventitious root number, root diameter (mm), root length (cm) and root dry weight (g). The root length was measured by selecting the longest root and the root diameter (the wider root was selected) was measured by using vernier callipers. Data on the root dry weight of individual plants were collected after the plant was separated into leaves, petioles, stem and root parts. The samples were measured using a digital balance after oven-drying at 60 °C for 48 hours.

Data analysis

The data obtained was analyzed using ANOVA in the SAS software (Version 9.4, S.A.S. Institute Inc. Cary, North Carolina, U.S.A.) and differences between treatment means were compared using Tukey's HSD at $P \leq 0.05$.

Results and Discussion

Successful cutting category (%)

Juvenility significantly ($P \leq 0.05$) affects the growth, shoot emergence, and rooting formation of cultivated cuttings. At 60 DAP, young cuttings showed a positive response on all dependent variables. The cuttings have been categorised based on the length of shoot emergence (Figure 4). The results showed that young cuttings have the highest percentage of successful cutting categorized in groups E (16.67) and D (14.16), where the cutting successfully produces shoot and root formation. The opposite pattern was apparent where the majority of the old cuttings were in group A (86.67) which was classified as unsuccessful cutting (Table 1). Old cuttings successfully produced shoots (category B) but failed to form roots (Figure 5). The emergence of new shoots by older mother plants could be explained by the utilization of the initial carbohydrate reserves (Druege et al., 2004). The ability of these cuttings to survive is uncertain because they did not produce any roots to ensure continuous uptake of water and nutrients. At 60 DAP, none of the old cuttings had successfully developed roots satisfactory and the cutting eventually rotted away.

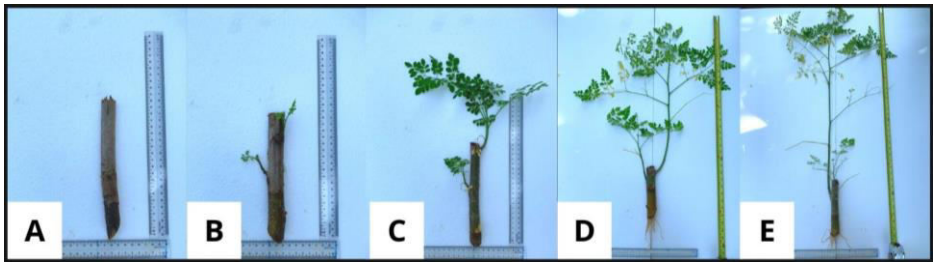

Figure 4: Five categories of *M. oleifera* L. cuttings and seedlings at 60 days after planting; A (unsuccessful cutting), B (shoot emergence, 0–10 cm), C (shoot emergence, 10–20 cm), D (shoot emergence, 20–40 cm) and E (shoot emergence, >40 cm).

Table 1: Successful cutting category (%) of *M. oleifera* at 60 DAP.

Factor	Successful cutting categories (%)				
Media (M)	A	B	C	D	E
Soil	77.5[a]	6.25[b]	1.25[a]	3.75[a]	11.25[a]
Sand	50.00[b]	30.00[a]	2.50[a]	11.25[a]	6.25[a]
Soil: Sand (1:1)	71.25[ab]	13.75[ab]	1.25[a]	8.25[a]	7.50[a]
Age (A)					
Old	86.67[a]	13.33[a]	0.00[b]	0.00[b]	0.00[b]
Young	45.83[b]	20.00[a]	3.33[a]	14.16[a]	16.67[a]
Media (M)	*	*	ns	ns	ns
Age (A)	**	**	*	*	*
M*A	ns	ns	ns	ns	ns

**Means followed with the same letter in the same column and source are not significantly different at P ≤ 0.05 by Tukey's HSD.*

Rooting performance

Mother plants juvenility significantly (P ≤ 0.05) affects the number of roots, root diameter, root length and stem and root dry weight. Only young cuttings had successfully generated roots (6.92) (Figure 5). Root diameter and root length also recorded the highest reading (3.23 mm) and (7.21 cm) respectively (Table 2). The findings of this study are comparable with those reported by Awang et al. (2011), who found the greatest root length and number produced by young mother plants. In fact, rooting inhibitor increases with growing age and thus inhibits the stimulation of rooting. The increased amount of endogenous rooting inhibitor may have contributed to the older mother plant cuttings failure to commence rooting (Awang et al., 2011; Kibblers et al., 2022). The phenolic level also decreases in older mother plant cutting material which contributes to the cutting success. The phenolic compound links with the accumulation of auxin and protects cutting from oxidation (De Klerk et al., 2011). In addition, internal factors such as anatomical structure, auxin level, rooting co-factors and rooting inhibitors may also affect the initiation and stimulation of rooting. Fouda et al. (2012) noticed that cuttings of lemon verbena that were difficult to produce root appeared to be mainly caused by anatomical alterations and the gradually forming vascular link between the new roots and the vascular tissues of the cuttings. Juvenile cutting material is promising to have good rooting formation, more vigorous, and provide a higher strike rate as compared to old cutting material.

Table 2: Effects of different growing media and juvenility of the mother plant on number of roots, root diameter, root length and root dry weight of Moringa plant.

Media (M)	No. of root	Root diameter (mm)	Root length (cm)	Root dry weight (g)
Soil	3.00[a]	1.69[a]	2.80[a]	0.04[a]
Sand	4.00[a]	2.22[a]	4.13[a]	0.07[a]
Soil: Sand (1:1)	3.38[a]	1.38[a]	4.01[a]	0.09[a]
Age (A)				
Old	0.28[b]	0.28[b]	0.08[b]	0.0[b]
Young	6.92[a]	3.23[a]	7.21[a]	0.13[a]
Media (M)	ns	ns	ns	ns
Age (A)	**	**	**	**
M*A	ns	ns	ns	ns

**Means followed with the same letter in the same column and source are not significantly different at P ≤ 0.05 by Tukey's HSD.*

Figure 5: Rooting formation of <6 months old cutting.

Figure 6: Unsuccessful rooting formation of >2 years old.

Conclusions

At 60 DAP, growing media had no effect on root growth in moringa stem cuttings. The findings revealed no significant effect on the initiation of new root growth. Sand shows a higher percentage of successfully rooted cuttings and can be used as a rooting media for moringa stem cuttings. The juvenility of the mother plants cuttings young (<6 months) and old (>2 years) significantly impacts overall rooting performance and successful cutting percentage. Juvenile mother plants enhance the cutting to initiate rooting and contributed to the cutting to success. Young mother plants; (<6 months) cutting have a tendency to be desirable characteristics of high-quality planting materials.

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Nitrogen Deficiency Effects on Chlorophyll Content, Normalized Difference Vegetation Index (NDVI) and Foliar Spectral Reflectance Properties of *Piper nigrum* L.

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Introduction

Black pepper (*Piper nigrum* L.) is a perennial climbing vine in the Piperaceae family that produces a hotly pungent spice from its fruits. It is indigenous to India's Malabar Coast and is one of the oldest spices known. Pepper is widely used as a spice all over the world, but it is also used in medicine as a carminative (to relieve flatulence) and as a stimulant of gastric secretions.

Early in history, pepper was widely cultivated in Southeast Asia's tropics, where it became popular as a condiment. Pepper became a significant element of overland trade between India and Europe, and it was frequently used as a medium of exchange. The ancient Greeks and Romans also called it “black gold” and pepper was commonly used as tribute among themselves. The crop was brought to East Malaysia in 1840 by Chinese settlers and is currently, one of Malaysia's most important commodities, contributing RM153.74 million to the country's GDP in the year 2021.

Spectral reflectance and chlorophyll fluorescence are rapid non-invasive methods that can be used to predict plant stress (Richardson and Berlyn, 2002; Sulok et al., 2012). In recent years, many plant parameters of interest have been estimated using multispectral reflectance measured with hand-held radiometers (Tarr, 2005). Leaf reflectance is determined by the biochemical components of the leaf, including photosynthetic pigment concentrations – some of these which include chlorophylls, carotenoids and xanthophylls are known to be affected by plant stress (Gitelson and Merzlyak 1996; Richardson et al., 2001; Sulok et al., 2012,).

Like any other crop, the growth and development of pepper are also affected by nitrogen (N), one of the most important nutrients for black pepper growth, development, and yield. According to Paulus (2011), nitrogen deficiency in black pepper is generally characterized by poor growth with pale and yellowish leaves. In severe cases, the entire canopy develops a distinctive yellow to orange-yellow discolouration, with the tip of the leaf becoming necrotic in some cases (Paulus, 2011). Rapid and non-destructive detection of nitrogen deficiency symptoms in pepper vines is needed for the effective management of black pepper farms across Malaysia.

Therefore, this study was conducted with the following objectives: (i) to investigate the foliar spectral reflectance of *P. nigrum* affected by nitrogen deficiencies (ii) to quantify the relative chlorophyll content (SPAD) and Normalized Difference Vegetation Index (NDVI) of *P. nigrum*, and (iii) to study the relationship between spectral reflectance indices and relative chlorophyll content as well as NDVI as an indicator to determine nitrogen deficiency symptoms in black pepper farms.

Materials and Methods

Field data collection

Field data collection was conducted in three different pepper farms located in Serian, Bau and Padawan, Sarawak for measurement of the spectral reflectance in black pepper vines affected with nitrogen deficiency symptoms. The Kuching cultivar was selected for measurement as this variety is among the recommended cultivar and is widely planted in Malaysia.

Foliar spectral reflectance, relative chlorophyll content and NDVI measurement

The experiment was conducted from January 2022 to July 2022 with mature leaves of *P. nigrum* freshly collected in their natural state. A total of 60 leaves from 10 sufficient nitrogen vines and 10 deficient nitrogen vines were taken for measurement. The leaves were sampled by selecting the ones displaying a yellow to orange-yellow discolouration from vines with a yellowish lower canopy but a greener upper canopy. These characteristics are the most prevalent nitrogen deficiency symptoms in pepper vines as described by Paulus, 2011.

Spectral reflectance measurements at wavelengths from 400 to 2500 nm were done using the PS-300 Apogee laboratory spectroradiometer. Consequently, three indices were derived from the data sets of percentage reflectance to monitor changes in chlorophyll absorption. The indices are R_{550} which is the percentage reflectance at 550 nm (Moran et al., 2000), photochemical reflectance index [PRI = $(R_{531}-R_{570})/(R_{531}+R_{570})$] (Penueles et al., 1997), and structure independent pigment index [SIPI = $(R_{800}-R_{445})/(R_{800}-R_{680})$] (Peneules and Inoue, 1999). Relative chlorophyll content and NDVI data of pepper leaves were determined by using a chlorophyll meter (SPAD-502, Minolta, Japan) and NDVI meter (Spectrum Technologies, USA).

Statistical analysis

Data were analyzed using a T-test with the SPSS software (Version 15) at $\alpha = 0.05$ level of significance to compare the means and to determine whether there were any significant differences on relative chlorophyll content between sufficient and deficient nitrogen on *P. nigrum* vines. A regression analysis of the relationships between spectral reflectance indices and relative chlorophyll content was performed.

Results and Discussion

Relative chlorophyll content and NDVI measurements

Results indicated that there was a significant difference in the mean relative chlorophyll content and NDVI measurement between nitrogen-sufficient leaves and nitrogen deficiency leaves. Higher relative chlorophyll content was observed in nitrogen-sufficient *P. nigrum* leaves compared to nitrogen deficiency leaves. Tomas and Caula (2003) found that nitrogen deficiencies had the most pronounced effect on chlorophyll concentration, height and reflectance. Meanwhile, for NDVI measurement, this study also indicated that a low NDVI value represents a deficiency of nitrogen in *P. nigrum*. This is consistent with the findings by Brayden et al. (2022) which stated that the NDVI value increased with increasing nitrogen rate application in maize, thus signifying that the lowest NDVI values tend to represent 0 N rate, while the highest NDVI value represented higher N rates.

Table 1: Average relative chlorophyll content and Normalized Difference Vegetation Index (NDVI) in sufficient and deficient N pepper vines.

Sample	Relative chlorophyll content	NDVI
Sufficient N	569.16 ± 72.62^{a}	0.83 ± 0.04^{a}
Deficient N	35.44 ± 12.97^{b}	0.35 ± 0.09^{b}

Means with the same letter superscript within the column are not statistically different at P > 0.05 probability level (mean±SD, n = 20).

Foliar spectral reflectance

The mean spectral reflectance for both groups showed a consistent increase in reflectance with nitrogen deficiency pepper vines at the visible wavelengths of 425–745 nm (Figure 1). A greater increase can be detected on nitrogen deficiency leaves from wavelength 425 nm until 749 nm compared to nitrogen-sufficient leaves' spectral reflectance. Findings by Tomas and Caula (2003) stated that the peak reflectance for nitrogen deficient plants was 75% greater in the visible (VI) range (400–700 nm) values and 97% greater in the infrared (IR) range (700–1100 nm). Wavelengths from 413 to 778 nm were correlated with macronutrient deficiencies in plants as these conditions are causing chlorosis and senescence of the leaves.

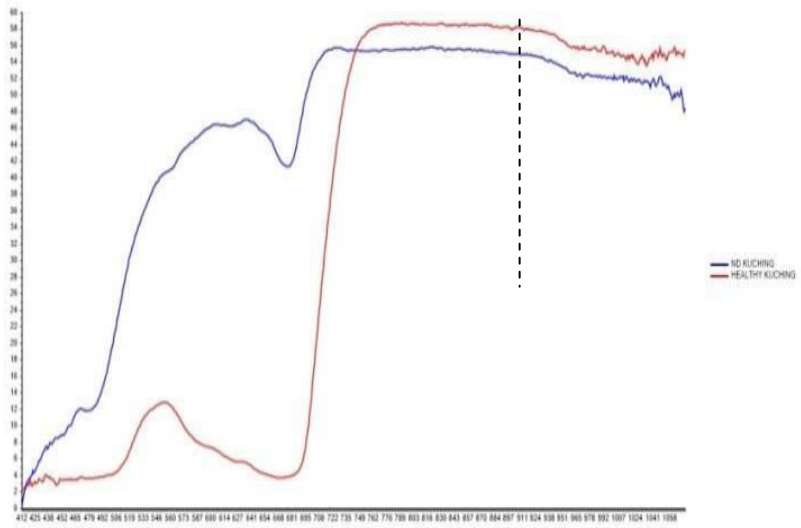
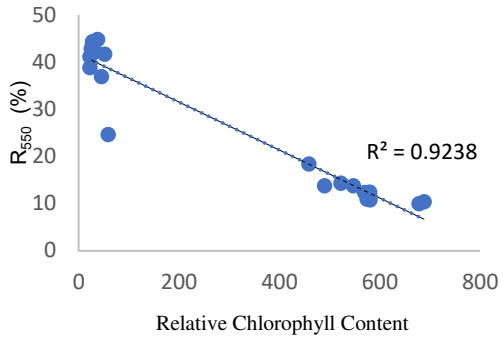


Figure 1: Mean spectral reflectance of *P. nigrum* leaves from nitrogen-deficient vines (■) compared to leaves from nitrogen-sufficient vines (■).

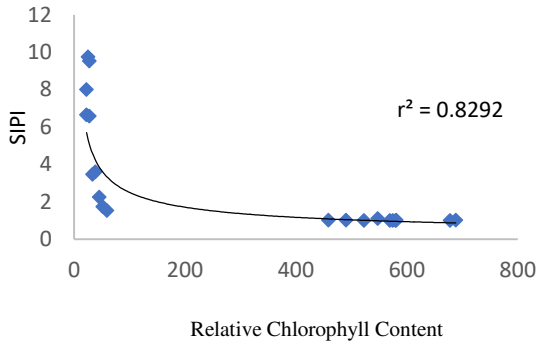
The relationship between relative chlorophyll content and three different spectral reflectance indices $R_{550,}$ PRI and SIPI is also described by regression analysis with correlation coefficient value (r^2) in Figure 2(a), (b) and (c).

Values of R_{550} decreased as foliar relative chlorophyll content increased and the relationship was described by a linear trendline in Figure 2(a). Correlation coefficient value of $r^2 = 0.92$ indicating a high correlation between the R_{550} wavelength and relative chlorophyll content in pepper leaves. Meanwhile, the SIPI values also decreased with increasing relative chlorophyll content as represented by a power trendline regression in Figure 2(b). The regression analysis shows a strong correlation between SIPI and relative chlorophyll content with $r^2 = 0.83$. In Figure 2(c), the PRI values increased with increasing relative chlorophyll content and the correlation was described by a polynomial inverse third-order regression with a correlation coefficient value of 0.82. Several studies have demonstrated a significant correlation between nitrogen concentrations and relative chlorophyll content for chlorophyll a and b, since nitrogen is associated with protein synthesis, which aids in the photosynthetic process. As a result, nitrogen deficiency disrupts the metabolic function of chlorophyll, the photosynthetic element responsible for electromagnetic energy absorption at specific wavelengths in the visible region (Ponzoni and Goncalves, 1999; Mutanga et al., 2004; Katz et al., 2006).

(a)



(b)



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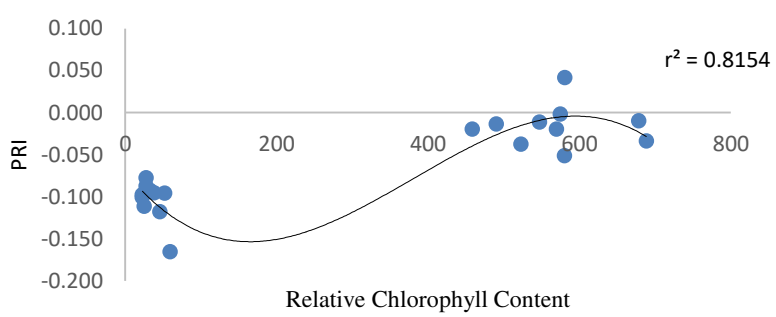

Figure 2: Relationship between three reflectance indices, (a) R_{550}, (b) SIPI, and (c) PRI and relative chlorophyll content in *P. nigrum* leaves subjected to nitrogen deficiency.

Normalized Difference Vegetation Index (NDVI)

Relationship between NDVI and three different spectral reflectance indices $R_{550,}$ PRI and SIPI were also evaluated by using regression analysis and the results are shown in Figure 3 (a), (b) and (c). Among the spectral indices, R_{550} (r^2=0.88) showed the strongest relationship with NDVI, followed by PRI (r^2=0.82) and SIPI (r^2=0.69).

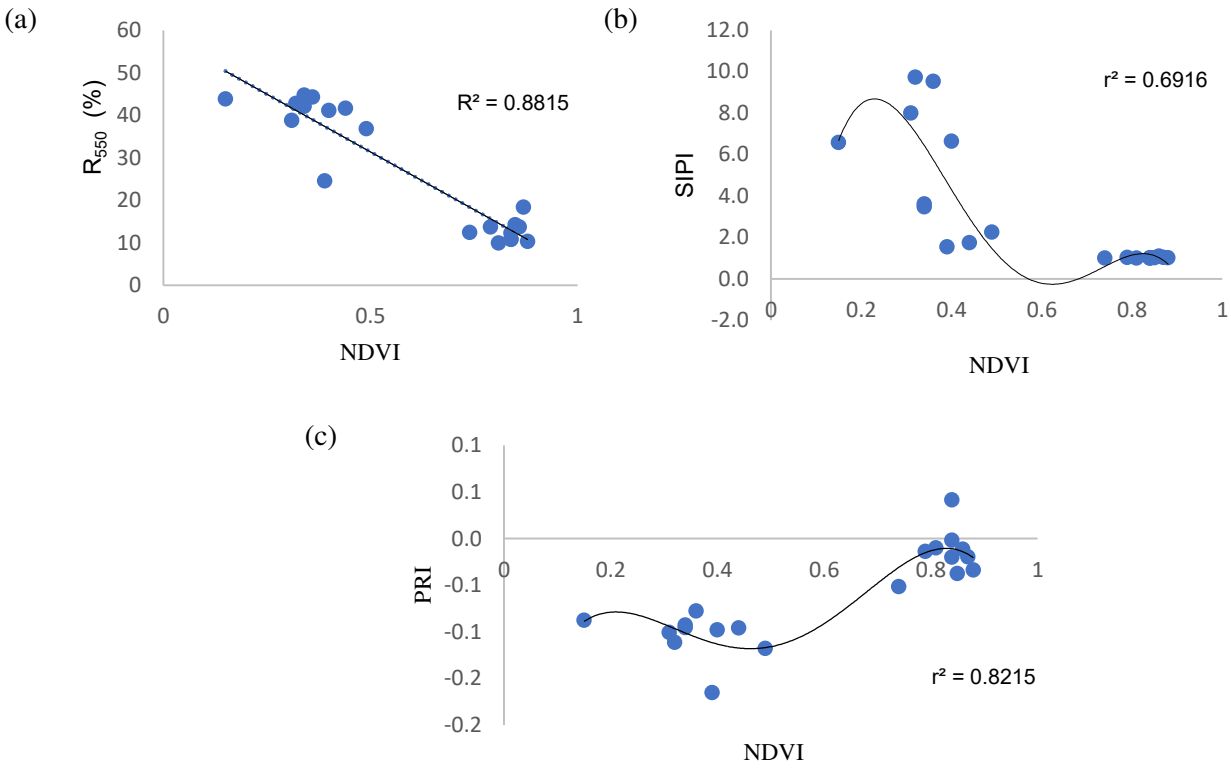

Figure 3: Relationship between three reflectance indices, (a) R_{550}, (b) SIPI, and (c) PRI and NDVI measurement in *P. nigrum* leaves subjected to nitrogen deficiency.

Regression analysis on R_{550} and NDVI shows a decreasing linear trendline as the NDVI value increased and the relationship was described in Figure 3(a). The correlation coefficient value of r^2 = 0.88 indicates a correlation between the R_{550} wavelength and NDVI of *P. nigrum*. Meanwhile, the SIPI values also decreased with increasing NDVI values as represented by a polynomial inverse four-order regression in Figure 3(b), with a moderate correlation at r^2 = 0.69. Meanwhile, in Figure 3(c), the PRI values increased with the NDVI value and the correlation was also described by a polynomial inverse four-order regression with a correlation coefficient value (r^2) of 0.82.

Conclusions

This study indicated that foliar spectral reflectance can be used as a non-destructive and rapid method for field assessment to identify nutrient deficiency problems in *P. nigrum.* According to the statistical and regression analyses in this study, it is evident that the three spectral indices, R_{550}, SIP and PRI can be regarded as the predictors of nitrogen deficiency problems in pepper vines, in relation to relative chlorophyll content and NDVI parameters.

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Growth and Morphological Response of Microgreens to LED Spectrum and Soilless Mediums under Microgreen Cube Grow Structure

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Introduction

The emphasis on urban agricultural activities such as farming in limited space has begun to be received by urban dwellers who are interested to practice farming in the cities. Urban agriculture can reduce kitchen expenditure, increase food and nutrition security and the economy of the urban poor. In line with the concept of urban agriculture, MARDI introduces microgreen cube grow (MCG) with a size of 128.8 cm length x 70.0 cm height x 60.3 cm width, an innovative technique for growing young seedlings inside the house. MCG is a cabinet structure or growth chamber of living fresh microgreens that can be placed in the kitchen, and harvested just before cooking, thus enabling the household to consume highly nutritious vegetables daily. They are also pesticide-free and very safe for consumption.

Microgreen plants are becoming trendy among Malaysia's healthy food enthusiasts. Microgreens are defined as tender immature green, produced from two fully developed cotyledon leaves with or without the development of a first true leaves which formed from the seeds of vegetables and herbs. They have a unique shape and color that is attractive and aromatic despite of rich source of iron, vitamins A, C (ascorbic acid) and E, as well as iron. The cultivation of microgreens is 100% fresh, healthy and free from the use of any pesticide and toxic-free (Vanninen et al., 2012; Hamdan et al., 2015). This gardening approach is environmentally friendly and sustainable.

Culturing under artificial light sources could regulate the growth, the phytochemical compound content and antioxidant capacity of microgreens (Zhang et al., 2020). The application of environmentally friendly light emitting diode (LED) has many advantages, including the flexibility to choose light wavelengths, change intensity and energy savings. The growth of plants using blue (B) and red (R) LEDs, which have the highest photon efficiencies, is the subject of numerous studies. It is well known that these lights are better absorbed by chlorophylls than light with other visible spectrum wavelengths (Zhang et al., 2020). Hence, the aim of this study was to determine the effect of different LED spectrum and planting mediums on the growth, physiochemical properties and yield of several selected microgreens using the MCG structure.

Materials and Methods

Microgreen cube grow components

Microgreen cube grow was developed using an artificial light source and plywood structure (Figure 1a). MCG consists of LED to provide light for the photosynthesis process and plant growth. In this structure, there is a fan to provide ventilation and maintain continuous ambient temperature (Figure 1b and c). It also comes with a glass door to facilitate management work such as monitoring, removing and placing containers. The MCG cultivation system can be applied using soilless media. Gardening activities by using the MCG improve air quality and produce cool and greenish effects in buildings or homes (Hamdan et al., 2015).

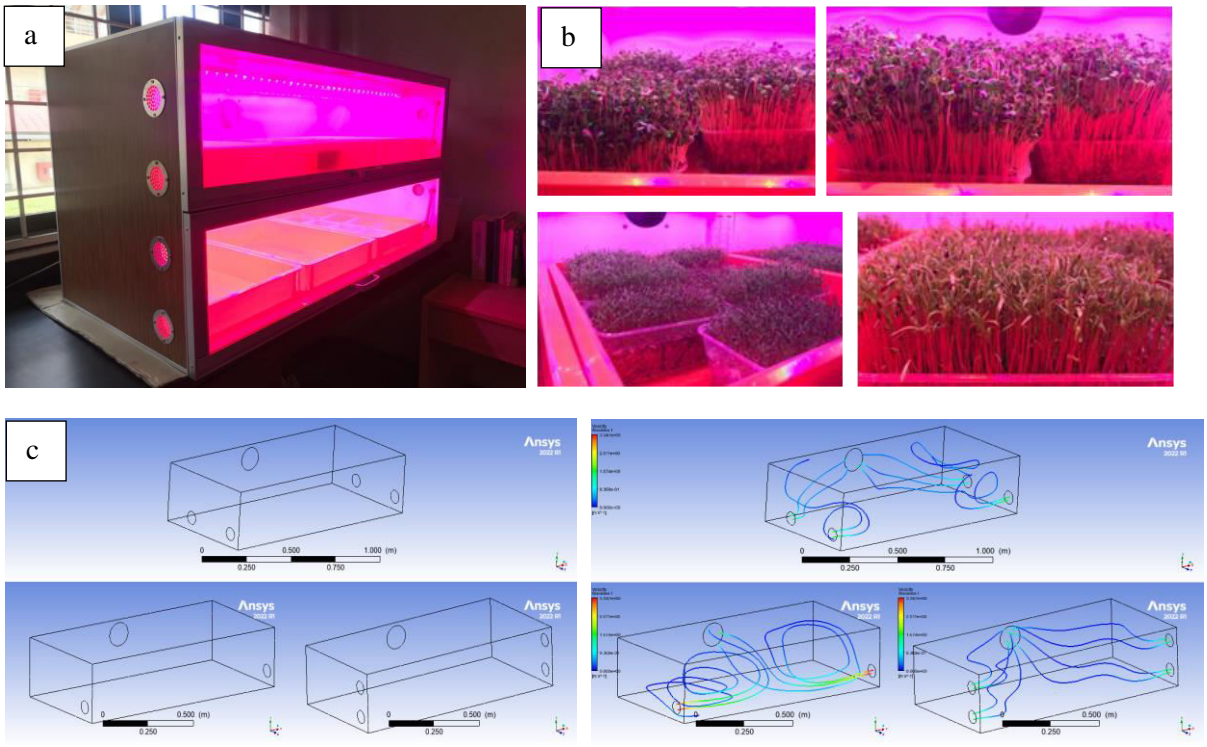

Figure 1: a) Microgreen cube grow (MCG) structure and microgreens in MCG structure, b) Hole position options for MCG structure and c) Airflow streamlines of MCG structure.

Microgreens preparation

Radish, wheat grass and sweet corn seedlings were grown in different soilless media combinations in plastic container (55x41x13 cm) for 10 to 14 days from sowing to harvest. The media combinations comprise of M1: peat moss + perlite + vermiculite (1:1:1v/v/v), M2: peat moss + perlite (1:1), M3: peat moss + vermiculite (1:1), M4: perlite + vermiculite (1:1), M5: perlite, M6: peat moss and M7: vermiculite. The containers were placed in MCG structure under custom made lighting comprises of LED combinations namely L1: Red + Blue (RB) and L2: Red + Blue + Green (RBG). Light intensity exposure was 211.8 µmol $m^{-2}s^{-1}$ for RB and 213.4 µmol$m^{-2}s^{-1}$ for RBG while the wavelength was 439 nm for RB and 656 nm for RBG. The experimental design was a randomized complete block design (RCBD) with three replications. Fertilization, pest and disease control were not practiced. Thoroughly observations were frequently conducted to identify the symptoms of early attacks and immediate preventive action.

Microgreens cotyledons with stems just above the ground were harvested 10 to 14 days after seeding, depending on types of species. Samples were taken from the central part of pot, leaving plants 1.5 cm from pot edges as guard plants. Plant height and fresh weight of plants were measured. Samples of dry matter microgreen from all containers per treatment were used for phytochemical analysis. Antioxidant activity, expressed as ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radicals and ascorbic acid were determined using spectrophotometric methods (Thermospectronic, USA). Data were subjected to analysis of variance (ANOVA) using the SAS software version 9.1 and means separation by the least significant difference (LSD) test at the 5% level.

Results and Discussion

There was significant effects of LED spectrum and media combinations on the fresh weight, plant height, ascorbic acid content and antioxidant activity of microgreens as shown in Table 1.

Table 1: Effects of different soilless mediums and LED light treatment on growth and physiochemical properties of microgreens.

Media (M)	Fresh weight (g)		Plant height (cm)		Ascorbic acid content (mg/100gFW)		% DPPH	
	LED		LED		LED		LED	
	RB	RBG	RB	RBG	RB	RBG	RB	RBG
Radish								
M1	169.39^{b}	138.38^{e}	5.78^{c}	7.72^{a}	4.89^{a}	4.87^{a}	64.97^{a}	66.33^{a}
M2	157.02^{d}	166.91^{c}	7.02^{b}	8.08^{a}	4.43^{a}	4.76^{a}	80.09^{a}	81.39^{a}
M3	164.60^{c}	173.24^{b}	7.99^{a}	7.94^{a}	4.27^{a}	4.60^{a}	65.85^{a}	65.34^{a}
M4	119.54^{f}	102.83^{f}	3.84^{e}	5.38^{b}	4.25^{a}	4.78^{a}	74.97^{a}	69.36^{a}
M5	117.24^{g}	71.42^{g}	3.63^{e}	4.20^{c}	4.64^{a}	4.56^{a}	74.99^{a}	71.85^{a}
M6	233.06^{a}	244.26^{a}	7.46^{ab}	7.97^{a}	4.56^{a}	4.47^{a}	51.81^{a}	50.55^{a}
M7	153.50^{e}	145.33^{d}	4.74^{d}	5.20^{b}	4.52^{a}	4.40^{a}	69.36^{a}	57.06^{a}
Wheat grass								
M1	81.94^{a}	62.14^{e}	15.92^{c}	14.56^{cd}	5.72^{b}	8.37^{d}	86.98^{ab}	86.79^{bc}
M2	58.63^{d}	56.95^{f}	16.28^{c}	14.61^{cd}	5.59^{b}	10.80^{b}	88.62^{a}	87.70^{bc}
M3	81.88^{a}	79.96^{b}	17.85^{b}	14.90^{bcd}	4.74^{cd}	6.47^{e}	85.30^{b}	87.44^{bc}
M4	61.97^{c}	66.71^{d}	12.98^{d}	16.77^{ab}	5.38^{bc}	10.82^{b}	85.78^{ab}	86.38^{c}
M5	28.71^{f}	45.20^{g}	15.39^{c}	13.32^{d}	7.31^{a}	9.54^{c}	87.78^{ab}	88.09^{ab}
M6	69.86^{b}	93.51^{a}	19.31^{a}	17.20^{a}	3.61^{e}	11.61^{a}	80.81c	89.22^{a}
M7	49.33^{e}	69.71^{c}	15.53^{c}	16.07^{abc}	4.59^{d}	8.33^{d}	84.54^{b}	84.87^{d}
Sweet corn								
M1	107.45^{b}	89.53^{c}	10.69^{b}	10.53^{ab}	3.50^{a}	1.52^{b}	80.18^{b}	78.63^{d}
M2	78.34^{d}	95.04^{b}	10.45^{b}	11.76^{a}	3.01^{a}	1.85^{b}	86.62^{a}	79.18^{c}
M3	97.17^{c}	95.71^{b}	11.46^{b}	9.72^{ab}	3.09^{a}	1.93^{b}	75.89^{d}	76.82^{e}
M4	44.60^{g}	28.88^{f}	6.03^{d}	6.61^{c}	3.43^{a}	3.53^{a}	77.68^{c}	80.82^{a}
M5	53.65^{f}	53.65^{e}	6.97^{d}	5.66^{c}	3.42^{a}	3.43^{a}	75.30^{d}	78.70^{d}
M6	137.77^{a}	149.82^{a}	13.39^{a}	12.08^{a}	3.43^{a}	1.87^{b}	79.80^{b}	79.65^{b}
M7	74.76^{e}	59.24^{d}	9.18^{c}	9.26^{ab}	3.83^{a}	4.09^{a}	76.25^{cd}	75.03^{f}

Means followed by different letters within same column are significantly different at $p < 0.05$ according to the LSD test
Notes: M1: peat moss + perlite + vermiculite (1:1:1), M2: peat moss + perlite (1:1), M3: peat moss + vermiculite (1:1), M4: perlite + vermiculite (1:1), M5: perlite, M6: peat moss and M7: vermiculite refer to the media combinations and RB (Red + Blue) and RBG (Red + Blue + Green) refers to LED combinations.

The significant highest fresh weight of radish and sweet corn were obtained from the seedlings that grown in peat moss (M6) under RB and RBG spectrum. However, wheat grass was higher in fresh weight under RBG spectrum in media M6 and RB spectrum in both media M1 and M3. Moreover, fresh weight of wheat grass under RBG spectrum is much higher as compared RB spectrum in media M6 by 14.5%. Plant height was significantly increased in radish cultivated under RB or RBG spectrum in media M3 or M6 as compared to other treatment. As for the wheat grass and sweet corn, plant height was higher in media M6 under RB and RBG spectrum.

There was significant effects of LED spectrum and media combinations on phytochemical analysis namely ascorbic acid and percentage of DPPH inhibition in microgreens. The highest ascorbic acid content was obtained on microgreens cultivated under RBG spectrum in media M6 and under RB spectrum in media M5 for Wheat grass which 11.61 and 7.31 mg/100g FW, respectively. As for the sweet corn, the highest ascorbic acid content was plants cultivated under RBG spectrum in M7 (4.09 mg/100g FW), M4 (3.53 mg/100g FW) and M5 (3.43 mg/100g FW). However, there is no significant different on ascorbic acid content for radish. As for the antioxidant activity, microgreens tested were significantly affected by LED spectrum and media except for radish. Antioxidant activity for wheat

grass is much higher under RB spectrum in media M2 and under RBG spectrum in media M6, 88.62 and 89.22%, respectively. Meanwhile, sweet corn cultivated under RB spectrum in media M2 (86.62%) and under RBG spectrum in media M4 (80.82%) is much higher as compared other treatment.

Johkan et al. (2010) reported that, at various stages of plant growth, red light had various effects. When compared to plants grown under fluorescent lights, the fresh weight of lettuce treated with red light (655 nm) increased by 25%. According to Orlando et al. (2022), the inclusion of green light at an irradiance level of 340 μmol m^{-2} s^{-1} enhanced biomass dry weight total carotenoid content and antioxidant capacity.

Conclusion

The response to LED spectrum and soilless media combination treatment varied between microgreen species on yield, plant height, ascorbic acid content and antioxidant activity. Based on results presented, radish is recommended to be cultivated in peat moss, M6 under RBG or RB spectrum. Whereas for the wheat grass and sweet corn, both crops are recommended to be grown in peat moss, M6 under RBG spectrum. Hence, the fresh-grown microgreens in the MCG can provide aesthetic impact in limited space as well as a source of food for the household.

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Effects of Number of Nodes and IBA Concentrations on the Vegetative Propagation of *Strobilanthes crispus* by Stem Cuttings

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Introduction

Strobilanthes crispus (L.) Bremek belongs to the Acanthaceae family and is one of Malaysia's most popular local herbal materials for both domestic and export uses. It is a woody shrub that can spread and grow up to 1.5 metres in height. The leaves' upper surface is rough, dark green in colour, and covered in short hairs. The flowers of *S. crispus* are yellow in colour and have short, dense, and panicled spikes (Sunarto, 1977). The plant can be found in the wild in scrublands and along river banks or it can be cultivated. Furthermore, it can grow alongside a river or in uninhabited fields (Noraida, 2005). Its native habitat is terrestrial, and the preferred climate zone is tropical.

To the local Chinese community, the plant is also known as “Hei Mian Jiang Jun” (Black-faced General), and the leaves are typically boiled and consumed as tea or mixed with other herbs. Whereas, in the Malay community, this plant is called “pokok pecah beling” or “pecah kaca”, and the water decoction is believed to be effective in treating gallbladder stones. Furthermore, *S. crispus* has been shown to have antidiabetic (Fadzelly et al., 2006; Norfarizan-Hanoon et al., 2009), antioxidant (Ismail et al., 2000; Qader et al., 2011), antiangiogenic (Muslim et al., 2010; Al-Henhena et al., 2011) and wound healing properties, as well as a hypolipidemic effect (Fadzelly et al., 2006). *S. crispus* products are available in Malaysia in the form of raw, crude powder (from leaves), capsules, and additives mixed with coffee or tea.

The commercial plantation of this plant is still lacking in Malaysia. Therefore, high quality planting materials need to be mass-produced to ensure a continuous supply of the raw materials. The macropropagation technique through cutting is a convenient and inexpensive method to obtain the raw materials in mass quantities. To date, there have been few reports on the macropropagation technique for *S. crispus*. Thus, this paper reported on the optimum number of nodes and indole-3-butyric acid (IBA) concentrations that can be applied to produce this plant on a large scale via stem cutting.

Materials and Methods

The experiment was carried out at the nursery of Herb and Tree Improvement Branch, FRIM, Kepong. The stock plants were obtained from Selangor accession and grown in the nursery. Two experiments were conducted to evaluate the rooting and shoot performance of *S. crispus* stem cuttings at different node numbers and IBA concentrations. The cutting material for experiment 1 was a leafless stem cutting. The stems were cut into different nodes (2, 3, and 4 nodes), and no rooting hormone was applied. The cuttings were planted in 100% fine sand media and grown in an enclosed mist propagation chamber. The cutting material for experiment 2 was leafy stem cuttings with two nodes. The base of the stem cuttings was immersed in different concentrations of IBA (0, 0.5, 1.0, and 2.0 mg/L) for 2 minutes before being quickly planted in rooting media.

This experiment was conducted in a Randomised Complete Block Design (RCBD) with 30 cuttings were used for each treatment and was replicated three times. The cuttings were misted three times daily for 10 minutes each session at 8:00 a.m., 12:00 p.m., and 4:00 p.m. Cuttings were evaluated at week 4 for experiment 1 and at week 2 for experiment 2. The variables measured were percentage of survival (survived cuttings divided by total cuttings and multiply by 100), number of roots, root length, shoot number, leaf length, and leaf width. All data were submitted for Analysis of Variance

(ANOVA) using SPSS. Differences between means were compared using the Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

Results and Discussion

In Experiment 1, the number of nodes showed a significant difference ($p \leq 0.05$) in the cutting survival percentage of *S. crispus*. Cuttings with two nodes had the highest percentage of survival (71.1%) (Figure 1). Except for root length, there were no significant differences ($p \geq 0.05$) in other rooting ability parameters and shoot production of *S. crispus* stem cuttings with different numbers of nodes (Table 1 and Figure 2). When compared to other treatments, stem cuttings with two nodes produced significantly longer roots.

Previous studies have reported that the rooting ability and survival percentage of the cuttings increased with an increase in the node number of the cuttings (Bhardwaj et al., 2017; Yesuf et al., 2021; Kitila et al., 2022). This might be due to the higher node number in cuttings producing more leaves and more carbohydrate accumulation in the cuttings for the early vegetative growth of roots and shoots (Adugna et al., 2015; Yesuf et al., 2021). However, a decreasing trend was also observed after an increase in a certain number of nodes. For example, an increase of mean values for all growth parameters was observed only up to node 4, while a decreasing order was recorded from node 4 or 5 of *Vanilla planifolia* cuttings (Hailemichael et al., 2012).

In contrast, an increase in the number of nodes could not enhance the rooting ability of *S. crispus* but the cuttings with fewer nodes performed better in terms of survival percentage and root length (Figure 1 and Table 1). This is supported by previous studies that found shorter cuttings rooted better and developed more roots than those of longer cuttings (Foster et al., 2000; OuYang et al., 2015). The relationship between the number of nodes and the rooting ability of cuttings varies between species. Many factors, such as cutting length and diameter, age and position of node/cuttings, endogenous auxin, and carbohydrate content, influence rooting effectiveness in general. In large-scale production, cuttings with two nodes are preferable, as more ramets can be produced from a mother tree.

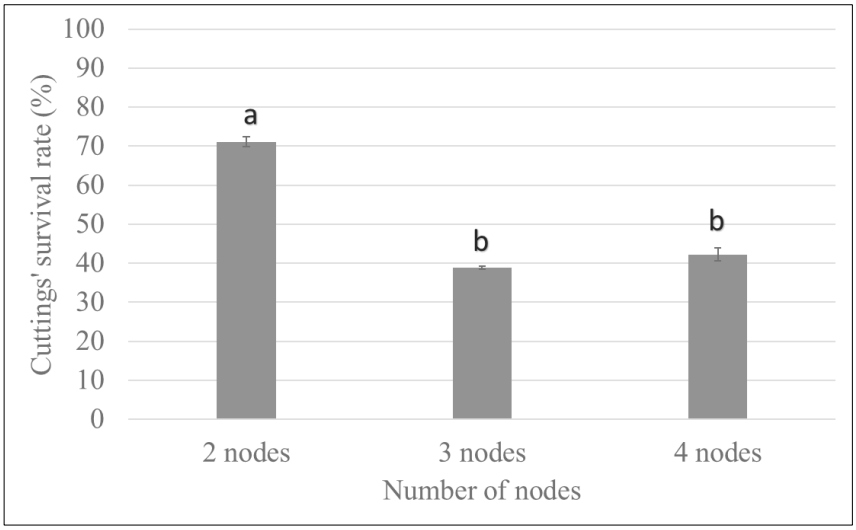

Figure 1: Effects of node numbers on the percentage of cuttings' survival rate. Means followed by the different letter(s) are significantly different at $p \leq 0.05$.

Figure 2: No significant difference was observed in the rooting ability and shoot performance of *S. crispus* stem cuttings treated with different node numbers.

Table 1: Effects of node number on rooting ability and shoot performance of *S. crispus* stem cuttings.

Samples	No. of roots	Root length (cm)	No. of shoots	Leaf length (cm)	Leaf width (cm)
2 nodes	1.20±0.21[a]	0.40±0.07[a]	2.36±0.35[a]	0.79 ±0.11[a]	0.38±0.08[a]
3 nodes	0.80±0.17[a]	0.18±0.04[b]	1.61±0.32[a]	0.65±0.13[a]	0.29±0.05[a]
4 nodes	0.88±0.17[a]	0.30±0.06[ab]	2.36±0.43[a]	0.86±0.15[a]	0.38±0.07[a]

Means followed by the same letter(s) are not significantly different at p ≤ 0.05.

In experiment 2, different IBA concentrations had no effect on the survival percentage of stem cuttings at p ≤ 0.05 (Figure 3). The survival rate of stem cuttings ranged from 76% to 83%. The number of roots and root length of *S. crispus* stem cuttings differed significantly (p ≤ 0.01) when IBA concentrations were varied (Table 2). The most roots and the longest root length were found in stem cuttings treated with 0.5 mg/L IBA. Stem cuttings in the control treatment (0 mg/L) had the fewest and the shortest roots. As the concentration of IBA increased from 0.5 mg/L to 2 mg/L, the number of roots and root length decreased.

Table 2: Effects of IBA concentrations on rooting ability of *S. crispus* stem cuttings.

IBA concentration (mg/L)	No. of roots	Root length (cm)
0 (control)	2.24 ±0.37[b]	1.54±0.23[c]
0.5	4.10±0.50[a]	2.66±0.28[a]
1.0	3.3 ±0.39[ab]	2.35±0.28[ab]
2.0	2.54±0.33[b]	1.89±0.22[bc]

Means followed by the different letter(s) are significantly different at p ≤ 0.05.

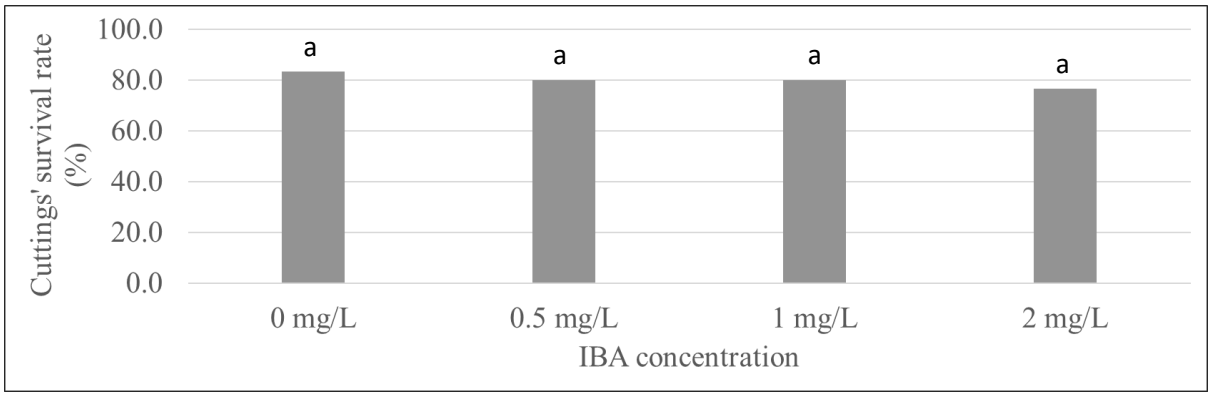

Figure 3: Effects of IBA concentration on the percentage of cuttings' survival rate. Means followed by the same letter are not significantly different at $p \leq 0.05$.

In the current study, it was discovered that almost all treatments, including the control, were capable of inducing rooting in cuttings (Figure 4). However, the percentage of rooting varied with treatment, and IBA was found to be effective in producing roots in *S. crispus*, especially in low concentrations (0.5 mg/L IBA). IBA has also been shown to be effective for root induction in stem cuttings of other medicinal plants, including *Andographis paniculata* (Hossain et al., 2021) and *Ginkgo biloba* (Aseesh et al., 2011).

Figure 4: Rooting ability of *S. crispus* stem cutting treated with different concentrations of IBA.

Conclusion

In this study, we found that the number of nodes and application of IBA significantly affect the survival percentage and root length of *S. crispus* stem cuttings. Stem cutting with two nodes and the use of 0.5 mg/L IBA as a rooting hormone are recommended for mass production of *S. crispus* as it is more productive and a better root system can be developed by the cuttings. This will facilitate the uptake of water and nutrients for proper growth and development of *S. crispus*.

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Effect of Drying Method for Regenerated Cellulose Membrane Incorporating Photocatalytic Zinc Oxide for Decolouration of Methylene Blue

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Introduction

Uncontrolled discharge of wastewater without proper treatment into rivers and other water sources can cause water pollution. Water pollution is a global issue that needs to be handled effectively and seriously. This will affect water-living organisms as well as humans if exceed the maximum residue limit as permitted by the local authorities. Methylene blue (MB) is a dye that is widely used in the textile and medical industries. Untreated MB wastes discharged into rivers might result in harmful issues such as nausea, headaches, chest pain, and confusion.

In the past few years, zinc oxide (ZnO) is among the favourable heterogeneous catalysts because of its non-toxic, cheap, low-corrosion, recyclability and minimum execution properties (Mu et al., 2011). When irradiated by UV light, changing of electron energy state from the valence band (VB) to the conduction band (CB) forms electron-hole pairs ($h+$) that lead to the excitation of radical forms for the decolouration of methylene blue (MB) as described below:

$ZnO + UV\ light \rightarrow h^+ + e^-$ …(1)
$e^- + O_2 \rightarrow O_2^-$ …(2)
$h^+ + OH^- \rightarrow OH^-$ …(3)
$O_2^{-} + H_2O \rightarrow HO_2^- + OH^-$ …(4)
$HO_2^- + H_2O \rightarrow H_2O_2 + OH^-$ …(5)
$H_2O_2 \rightarrow 2OH^-$ …(6)
$2OH^- + MB \rightarrow CO_2 + H_2O$ …(7)

Photocatalysis is an environmentally-friendly method in which photons from a suitable light source are leveraged to generate hydroxyl radicals during the catalysis process. In an aqueous solution, the hydroxyl radicals enable the degradation of toxic organic compounds into simple and harmless inorganic molecules without the derivation of secondary waste (Vidal and Sanchez, 1994). Consequently need for environmentally friendly heterogeneous catalysis has led to much research on metal oxides, whose redox and acid-base properties make them excellent candidate catalysts (Boon et al., 2018).

In this study, regenerated cellulose nanofibril (CNF) from cotton linter was used as a matrix in the development of bio-composite membranes embedded with zinc oxide (ZnO) as a catalyst. ZnO was embedded into the CNF matrix by direct mixing into the membranes and aerogels cellulose solutions, followed by drying via a freeze-drying process to obtain a porous structure while maintaining its shape. The main purpose of this study is to focus on the preparation and characterization of the CNF aerogel and membrane bio-composites, followed by the decolouration of MB using the developed aerogels and membrane. The photocatalytic process was illustrated in Figure 1.

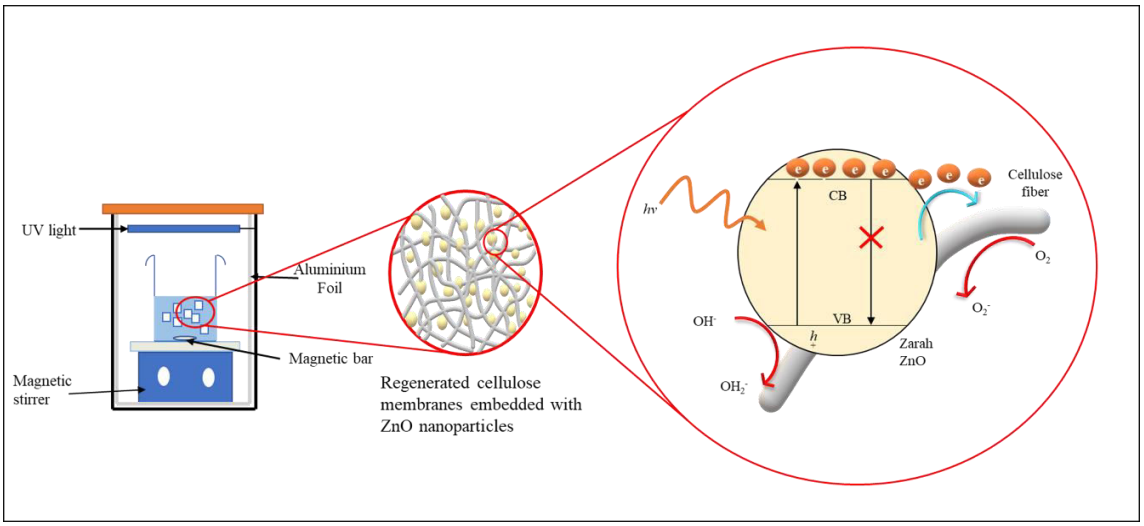

Figure 1: Schematic diagram of a photocatalytic process using regenerated cellulose membranes.

Materials and Methods

Materials

Commercial zinc oxide (ZnO) powder (average particle size 21±5 nm) and methylene blue (MB) were purchased from Sigma Aldrich. A cotton linter with an average molecular weight (Mw) of 90,000 was obtained from Hubei Chemical Fiber Co. Ltd., China. Other chemicals such as sodium hydroxide (NaOH) and urea (CH_4N_2O, 99%) were purchased from R&M Chemicals. All chemicals were analytical grade and used without further purification.

Preparation of regenerated cellulose

The regenerated cellulose membrane was prepared according to a previous report (Kaco et al., 2014). Briefly, an aqueous solution of NaOH/urea with a weight ratio of 7:12:81 was prepared and frozen at -20 °C for at least eight hours. The solution was then thawed to -13 °C, after which cotton linter (3.5 g) was added and the solution was stirred vigorously using an overhead stirrer until the linter dissolved. The resulting cellulose solution was centrifuged at 12,000 rpm for 10 min at 5 °C to separate unreacted cellulose and air bubbles. Finally, the supernatant was collected and stored at 4 °C until further analysis.

Membranes

The cellulose solution membrane was prepared by adding 1.6 wt% (w/v) of ZnO to the cellulose solution and labelled it as CMZ. The soluble cellulose solution containing ZnO was cast on a glass plate to form a thin layer of membrane and immersed in distilled water as a coagulating bath to regenerate the cellulose. The formed membrane was soaked in distilled water to remove excess and unreacted chemicals and was dried using two different methods: oven drying at 40 °C and freeze-drying is freezes for 24 hours.

Photocatalytic activity for decolouration of MB

All MB solutions were prepared according to the initial MB concentrations required. Concentrations were measured using a UV-vis spectrophotometer (OPTIZEN POP) at λ_{max} 662 nm. A calibration curve was constructed using MB solutions with absorbances ranging from 0.1 to 1. Membranes were cut to dimensions of 1×1 cm and aerogel dimensions of 1×1×1 cm weighing about 0.4 g. The cut membranes were immersed in 100 mL of MB solution (5 mg L^{-1}) in a beaker with a magnetic stirrer, which beaker was in turn placed in a chamber walled with aluminium foil and having a lid equipped

with a 26-watt UV lamp (λ ≤ 390 nm). The solution was stirred at 250 rpm in the dark for one hour to allow equilibrium between the organic molecules and the catalyst. After an hour, the solution was exposed to light and the photocatalytic activity was measured at five-minute intervals for another 100 minutes using a UV-vis spectrophotometer (OPTIZEN POP). The amount of decolourized MB was determined according to the following equation:

$$Removal\ percentage\ (\%) = \frac{C_0 - C_e}{C_0} \times 100 \qquad \ldots (8)$$

where C_0 and C_e are MB's initial and equilibrium concentrations, respectively.

Results and Discussion

Natural-sources-derived nanocellulose membranes are regarded as among the bio-smart materials because of their structure and adaptable properties, which include resistance to temperature changes and mechanical stimulation (Awang et al., 2018). To enhance the photodegradation reaction, a good and stable pore size is essential for the insertion of a catalyst. Therefore, the ideal circumstances such as the effect of the drying method are essential for nanocellulose membranes to achieve a consistent pore size.

Figure 2 portrays the effect of the cellulose membrane drying process, which is freeze-drying and oven-drying using the CMZ sample. The graph clearly shows that the freeze-dried process performs better with 87.6% decolourization compared to the oven-dried process which stated 80.3% decolourization. This is because the pores resulting from the freezing of water drawn during the freeze-drying process are larger and more stable compared to oven drying which shrinks slightly due to heat factors (Cardea et al., 2009). Due to the material's tiny pore structure, the photodegradation process can be carried out while the catalyst is held more effectively and prevented from leaching into the aqueous solution. This data is needed to identify the best drying method taking into account the most cost-effective if it needs to be done on a larger scale.

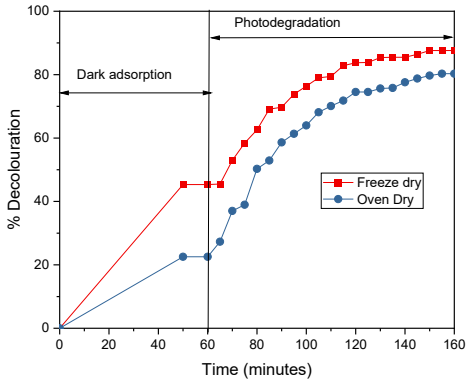

Figure 2: Comparison of two different drying methods for regenerated cellulose membranes.

Conclusions

This study has shown a success story of the development of cellulose membranes embedded with ZnO as a catalyst for the decolouration of MB. As a result, a comparison study between both drying methods shows freeze-dried has a better performance compared to oven-dried. This may be caused by no heat has been introduced during the freeze-drying process in comparison with oven-dried which involved heat that may lead to the degradation of membranes' pore structure (Cardea et al., 2009). This study has proven that regenerated cellulose membranes can be a model for scaffolding catalysts for other uses.

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Effects of Elevated Temperature on Vegetative Growth of Papaya Variety Sekaki

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Introduction

Global climate change which causes heat stress has become a major abiotic challenge (Kourani et al., 2022) due to the rise in temperature beyond a threshold level for a period of time will contribute irreversible damage to growth and development of various crops and reduction in crop productivity (Okunlola and Adelusi, 2013). An elevated temperature can physiologically affect plant growth, photosynthesis, respiration and stomatal conductance (Firmansyah and Argosubekti, 2020). The temperature range for papaya optimal growth is 21 °C to 33 °C where temperature above 35 °C can limit photosynthesis and hinder the fertilization of the flowers, thus causing diverse flower malformations which will reduce yield and fruit quality (Salinas et al., 2021). Data obtained from the previous study showed that elevated temperature had a significant effect on the petiole length, shoot height, leaf length, leaf width, leaf area, fresh weight and dry weight of *Carica papaya* (Okunlola and Adelusi, 2013). A study was conducted with an objective to determine the effect of simulated high temperature (38 °C) on the vegetative growth parameters of papaya (variety Sekaki) grown inside a phytotron and to compared with those grown inside a greenhouse having an ambient temperature ranging from 28 °C to 32 °C during the day.

Materials and Methods

Two months old of papaya seedlings variety Sekaki were transplanted into the 50.8 cm x 50.8 cm polybags containing a mixture of soil, peat moss, perlite and vermiculite (2:1:1:1 v/v). Four plants that had recovered from transplanting shock after 30 days were randomly arranged for each treatment in a Nested design. For the heat stress treatment, plants were grown inside the phytotron (ThermoStable GC-1000, Daihan Scientific, Korea) where the temperature was set at 38 °C for 6 hours daily (0800 h to 1400 h) with 12 hours of daylight at 400 µmol photon/m^2/s. For another 12 hours (light off), the temperature of the phytotron was set at 25 °C. The papaya plants grown inside the greenhouse (ambient environment) were managed as control treatment. Relative humidity was maintained at 70%. Stringent crop management in terms of irrigation, fertilization and pest control was done according to standard practice to reduce experimental errors.

The effect of simulated high temperature on the relevant plant growth parameters were measured at weekly intervals for a period of one month. All the recorded data were analyzed appropriately by using Statistical Package for the Social Sciences (SPSS) software.

Results and Discussion

Heat stress causes dehydration and generally reduces photosynthetic efficiency, thus shortening the plant life cycle and diminishing the growth and productivity of the plants (Zhao et al., 2021). From the observation, the papaya plants (variety Sekaki) grown inside the greenhouse were bigger and healthier compared to those in the phytotron (Figure 1). The changes in the plant growth parameters of plant height and canopy size due to heat stress were more responsive and significant ($p=0.05$) compared to other parameters (canopy width, canopy length, leaf length, leaf width, leaf number and stem diameter).

The analysis of covariance that compared the linear regression lines of the treatments showed that papaya plants receiving continuous heat at 38 °C for 6 hours significantly reduced plant growth and canopy size ($p < 0.05$). During the growing period of four weeks, the daily change in the rate of plant height of papaya plants receiving heat treatment was declining (negative) as opposed to those plants growing under ambient temperature (Figure 2). Similarly, the daily rate change of canopy size of heat treated papaya plants also significantly experienced a negative change with time unlike plants in the ambient treatment (Figure 3).

Figure 1: The difference in the size of four months old papaya plant grown in ambient temperature (left) versus elevated temperature (right).

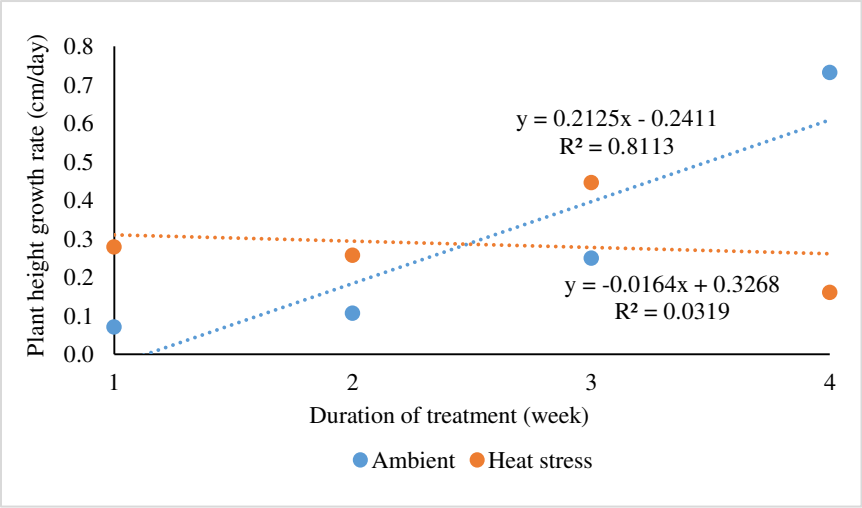

Figure 2: Growth rate for plant height of papaya plants grown under heat treatment and ambient environment.

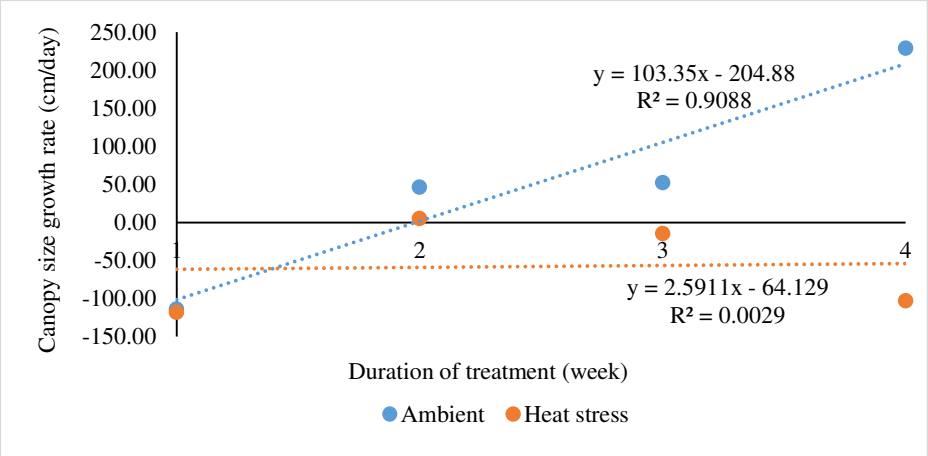

Figure 3: Growth rate for canopy size of papaya plants grown under heat treatment and ambient environment.

Conclusion

The experiment proved that the heat treatment significantly affects growth of papaya plants mainly by declining the growth rate in plant height and canopy size as compared to other parameters (canopy width, canopy length, leaf length, leaf width, leaf number and stem diameter). These two plant growth parameters should be used as indicators to monitor the effect of heat stress on papaya plants (variety Sekaki) in future experiments.

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Effects of Sowing Media and Cultivar on Rooting Ability and Early Growth of Strawberry Daughter Plant

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Introduction

Strawberry (*Fragaria* x *ananassa* sp.) is a high value crop due to its aroma, taste and high nutritive value. Production of strawberry (*Fragaria* x *ananassa* sp.) is expected to rise due to a significant number of demands worldwide. This plant requires temperature between 20 to 28 °C during day time and 12 to 18 °C during night time (Uselis et al., 2008). Strawberries in Malaysia are planted in Cameron Highland at an elevation of 1,500 meters above sea level (Rosemary et al., 2016). Due to limitation of agricultural areas in the highland, the shift to more modern and sustainable productivity known as Controlled Environment Agriculture (CEA) has become more urgent now than ever. This technology has the ability to control conditions that can imitate the highland environment such as temperature, humidity, CO_2, irradiance and others.

Recently, growing strawberry in CEA with artificial lighting has attracted attention among scientists for year-round production (Kim et al., 2010; Wu et al., 2011). Botanically, strawberry is a hybrid species that propagates by either seeds, or vegetatively by runners that produce daughter plants that are identical to mother plants. Runner propagation is an important method in strawberry plants. Thus, we need to produce healthy runners, and ready to be transplanted to CEA condition, which influences plant survival and fruit yield during the commercial production.

A successful rooting of daughter plants depends on various factors such as the growth media, air humidity and quality of plant-lets (Kazemi and Mohorko, 2017). The sowing media plays an important role in the rooting process of runners. The sowing media during propagation helps to provide moisture, support, nutrient and aeration for growing daughter plants and aids in the proper growth and development of plants after transplant (Joeng et al., 2006). Thus, it is important to know an efficient method of producing strawberry daughter plants in lowland that can adapt to the hydroponic system under CEA conditions. The purpose of this study is to investigate the influence of sowing media on rooting and growth of daughter plants on four different cultivars.

Materials and Methods

Experimental locations

This experiment was carried out in Controlled Environment Agriculture at Malaysian Agricultural Research and Development Institute, Serdang, Selangor, Malaysia. The environment conditions are as follows: air temperature: 20-22 ^{o}C, CO_2: 400-500 μmol mol^{-1} and relative humidity was controlled at 80-85%. Light intensity of 250 mol m^{-2} s^{-1} and the photoperiod of 12h d^{-1} were provided by LED lights consisting of red blue and green chips.

Plant materials and propagation methods

Uniform size of four different cultivars: 'Festival', 'Monterey', 'Snow White' and 'Angel Eight' daughter plant were selected with at least had three leaves. Five strawberries were grown for each cultivar in (a) sowing mixture (cocopeat and perlite, 1:1) and (b) sowing substrate (sponges, size 25mm x 25mm x 25 mm) (Figure 1). The medium was drenched completely with nutrient solution (in mmol L^{-1} NH_4^+ 1.0, NO_3^- 10.6, $H_2PO_4^-$ 1.2, K^+ 5.1, Ca^{+2} 1.0, and SO_4^{-2} 0.3, and in n µmol L^{-1} Fe^{+3} 43.6, BO_3^{-3} 22.6, Mn^{+2} 9.4, Zn^{+2} 1.5, and MoO^{4-} 0.5) with electric conductivity 1.6 $dS.m^{-1}$, adjusted pH 5.8 at 3 days interval. The nutrient solution was renewed every 3 days during the experiment.

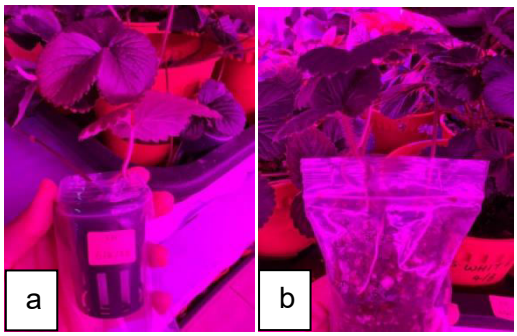

Figure 1: Sowing media of strawberry daughter plant by using (a) sowing mixture (cocoopeat and perlite, 1:1) and (b) sowing substrate (sponge).

Measurement of the growth parameter

The growth for shoot and root were recorded after one month of propagation. Fifteen daughter plants from each sowing media and variety were harvested and the roots were gently washed with running water. The fresh weight of shoots and roots were recorded immediately after removing the free surface moisture with soft paper towels. Root number was counted, and root and shoot length were measured using a ruler from the initiation crown area until the shoot and root tip. Shoot and root samples were then oven dried (FO-450M, Jeio Technology Co. Ltd, Daejeon, Korea) at 60 °C for 72 h, and weighed for dry weight on a digital balance (B303-S, Mettler Toledo, USA). Absolute growth rate of root was measured in soilless media every 5 days interval of propagation.

Statistical analysis

The experiment was carried out with 3 replicates in a randomized complete block design, using 15 runners in each observation unit. A multiple way analysis of variance (ANOVA) with the SAS program (Statistical Analysis System, V.9.1, Cary, NC, USA) was applied statistically to the sowing media and variety factors. Turkey's multiple range test was adopted to test the significant difference between the difference means. Relationships between the root length and propagation duration were analyzed using quadratic and exponential functions whenever appropriate. The relationships were calculated by using $y = ax^2 + bx + c$ for the quadratic functions and $y = ae^{bx}$ for the exponential functions.

Results and Discussion

Growth measurement

Root and shoot development were significantly affected by cultivars and different media treatment as shown in Figure 2 and Table 1. Cultivars 'Festival' and 'Monterey" respond very well as compared to Snow White and Angel Eight. The shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of Festival runner in sowing mixture higher by 3.4%, 8.8%, 23% and 32% as compared to snow white respectively. The adventitious root production of cuttings depends on carbohydrate sinks,

requiring low energy for root development (Costa and Challa, 2002). This result indicated that Festival and Monterey was in favour of producing strong plants.

The difference in root number were obvious which better developed in sowing mixture than those in sponge media, which increased higher root length and number and maximized the fresh weight and dry weight of the below ground runner biomass. The primary roots of daughter plants in sponge media are shown slender than in sowing mixture. One possible observation of plant root performance is the media have good porosity, water holding capacity and aeration characteristics (Wei et al., 2020). In sponge media, when the root interacts with water (100% water holding capacity characteristics causes the plant roots to be under water stress thus reducing the rooting performance. As reported by Tehranifar et al. (2007) strawberry grown in media with peat and cocopeat showed less malformed fruit as compared to soiless cultivation. Liao and Lin (2001) reviewed the physiological adaptation of crop to flooding stress (soiless). Excess water produces anoxic soil conditions, plant roots, consequently suffer hypoxia and anoxia.

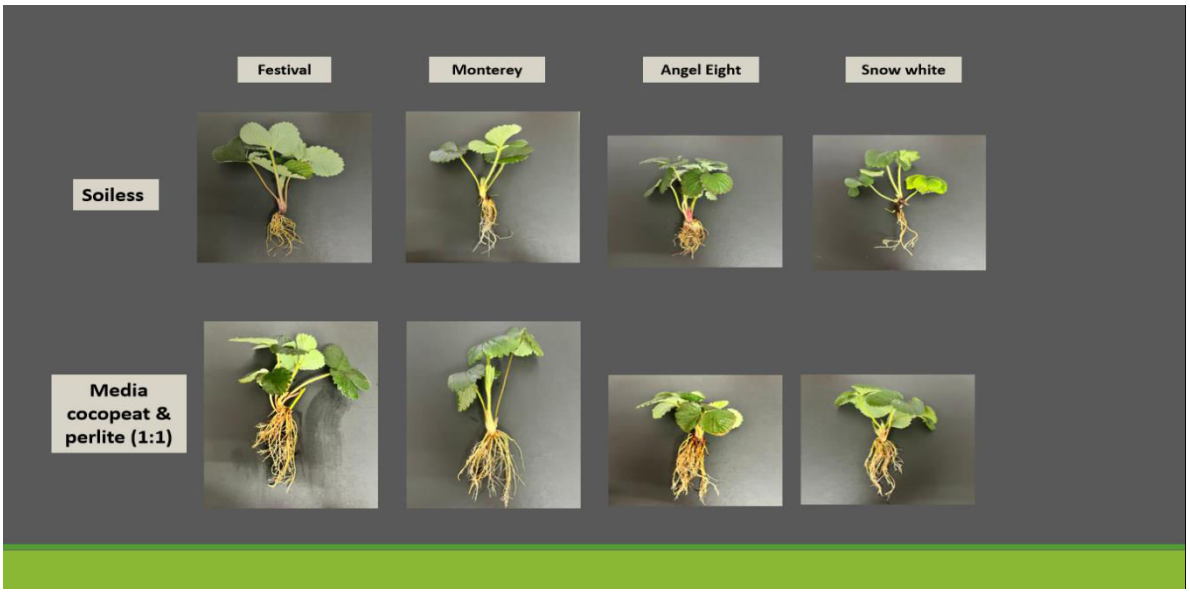

Figure 2: The effects of sowing media and cultivar of the strawberry daughter plant after 30 days of propagation.

Table 1: The effects of growth media and cultivar on the length, fresh weight and dry weight of the strawberry daughter plant after 30 days of propagation.

Cultivar	Media	Shoot			Root			
		Length	Fresh Weight (g)	Dry Weight (g)	Length	Fresh Weight (g)	Dry Weight (g)	Number
Festival	S. Mixture	15.19^{a}	4.34^{a}	2.82^{a}	12.99^{a}	5.33^{a}	3.83^{a}	41^{a}
	S. substrate	15.09^{a}	4.31^{a}	2.80^{a}	6.90^{c}	3.30^{c}	1.8^{c}	21^{bc}
Moterey	S. Mixture	16.34^{a}	4.37^{a}	2.79^{a}	11.61^{a}	4.87^{a}	3.37^{a}	38^{ab}
	S. substrate	14.42^{a}	4.22^{a}	2.72^{a}	10.23^{a}	4.41^{a}	2.91^{ab}	16^{c}
Snow white	S. Mixture	8.75^{c}	4.19^{b}	2.57^{b}	9.3^{b}	4.10^{b}	2.6^{b}	45^{a}
	S. substrate	12.22^{b}	4.11^{b}	2.42^{b}	7.32^{bc}	3.44^{c}	1.94^{c}	21^{bc}
Angel Eight	S. Mixture	8.72^{c}	4.11^{b}	2.51^{b}	8.01^{bc}	3.67^{b}	2.17^{b}	32^{ab}
	S. substrate	9.14^{c}	4.19^{b}	2.40^{b}	9.36^{b}	4.12^{ab}	2.62^{ab}	12^{c}

Means with different letters within the column indicate significant differences at $p \leq 0.05$ level according to Tukey Analysis.

Root performance

Further analysis on the root growth rate performance projected a strong exponential relationship between propagation and root length in Festival and Monterey cultivar (Figure 3) with R^2= 0.98. However, there was no significant relationship between root length and propagation duration for Snow White and Angel Eight with R^2= 0.53 and R^2=0.63, respectively. The results showed that root elongation started after day 10 followed by gradual growth until day 20. Root length increased rapidly from day 20 to 30. Similar growth trends were observed for Festival and Monterey. Higher performances were noticed for Festival and Monterey cultivar as compared to Snow White and Angel Eight. The nodal root system started to elongate from day 10 onwards. A set of nodal roots started maturation at each progressively higher node on the cutting (Yamashita and Immamura, 2007).

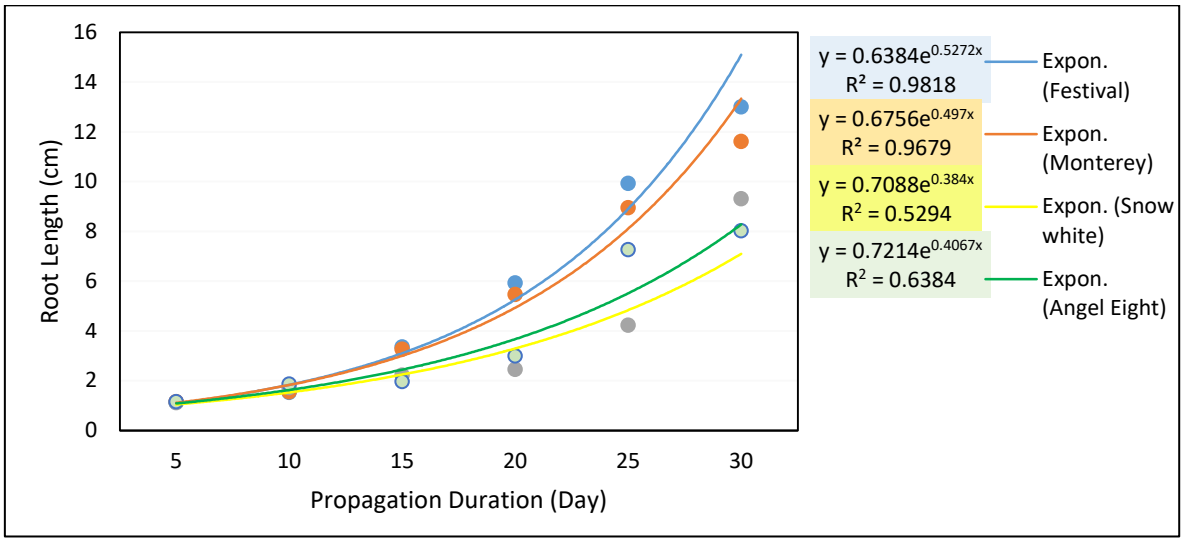

Figure 3: Root length of runers for strawberry Festival (▬), Monterey (▬), Snow white (▬) and Angel (▬) cultivars during 30 days of propagation. Solid line indicates significant exponential relationship. Each point corresponds to the mean of four runners.

Conclusions

Root development of runners was best in Festival and Monterey cultivar at the seedling stage. Propagation of strawberry runners in sowing mixture increased the survival rate after transplanted to real growth condition. Therefore, Festival and Monterey cultivars that suit with sowing mixture are recommended for rapidly producing hydroponically strawberry plants in CEA conditions. This study provides a reference for strawberry growers to improve the quality of planting materials and efficiency of sowing media during the propagation period.

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Chapter 2

Ecophysiology and Stress Biology

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Effects of High Temperature and Water Stress on Leaf Physiological Responses and Yield During Flowering Stage of Rice (*Oryza sativa* MR 253)

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Introduction

Rice is the most important crop in Asia. This plant is one of the staple foods for almost 4 billion people on earth. The production of rice is always insufficient due to the increase of human population. Therefore, various efforts and studies have been carried out to increase the yield of rice production such as production of high-yield varieties, fertilizer technology, crop and field management, and effective harvesting methods (Horie, 2019). The expected growth of the world's population will reach 10 billion by the year 2050. Therefore, the demand for rice will be higher than other crops. Among the factors that have been identified as challenges in achieving higher and sufficient rice productivity in the future is climate change (Krishnan et al., 2011). The optimum temperature for rice cultivation in Malaysia is between 24-34 °C and the optimal average annual rainfall is not less than 2,000 mm per year (Lin et al., 2010). Temperatures above 25 °C have the potential to reduce the mass of rice grains by 4.4% for every 1 °C increase in temperature, while production yields decrease by 9.6-10% for each 1 °C increase in temperature under the current climate change scenario in Malaysia (Alam et al., 2014). However, due to the effects of high temperature and water stress, this value is expected to decrease more with an increase in temperature around 0.3-4.5 °C (Rahman, 2018). High daily temperature will lower the percentage of rice pollen germination and abnormal pollen formation (Singh et al., 2011). High temperatures and drought during the flowering and grain-filling phase reduce yield due to spikelet sterility and shorten the duration of the grain-filling phase. High temperatures above 30 °C also are generally not suitable for plants to mature (Krishnan et al., 2011). So, in this study, the effect of high temperature (38 °C) and water stress on leaf physiological responses and yield were investigated during flowering stage using rice variety MR 253. This stage was chosen because it is the stage that is most critical in determining the yield.

Materials and Methods

The experiment was carried out under a glasshouse and plant physiology laboratory at MARDI, Serdang. Randomized complete block design (RCBD) with five replications was used in this study. MR 253 variety was used as a seed source. Rice plants were planted in containers (45 cm in diameter and 30 cm in height) according to the different treatments which are Control (C), Water Stress-Drought (WS), High Temperature (HT), and High Temperature (HT) + Water Stress (WS) (Figure 1). Plants in the control condition were conducted based on current farmers' practices including fertilizer rate, pest and disease management. The plant was planted at a normal temperature (30 °C during the day, 23 °C at night), with a relative humidity of 75-85% under natural sunlight conditions. Whilst, the water-stressed plants (WS) were conducted in accordance with the control plot's management except for water management. The water in the container of WS container was drained out 14 days before flowering create drought conditions until temporary wilting was witnessed (Hussain et al., 2022). A constant volume (500 mL) of water was added back daily until the tiller completed anthesis followed by complete flooding. Plants in (HT) were exposed to HT for 6 h (0830-1430) using a phytotron (ThermoStable GC-1000, Daihan Scientific, Korea), on the first day of anthesis (i.e. the appearance of anthers). This process was repeated for three consecutive flowering days (Figure 2). Finally, the condition for HT+WS treatment followed the same treatment as those in HT and WS but was carried

out simultaneously. Crop care management such as fertilization, pest control and irrigation followed normal farmer's practices. (Hashim et al., 2008)

The effects of high temperature on leaf physiological parameters at the flowering stage were observed every day for three consecutive days. The measurements of net photosynthetic rates, stomatal conductance and transpiration rate were taken using a portable photosynthesis system (LI6400XT, LICOR Inc., Nebraska, USA). The chlorophyll fluorescence measurements were made using a portable Plant Efficiency Analyzer (PEA) (FMS 2, Hansatech Instruments Ltd, U.K.). The Fv/Fm ratio was used to determine the leaf chlorophyll fluorescence responses. The relative content of chlorophyll in rice leaves was determined using a portable chlorophyll meter (SPAD-502, Konica-Minolta, Japan). Measurements were made on the mature leaves of each plant sample. Analysis of chlorophyll 'a' and 'b' was done to see the amount of chlorophyll activity after being subjected to treatment using the methodology from Arnon method (Pappas et al., 2016). An area of 3 cm^2 of leaves was taken from each treatment and soaked in 80% acetone solution for 7-10 days until all the leaves decompose. The solution was read using a Spectrophotometer (Genesys 10S UV-VIS) with wavelengths of 647 nm and 664 nm. Plant harvested on 105 days and yield was determined using grain counter Model INDOSAW S6709 and weighed using a digital scale (Precisa XB220A, Switzerland).

Analysis of variance (ANOVA) was used to determine the significant differences between treatments for several study parameters. Mean differences were conducted using Duncan Multiple Range Test (DMRT) at the 5% level with statistical analysis system (SAS) version 9.4.

Figure 1: Plants were grown in containers of 45 cm in diameter and 30 cm in height.

Figure 2: The high temperature treatment of 38 °C was simulated from 0830 to 1430 during flowering (6 h) inside a phytotron.

Results and Discussion

Photosynthetic rate

Plants with WS and HT+WS treatments showed a significant decrease in photosynthetic rate compared to those of C and HT. Lack of water at the flowering stage causes the plant to be in a state of stress which contributes to a decrease in the rate of photosynthesis (Figure 3). Stomatal conductance increased with rising temperature despite a decrease in leaf water potential, an increase in transpiration, an increase in intercellular CO_2 concentration, and the fact that it became uncoupled from photosynthesis (Urban et al., 2017)

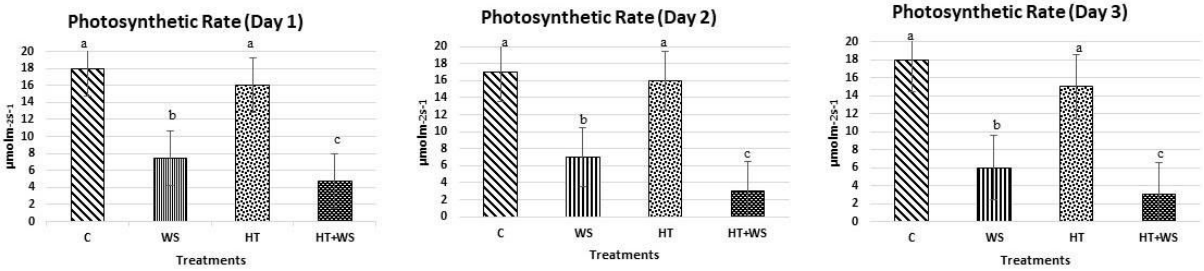

Figure 3: Photosynthetic rate for three consecutive days during the flowering stage. Means followed by the same letter are not significantly different by DMRT at $P \leq 0.05$.

The effect of water stress on leaf gas exchange is due to stomatal closure due to increased ABA production on the leaves. Water stress induces stomatal closure due to plants intercellularly using a lot of CO_2 which leads to a state of photoinhibition or light inhibition. A study by Zain et al. (2014) has shown that drought stress on rice for 15 days at the reproductive stage gave the lowest net photosynthesis (14.28 µmol/m^2/s) and stomatal conductance (0.31 mmol/m^2/s). Other than ABA, various other factors that accumulate during drought and affect the function of the stomata include plant hormones (auxins, MJ, ethylene, brassinosteroids, and cytokinins), microbial elicitors (salicylic acid, harpin, Flg 22, and chitosan), and polyamines. Stomatal function is altered as a result of the accumulation of these various factors. The function of the many different signalling components and secondary messengers that are present during stomatal opening or closing has been the subject of extensive research. During the process of stomatal closure, some of the well-documented signalling

components include calcium, cytosolic pH, reactive oxygen species (ROS), nitric oxide (NO), and reactive oxygen species (ROS). These signalling components, such as ROS and NO, as well as cytosolic pH and free Ca^{2+}, have quite complicated interrelationships and interactions with one another, which call for more in-depth research (Agurla et al., 2018).

Chlorophyll fluorescence

Fv/fm represents the maximum quantum yield of photosystem II, which is related to the quantum yield of photosynthesis. It is usually used as an indicator of photoinhibition or other injuries affecting photosystem II. The values of 0.78-0.84 indicate the plants under non-stressed conditions, below this range plants are classified as under stress (Maxwell and Johnson, 2000). The results showed that Fv/Fm values of (WS) and (HT+WS) treatments decreased the most, which indicated that the PSII reaction center of rice leaves was damaged by drought stress resulting in the potential energy conversion efficiency being weakened due to photoinhibition (Figure 4).

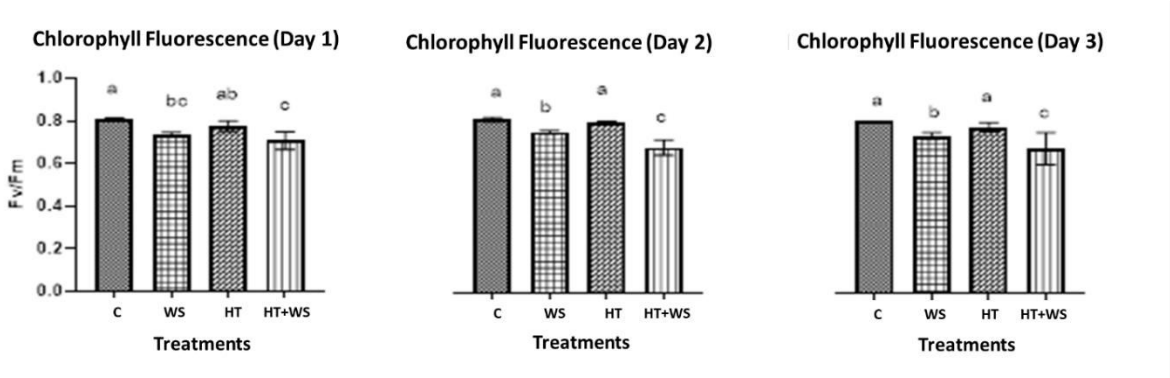

Figure 4: Chlorophyll fluorescence during flowering stage of all treatments. Means followed by the same letter are not significantly different by DMRT at $P \leq 0.05$.

Chlorophyll 'a' and 'b'

The decrease in the rate of photosynthesis is also caused by the damage of chloroplasts which are the main organelles of the photosynthesis process. The chlorophyll content in the leaves subjected to the WS and HT+WS treatments was very low compared to those of C and HT. This proves that poor water conditions contributed a great impact on plant growth physiologically. Chloroplasts deteriorate and affect the photosynthesis process (Figure 5).

Water stress and high temperature cause the ability of mesophyll cells to use available CO^2 in the leaf to decrease. As a result, the amount of chlorophyll continues to decrease (Kadioglu et al., 2012).

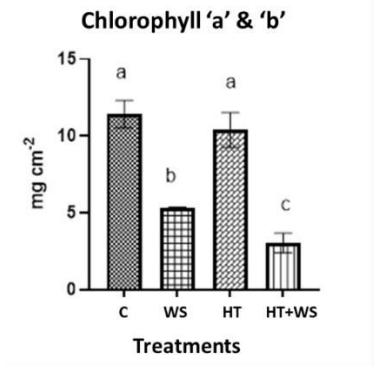

Figure 5: Chloroplast content as affected by high temperature and water stress during flowering stage. Means followed by the same letter are not significantly different by DMRT at $P \leq 0.05$

Yield

Lack of water causes a decrease in yield more than high temperature. Nevertheless, the combination of water stress and high temperature caused a worse decrease in yield even though it did not show a significant difference with water stress alone (Table 1). Physiologically, the chlorophyll content in leaves reacts with the photosynthetic activity and potential yield to be harvested. Chlorophyll synthesis will be inhibited if the plant is under water stress (Yıldırım et al., 2016).

Table 1: Harvested yield on 105 days after sowing as affected by different treatments.

Treatment	Yield (tonne/ha)
Control	5.5[a]
Water Stress	1.5[c]
High Temperature	3.0[b]
Water Stress + High Temperature	0.8[c]

**Means followed by the same letter are not significantly different by DMRT at P ≤ 0.05.*

According to Moonmoon and his colleague (2017), since the treatment is applied at the flowering stage, it is largely caused panicles to be less fertile and the percentage of filled grains to decrease. Water stress in rice plants at the flowering stage can disrupt floret initiation causing spikelet sterility and poor grain filling resulting in lower grain weight and ultimately reducing rice yield.

Conclusion

Water stress that causes drought affected physiological activity on leaves and reduced yield. However, high temperatures during flowering also cause a decrease in yield due to pollen injury. Combination between water stress and high temperature was the worst scenario that affected yield if occur during flowering stage.

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Spermine Foliar Application on Physiological and Yield Responses of Rice under Water Stress using UAV-based Multispectral and Vegetative Indices

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Introduction

Rice paddies production in Malaysia, as in other parts of the world, is extremely exposed to weather changes and extreme conditions such as drought. Several measures are available to mitigate the impact of drought on rice which includes improved irrigation facilities in all granaries, breeding programme and simple agronomic manipulation. Undoubtedly, managing rice under limited water stress is highly desired to ensure crop survival while enhancing yield in general for the given situation. Foliar application of growth enhancer such as spermine that can be economical, feasible, easy to apply and readily available to the farmers could be a possible solution for rice growing in water stress environment (Farooq et al., 2009; Zulkarami et al., 2021).

Spermine, also a growth enhancer, improved plants subjected to water deficit by increased dry matter yield and net photosynthesis that was associated with the maintenance of leaf water status, membrane properties and improved water use efficiency in rice (Farooq et al., 2009; Jonas et al., 2012; Zulkarami et al., 2021). It was also involved in different cellular processes ranging from growth promotion and cell division to inhibition of ethylene production and senescence (Jonas et al., 2012).

Integrating advanced technology like Unmanned aerial vehicle (UAV) in agriculture sector to increase rice productivity without damaging the environment is the best practice to implement. UAVs are appropriate for assessing rapid changes in crop phenology, stress assessment and crop heath in near real-time. By using remote sensing and Geographical Information System (GIS), farmers can monitor the rice field in an effective way and the productivity will increase by the good monitoring of the field. Sensors such as multispectral camera, RGB camera, hyperspectral camera and thermal sensor are very useful for crops monitoring (Ya et al., 2019). Among the remote sensing techniques, we can highlight the vegetation indices (VI's), which are mathematical models for different wavelengths. They are characterized as reliable algorithms for the evaluation of vegetation cover, vigor and growth dynamics, nutritional status, among other applications (Da Silva et al., 2020). The multispectral and vegetative indices approach is the best and reliable approach to monitor rice plant in large area.

Thus, this study was undertaken to elucidate the response of spermine on physiological feedback in relation to the aerial imagery and yield of rice under water stress condition.

Materials and Methods

Experimental site and soil properties

An experiment was conducted under the rain shelter facilities at Field 16, Faculty of Agriculture, Universiti Putra Malaysia. The soil series having a clay loam texture (29% sand, 21% silt and 50% clay) with pH 6.1 and 1.9% organic carbon was used as cultivation medium. The soil was composed of 0.81% total N, 24 mg kg^{-1} available P and 15 mg kg^{-1} available K. The soil was obtained from rice growing area Kemubu Agricultural Development Authority (KADA) Kelantan, Malaysia (East Coast of Peninsular Malaysia). The second largest granary area is KADA, Kelantan which is the driest place of rice planting in Malaysia especially during off season.

Planting materials and plant establishment

The seeds of MR 219 rice variety were obtained from Seri Merbok Sdn. Bhd., Kangkong, Alor Setar, Kedah, Malaysia. The seeds were soaked in water containing seed priming product from ZAPPA-PLUS (PeladangTech, Bangi, Selangor, Malaysia) and spread on wet tissue in a flat tray overnight. After two days of soaking, three seeds were sowed in each pot (390 width × 390 diameter × 350 mm height) containing approximately 17 kg of soil by direct seeding technique. Compound fertilizer that contained N:P:K in equal ratio was applied at 140 kg ha^{-1} (1.5 g pot^{-1}) at 15 days after sowing (DAS) while urea was applied at 80 kg ha^{-1} (0.4 g pot^{-1}) at 35 DAS. In addition, at 50 and 70 DAS, N:P:K blue (12:12:17:2TE) was applied at 100 kg ha^{-1} (0.7 g pot^{-1}) (MADA, 2015). Plant protection measures were necessary to avoid yield loss due to weeds, pests and diseases. In this experiment, visual inspection was carried out regularly while weeding was done manually by hand. The rain shelter wire mesh with the addition of hard wire mesh surrounding the rice plant served as barrier against the entry of rodents and birds. The water level was maintained at 5 cm to reduce weed infestation.

Experimental design and statistical analysis

The plants were arranged in a Randomized Complete Block Design (RCBD) with three replications. In this experiment, treatments comprised two sources of water availability; well-watered (WW) and 10 days' cyclic water stress (WS) and two sprays; distilled water as foliar control and foliar spermine (modified from Farooq et al., 2009). The water-stress treatment was started on the 30^{th} DAS for seven cycles (lasted 10 days in each where re-watering was done on the first day of each cycle) (Nurul Amalina and Mohd Razi, 2015). Well water was maintained at 5 cm by irrigation throughout the rice cultivation periods. The Statistical Analysis System (SAS 9.2) using a two-way ANOVA and Least Significant Different (LSD) at $P \leq 0.05$ was performed.

Leaf gas exchange and stomatal conductance

The photosynthetic rate was measured on fully expanded young leaves (the third leaf from the top) at 0900-1100 am (limited time when stomata opening for photosynthesis occur due to C3 plant) on a day with clear sky using a portable photosynthesis system (Li-6400XT, LI-COR, Lincoln, Nebraska, USA). The measurements were taken on the abaxial surface at a CO_2 reference rate of 400 μmol m^{-2} s^{-1} at 45, 55 and 90 DAS. The photosynthetic photon flux density (PPFD) was 1000 mmol m^{-2} s^{-1}. The stomatal conductance was derived from the same photosynthesis measurements described earlier.

Rice crop monitoring using a multirotor unmanned aerial vehicle (UAV) with red, blue and green (RGB) and multispectral digital camera

For RGB imaging, a multirotor UAV DJI Phantom 4 Pro V2.0 with a gimbal-stabilised 4K60 and 20 megapixel RGB digital camera attached was used to fly at 20 m (Ground Sample Distance (GSD) = 3.47 cm/pixel) above the experimental field area. The flight plan was designed before the data

acquisition by using DroneDeploy software on a tablet. For multispectral imaging, a multirotor UAV DJI Inspire 2 with MicaSense RedEdge-M multispectral sensor attached was used. The flight plan was designed using DJI Pilot apps on smartphone. Agisoft Metashape Professional software was used to performs photogrammetric processing of digital images, align the imagery mosaic and generates orthophoto and 3D spatial data to be used in GIS applications. All images data were imported to ArcGIS 10.2 software for visualization and analysis. The methodology used in this study is shown (Figure 1).

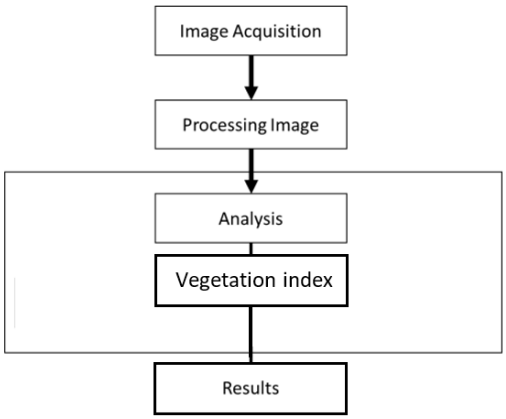

Figure 1: The methodology used for RGB and multispectral analysis in this study.

Yield components

At 115 DAS, all plants were harvested. The grain weight and yield components were determined after drying (72 h, 60 °C). The grain yield was based on the weight of filled grains per pot and expressed in grain per pot (g pot^{-1}). The grain yield was determined using digital balance (QC 35EDE-S Sartorius, Germany). Panicle per hill, grain number per panicle and percentage of filled grain per panicle were counted and calculated manually. The thousand grains weight (g) was also obtained using the same balance. Ten panicles bearing tillers from each treatment were sampled. Prior to weighing the grains, fully filled grains were separated from the unfilled grains manually. The percentage of filled grains per panicle was derived from the ratio of the number of fully ripened grains (filled grains) to the total number of grains per panicle per average hill. Thus, the total percentage of filled grains was calculated using the following formula: Percentage of filled grain per panicle = Number of filled grains (Filled + Unfilled grains) × 100% (Yoshida, 1981).

Results and Discussion

The interaction of types of foliar spray and water regimes were not significantly difference on photosynthetic rate and stomatal conductance of rice plants (Table 1). Interestingly, spermine foliar treatments enhanced photosynthesis rate by 19, 26 and 21% at 45, 55 and 90 DAS, respectively, compared to control. Similar results in water regimes where water stressed treatments improved by 14, 22 and 13% as compared to well-watered treatments. Spermine foliar sprays significantly improved stomatal conductance at 45 (374 mmol H_2O m^{-2} s^{-1}) and 55 (374 mmol H_2O m^{-2} s^{-1}) DAS compared with control. In water regimes treatments, water stressed treatments were significantly higher by 24% at 45 DAS but relatively lower at 90 DAS as compared to those in well-watered treatments. The results indicate that foliar spermine application is one of the short-term strategies to mitigate water stress. This strategy was the economical, easy to apply, and quicker way to alleviate water stressed in the plants (Zulkarami et al., 2021). Interestingly, spermine foliar treatments improved tremendously in photosynthesis rate at all days after sowing. It also enhanced maximum photosynthesis rate during reproductive stages for maximizes yield production as well even during water stressed occurred. These results similarly with Farooq et al. (2009) and Zulkarami et al. (2019) discovered that the exogenous application of spermine improved the drought tolerance of rice plants, as measured by photosynthetic

capacity under water stress (Zhang et al., 2008; Farooq et al., 2009) which corroborated with results obtained in the current study.

Table 1: Photosynthesis rate and stomatal conductance of rice plants at 45, 55 and 90 DAS in different foliar sprays and water regimes.

	Photosynthesis rate (μmol CO_2 m^{-2} s^{-1})			Stomatal conductance (mmol H_2O m^{-2} s^{-1})		
Days after sowing (DAS)	45	55	90	45	55	90
Foliar sprays						
Control	11.0^{b}	10.6^{b}	18.0^{b}	292^{b}	341^{b}	506^{a}
Spermine	13.1^{a}	13.4^{a}	21.7^{a}	374^{a}	374^{a}	524^{a}
Water regimes						
Well-watered	11.2^{b}	10.8^{b}	18.6^{b}	297^{b}	311^{a}	628^{a}
Water stressed	12.8^{a}	13.2^{a}	21.1^{a}	369^{a}	305^{a}	402^{b}
LSD $_{(P=0.05)}$	1.32	1.53	1.65	0.05	0.07	0.18
CV	7.79	9.05	8.44	10.83	16.78	11.20

Mean values followed by the same letters within a column are not significantly different at P ≤ 0.05 by the LSD test. CV: Coefficient of variation.

Crop growth map using Unmanned Aerial Vehicle (UAV) and RGB digital camera

Figure 2 and 3 showed RGB and multispectral image from Unmanned Aerial Vehicle (UAV) at 55 and 65 DAS with Normalized Difference Vegetation Index (NDVI) values. The observation was based on 10 days after applying spermine foliar for two times. Interestingly, with Unmanned Aerial Vehicle (UAV) monitoring using multispectral sensor, there are higher in Normalized Difference Vegetation Index (NDVI) values between treatments, especially with foliar spermine applications. Spermine foliar treatments enhanced value of NDVI in water stressed by 1.76% compared to control at 55 DAS. Interestingly, NDVI values were improved from spermine foliar applications in both reproductive stages on both water regimes. These results indicated that multispectral and RGB cameras have been indicated to be appropriate tools for growth and yield monitoring or prediction (Zhang et al., 2021; Elshika et al., 2022). Similar from previous findings on spermine foliar applications enhanced photosynthetic rate.

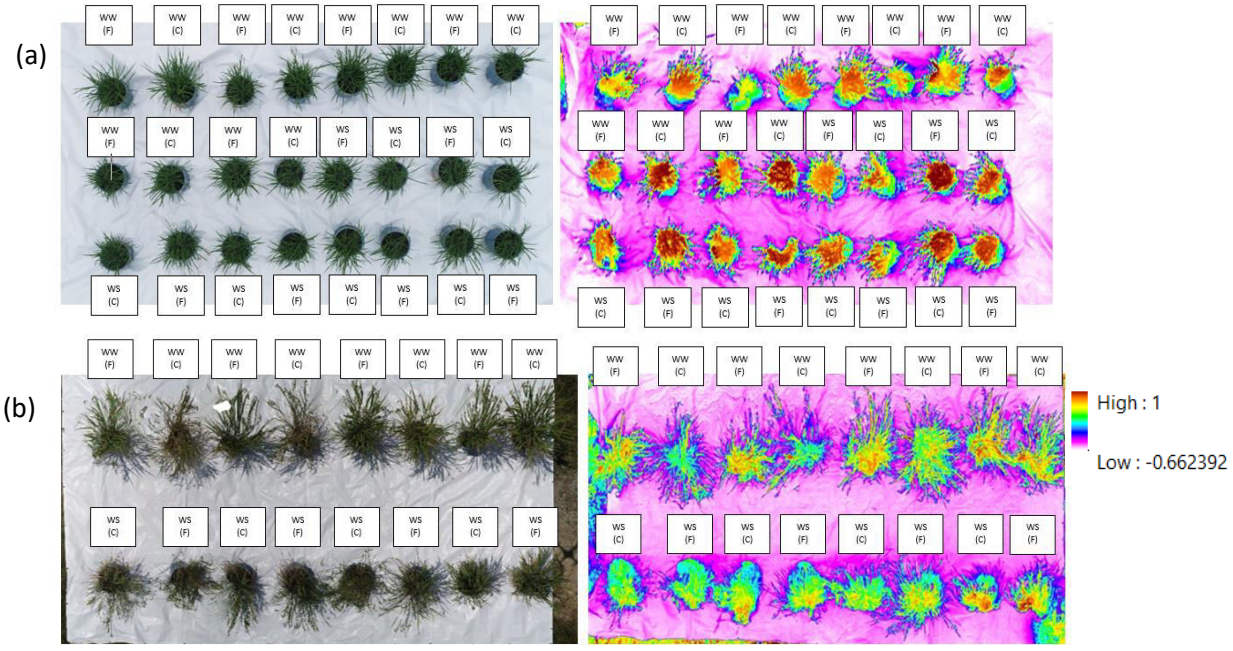

Figure 2: RGB and multispectral image from drone at (a) 45 DAS and (b) 55 DAS.

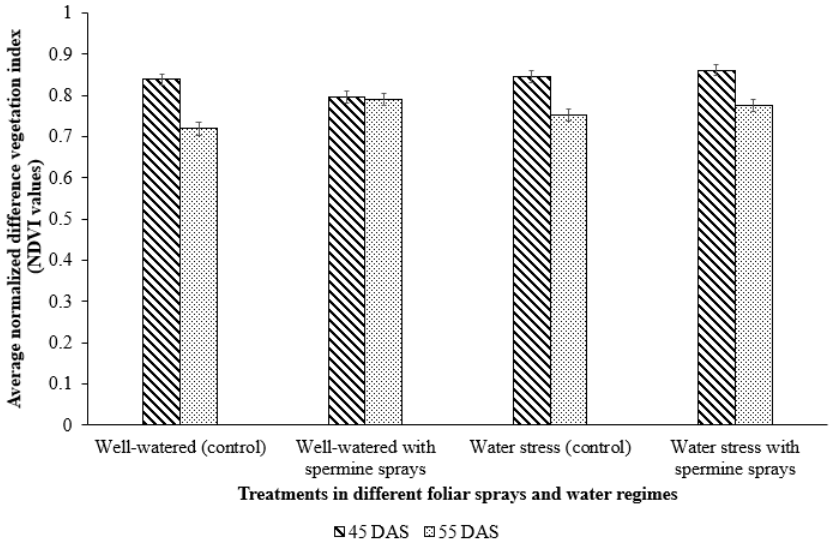

Figure 3: Average normalized difference vegetation index (NDVI) values in different treatments at 45 and 55 DAS in different foliar sprays and water regimes. DAS: Days after sowing.

Yield attributes

At 120 DAS, all plants were harvested and the grain weight of spermine treated plant (55.3 g per pot) was remarkably higher than control (48.6 g per pot) (Table 2). There was also significant increase in the number of grains per panicle (17%) and grain filling percentage (11%) under SPM treatment. Under water regimes, well-watered treatments showed highest in all yield attributes compared to water-stressed treatments. It showed that number of grains per panicle, percentage of filled grains and grain weight per pot were all significantly reduced in water stress treatment by 9, 9, 35, 10 and 49%. Remarkably, grain weight and grain filling were the most affected during water stress. It is obvious that the application of spermine sprays on rice plants leads to improved yield components that are similar to results reported by Lemoine et al. (2013) and Sekhar et al. (2015).

Table 2: Yield components of rice plants at 120 DAS in different foliar sprays and water regimes.

Treatment	Panicle length (cm)	Number of grains (per panicle)	Filled grains (%)	1,000-grain weight (g)	Grain weight (g/pot)
Treatments					
Control	22.8[a]	138[b]	63.4[b]	22.0[a]	48.6[b]
Foliar spray	24.1[a]	161[a]	74.8[a]	20.3[b]	55.3[a]
Water regimes					
Well-watered	24.5[a]	212[a]	79.5[a]	22.4[a]	62.1[a]
Water stressed	22.4[b]	194[b]	58.7[b]	20.3[a]	41.7[b]
LSD $_{(P = 0.05)}$	2.08	4.12	10.91	3.64	3.58
CV	3.18	2.45	3.18	3.18	3.18

Mean values followed by the same letters within a column are not significantly different at $P \leq 0.05$ by the LSD test. CV: Coefficient of variation. DAS: Days after sowing.

Conclusions

As conclusion, spermine foliar application significantly increased growth performance and rice yield, and monitoring of rice using UAV multispectral sensor and NDVI values was capable for prediction of rice yield.

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Physiological Changes of Irrigated and Rain-fed Grain Corn as Affected by Different Planting Seasons

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Introduction

Maize (*Zea mays* L.) is a multipurpose crop with wide adaptability to different agro-climatic conditions. Maize is categorized as industrial crop, as 70-80% of its production is used as feed while only 12-13% is used for human consumption globally. In Malaysia, maize produced for human consumption is known as sweet corn whereas maize for animal feed is known as grain corn. In order to achieve food security, Malaysia is progressing towards local production of grain corn. In many countries, maize is grown in areas that receive 300-500 mm of precipitation, which is near or below the critical level for obtaining a good yield (Hanjra and Qureshi, 2010).

Even though Malaysia has the suitable climatic condition for growing grain corn, production of grain corn during the dry seasons is highly dependent on irrigation system. During the dry seasons, daily rainfall is expected to be less than 10 mm continuously for a period of time (Chenoli et al., 2018) leading to drought stress on rain-fed grain corn crops.

Drought is one of the major causes for crop loss worldwide, reducing average yields by 50% and more (Wang, 2003). Responses to drought are multiple and interconnected. It is well established that drought stress impairs numerous metabolic and physiological processes in plants (Levitt, 1980). It leads to growth reduction, reduction in the content of chlorophyll pigments and water, and changes in fluorescence parameters. In addition to that, many plants cope with drought stresses by synthesizing and accumulating some substances such as proline. The reaction of the plants to drought differs significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and the stage of development.

Farmers who solely depend on rainfall to irrigate their crops have to be equipped with information on the critical dates or suitable planting seasons in producing rain-fed grain corn. Under rain-fed condition, decision support tools of critical planting dates derived from agro-climatic information can significantly contribute to optimum growth and subsequently higher yield. Understanding the physiological changes of grain corn plants grown during different planting seasons and rainfall variabilities will maximize yield in growing rain-fed grain corn.

The present study was aimed at determining the best planting seasons for producing rain-fed grain corn in North Peninsular Malaysia by exploring the physiological and biochemical responses of the plants when subjected to different rainfall availability.

Materials and Methods

Study area and plant materials

The field experiment was conducted at MARDI Seberang Perai, Pulau Pinang. The geographic position for climate station is: 5.54066, 100.47117. The soil of the experimental site was the alluvial soil. Seeds of hybrid grain corn Dupont P4546 were obtained from a local seed supplier and were kept in temporary storage at 5 °C prior to planting.

Land preparation and agronomic practices

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 at a rate of 3 tonnes ha^{-1} was applied at 7 days before planting. The seeds were sown at a depth of 3-5 cm with spacings of 75 x 20 cm. Chemical fertilizer 15:15:15 (N: P: K) was applied at 14 days after sowing (DAS) while UREA (46% N) was applied at 30 DAS, at rates of 400 and 130 kg ha^{-1}, respectively.

Irrigation treatments and growing seasons

Irrigated plots were irrigated twice daily using 7-feet sprinkler system whereas rain-fed plots depended solely on rainfall availability. Both plots were grown for three seasons from April 2020 to April 2021 (Table 1).

Table 1: Duration, average monthly rainfall and average temperature recorded for different planting seasons.

Season	Duration	Average monthly rainfall (mm)	Average temperature (°C)
S1	April - July 2020	321.75	Max 33.1, Min 24.3
S2	August - November 2020	263.2	Max 32.4, Min 24.0
S3	January - April 2021	125.25	Max 34.1, Min 24.4

Relative water content (RWC)

At 45 days of sowing, samples from the top fully expanded leaves were taken for RWC determination. Fresh weight (FW) of five leaf discs were measured before floated on deionized water for 4 hours. The wet surface of the turgid leaf discs were blot dried quickly before weighing (TW). The leaf discs were then dried for 72 hours at 70 °C in oven and dry weight (DW) was then measured. The RWC was calculated and expressed in percentage based on the formula below.

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

Photosynthetic pigment extractions

At 45 days of sowing, leaf discs were taken from the leaf samples by using a single hole paper punch (6 mm diameter). Leaf discs were placed in 20 mL screw cap amber glass. Pigments were extracted from the leaf discs with 10 mL dimethyl sulfoxide (DMSO) followed by incubation at 65 °C for 4 hours (Hiscox and Israelstam, 1979). A 3.0 mL chlorophyll extract was transferred to a cuvette, and absorbance readings were taken at 649, 665, 480 and 510 nm using K-LAB Single-Beam UV-Vis Spectrophotometer Model OPTIZEN-POP (Korea). Chlorophyll and total carotenoid content were calculated based on the following equations (Hendry and Price, 1993; Wellburn, 1994).

$$Chlorophyll\ a\ (\text{nano mol}/cm^2) = \frac{\left(12.47 \times (E665) - 3.62 \times (E649)\right) \times \text{ DMSO volume, ml } \times 1.119}{\text{total area of leaf disks}, cm^2}$$

$$Chlorophyll\ b\ (\text{nano mol}/cm^2) = \frac{\left(25.06 \times (E649) - 6.50 \times (E665)\right) \times \text{ DMSO volume, ml } \times 1.102}{\text{total area of leaf disks, } cm^2}$$

$$Caratenoid\ (\text{nano mol}/cm^2) = \frac{\left(7.60 \times (E480) - 1.49 \times (E510)\right) \times \text{ DMSO volume, ml } \times 1.102}{\text{total area of leaf disks, } cm^2}$$

$$Total\ chlorophyll\ (\text{nano mol}/cm^2) = Chlorophyll\ a\ +\ b$$

Proline assay

At 45 days of sowing, free proline was extracted from the leaf tissues according to the method described by Bates et al. (1973). The samples were frozen in liquid nitrogen prior to analysis. The samples were weighed for 0.5 g and homogenized in 10 mL of 3% aqueous sulfosalicylic acid. The mixture was then filtered through filter paper. Two mL of the filtrate was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid in a test tube. The reaction mixture was extracted with 4 mL toluene and the chromophore containing toluene was aspirated. The absorbance was quantified spectrophotometrically at 520 nm and proline concentration was estimated using the standard curve.

Experimental design and statistical analysis

The treatments comprising three planting seasons and two irrigation systems were arranged in a split-plot with three replicates, each with 5 plants. 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 Tukey's Honest Significant Difference (HSD) at $P \leq 0.05\%$.

Results and Discussion

Effects of different water availability and planting seasons on chlorophyll content

Chlorophyll content of leaf is indicator of photosynthetic capability of plant tissues (Nageswara Rao et al., 2001). Reduction or no-change in chlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress severity and duration (Jagtap et al., 1998). In this study, irrigation significantly affected all extracted pigment contents ($P < 0.05$) while seasons significantly affected all parameters taken except carotenoids ($P < 0.01$). Significant interactions between the main effects were recorded only for chlorophyll b ($P < 0.05$) (Table 2).

Table 2: Main and interaction effects of two irrigation systems and three planting seasons on extracted pigment contents.

	Chlorophyll a (nmol/cm^2)	Chlorophyll b (nmol/cm^2)	Total Chlorophyll (nmol/cm^2)	Carotenoids (nmol/cm^2)
IRRIGATION, I				
Rain-fed, P1	39.54^b	9.21^b	48.75^b	16.32^b
Irrigated, P2	46.64^a	11.44^a	58.08^a	18.69^a
SEASON, S				
S1	39.50^b	10.86^a	50.36^b	16.87^a
S2	52.30^a	11.18^a	63.48^a	18.94^a
S3	37.48^b	8.93^b	46.41^b	16.70^a
IRRIGATION, I	*	**	*	*
SEASON, S	**	**	**	ns
I X S	ns	*	ns	ns

*** Significant at 1 % probability level, * Significant at 5% probability level, ns: Not significant. Means in each column with the different letters within each factor indicate significant differences at $P \leq 0.05$ according to Tukey's HSD (Mean ± SE, n=3).*

As compared to irrigated plants, rain-fed plants had significantly lower extracted pigment contents, regardless the growth seasons. Extracted pigments; chlorophyll a, b, total chlorophylls and carotenoids were 15%, 19.5%, 16.1% and 12.7% lower, respectively, when compared to irrigated plants. Regardless of irrigation system, plants grown during season 3 recorded significantly lower chlorophyll a content as compared to season 2. However, no significant difference in chlorophyll a was recorded between season 3 and season 1. The same pattern was also observed in total chlorophyll.

Significant interaction between the main effects for chlorophyll b indicated that reduction of chlorophyll b was highly dependent on the growth season. When plants were grown during season 1 and 2, no significant difference of chlorophyll b was recorded between irrigated and rain-fed plants. However, when plants were grown during season 3, the chlorophyll b of rain-fed plants was significantly reduced by 36.75% as compared to irrigated plants (Figure 1). Water deficit can destroy the chlorophyll and prevent its biosynthesis (Lessani and Mojtahedi, 2002). Also, some researchers have reported damages to leaf pigments as a result of water deficit (Montagu and Woo, 1999). A reason for decrease in chlorophyll content as affected by water deficit is that drought or heat stress producing reactive oxygen species (ROS) such as O_2^- and H_2O_2 can lead to lipid peroxidation and consequently, chlorophyll destruction (Mirnoff, 1993). Also, with decreasing chlorophyll content due to the changing green color to yellow in the leaves, the reflectance of the incident radiation is increased (Schlemmer et al., 2005). It seems that this mechanism can protect photosynthetic system against stress. According to Lawlor and Cornic (2002), reduction of carbon assimilation confronting water deficit is due to limitation of Rubisco synthesis and ATP storage. Studies done in vivo showed that water deficit resulted in destruction of D1 protein of photosystem 2 (Xian-He et al., 1995) but the reasons have yet to be known.

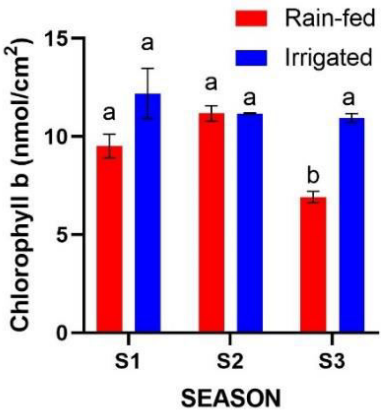

Figure 1: Effects of different irrigation sources on chlorophyll b content of grain corn leaves planted at different seasons. Means in each season with different letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD.

Effects of different water availability and planting seasons on osmoregulation

Season is the only main effect that significantly affected ($P < 0.01$) proline and RWC. Significant interactions between the main effects were recorded for both proline and RWC ($P < 0.05$) (Table 3).

Leaf RWC is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate (Lugojan and Ciulca, 2012). Generally, osmoregulation is one of the main mechanisms preserving turgor pressure in most plant species against water loss from tissues therefore causes the plant to continue with water absorption and retain metabolic activities. Results of significant interactions showed that reduction of RWC was highly dependent on the growth season. When plants were grown during season 1 and 2, no significant difference in RWC was recorded between irrigated and rain-fed plants. However, when plants were grown during season 3, the RWC of rain-fed plants was significantly reduced by 21.59% as compared to irrigated plants

(Figure 2). Reduction in leaf RWC indicated that the plants grown during season 3 were under water stress conditions and the plants lost their turgor.

Table 3: Effects of two irrigation systems and three planting seasons on proline content and relative water content (RWC).

	PROLINE (µgram proline/g)	RWC (%)
IRRIGATION, I		
Rain-fed, P1	107.61[a]	79.32[a]
Irrigated, P2	101.97[a]	84.71[a]
SEASON, S		
S1	85.78[b]	90.38[a]
S2	86.39[b]	81.62[b]
S3	142.21[a]	74.05[b]
IRRIGATION, I	ns	ns
SEASON, S	**	**
I X S	*	*

*** 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 according to Tukey's HSD (Mean ± SE, n=3).*

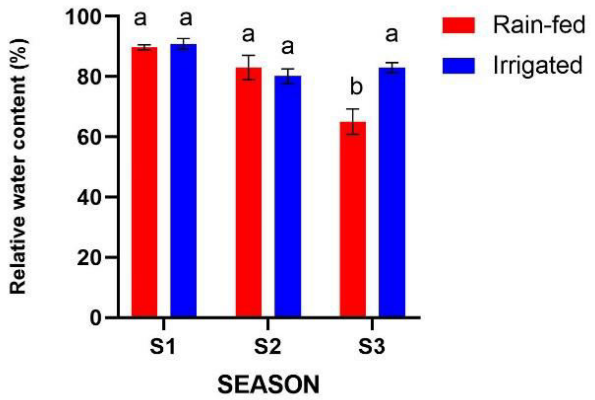

Figure 2: Effects of different irrigation sources on RWC of grain corn leaves planted at different seasons. Means in each season with different letters indicate significant differences at P ≤ 0.05 according to Tukey's HSD.

For proline, even though no significant differences were recorded between rain-fed and irrigated plants in each season, proline content was the highest in season 3 plants, regardless of irrigation treatments as studied. Plants grown during season 3 had 65.79% and 64.6% higher proline content as compared to plants grown during season 1 and 2, respectively (Figure 3).

The synthesis of osmolytes including proline is widely used by plants to stabilize membranes and maintain the conformation of proteins at low leaf water potentials. The synthesis and accumulation of osmolytes vary among plant species as well as among different cultivars of the same species. Proline is also known to be involved in reducing the photo damage in the thylakoid membranes by scavenging and/or reducing the production of O_2^- (Reddy et al., 2004).

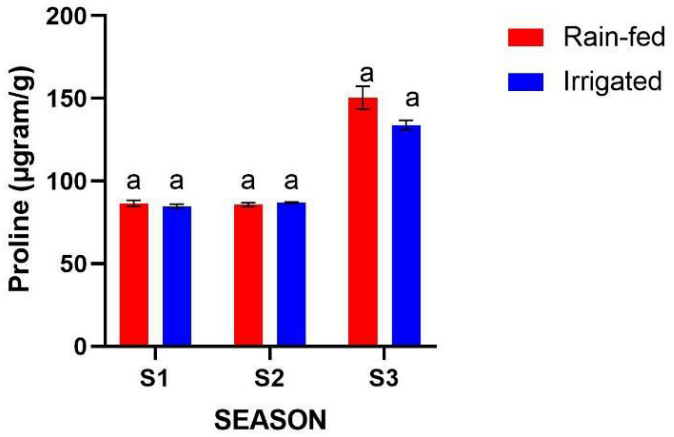

Figure 3: Effects of different irrigation sources on proline content of grain corn leaves planted at different seasons. Means in each season with different letters indicate significant differences at $P \leq 0.05$ according to Tukey's HSD.

Conclusions

In the agro-climatic zone one region, it is not recommended to produce grain corn at season 3 (January-April) without irrigation system due to the unfavorable climatic conditions for the crop's growth and physiological development.

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Growth and Yield of Chilli (*Capsicum annuum* L.) in Response to Irrigation Interval in Superabsorbent Biodegradable Hydrogel Amended Medium

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Introduction

Chilli (*Capsicum annuum* L.), among the top 10 vegetables in 2019, with over 38 million metric tons produced globally (Shahbandeh, 2022), is a high-valued important vegetable widely cultivated for its spice content (Madala et al., 2020). The spice that favours optimal daytime temperatures between 20 to 30 °C for growth can be consumed raw, cooked, or dried, depending on preference (Baenas et al., 2019).

Water stress is the most prevalent abiotic stress in chili production, which becomes the major constraint in chilli production (Michelon et al., 2020). Water scarcity can lead to water stress which might impair photosynthesis, resulting in diminished food supplies crucial to other processes, such as inhibiting the enzymes and hormone activities in the plant (Chehab et al., 2017). Hence, overcoming the issues that arise from water stress requires programs that can conserve water and nutrients to increase efficiency and yield to meet the growing demands.

One of the innovative ways to overcome the water issues in agriculture is by incorporating the materials or substrates into a planting media within the irrigation system to retain water for plant growth. This includes mixing the planting media with a superabsorbent material that minimizes the leaching fraction (Hemvichian et al., 2014) and maintains water and nutrient while increasing water holding capacity (Zhang et al., 2020). The term "hydrogel" is a generic one that is used to designate the three-dimensional (3D) matrices that are utilized in the fields of medicine (Zeng et al., 2023), engineering, wastewater treatment (Das et al., 2020), food processing (Stephen et al., 2021), and agriculture. Researchers' curiosity has been piqued because of the capacity of hydrogel polymers to hold water, which has led to the exploration of the material's possible use in agriculture. Consequently, the current study aimed to determine the effects of fertigation intervals on the growth and yield of chilli germplasm in response to the amended hydrogel as a planting medium. The methods of irrigation or fertigation [utilized interchangeably throughout this article] were devised to conserve water and nutrients to boost efficiency and productivity (Rehman et al., 2022) to satisfy the expanding demand.

Materials and Methods

Plant materials and experimental design

The study was conducted at the Institute of Tropical and Food Security (ITAFoS) Complex, Universiti Putra Malaysia, Serdang, with two planting cycles. The 21-day seedlings were transplanted into polybags in the glasshouse. The experimental trial was laid out based on a randomized complete block design (RCBD) consisting of five irrigation intervals *viz*: (T1) daily with cocopeat as media, (T2) one-

day interval with hydrogel in cocopeat, (T3) two-day interval with hydrogel in cocopeat, (T4) three-days interval with hydrogel in cocopeat, and (T5) six-days interval with hydrogel in cocopeat.

Cultural practices

The fertilizer formulation and management for fertigation and hydrogel-adjusted planting medium were carried out following the guidelines set forth by the Malaysian Agricultural Research and Development Institute (MARDI) (Mohd et al., 2012). Confidor, Solomon, and Decis were insecticides that controlled the most common pests and diseases. At the same time, a fungicide named Kencozeb (Kenso, Australia) was sprayed on the crops twice monthly to maintain a healthy plant.

Data collection

Growth parameters measured were plant height (cm), shoot and root dry weight (g) taken at 120 days after transplanting. The cell membrane stability test (%) was conducted according to the protocol described by Martineau et al. (1979). The yield per plant (g) was taken when the ripe fruits turned red until 120 days after the transplant.

Statistical analysis

The collected data were analysed using R studio statistical software. The least significant difference (LSD) test was used to separate the means for each treatment at a 5% significance level ($p \leq 0.05$).

Results and Discussion

Growth and yield

Results showed that the irrigation intervals significantly influenced the growth and yield per plant in the hydrogel-amended planting medium (Figure 1a). The trial plant that received daily irrigation (T1) and one-day irrigation interval (T2) showed the highest means for height, in agreement with the finding by Galeş et al. (2016) and Azizi (2018). The highest yield per plant was observed in the trial plants that received a one-day irrigation interval (Figure 1b). In contrast, the six-day irrigation interval resulted in the lowest yield per plant with a significant yield reduction of up to 77% compared to the control. These findings align with the previous literature, where increased irrigation intervals in the hydrogel medium significantly increased the yield attributes (Ray et al., 2021). These might be due to the gradual and uniform water availability, which synchronized with the crops' demand while minimizing nutrient leaching, runoff, volatilization, and immobilization (Tan et al., 2021).

Similar shoot and root dry weight patterns demonstrated that one-day irrigation was the best among all treatments (Figure 1c and 1d). The nitrogen (N) uptake, vegetative growth, and biomass production of plants exposed to water stress following long-interval irrigation were considerably reduced, as demonstrated by the decreased root growth, biomass, number, and apical structures (Mahdavi et al., 2020). The results in this study were also in line with the findings obtained by Orikiriza et al. (2013) and Azizi (2018), who explained that exposure to longer fertigation intervals would diminish the root dry matter partitioning in plants.

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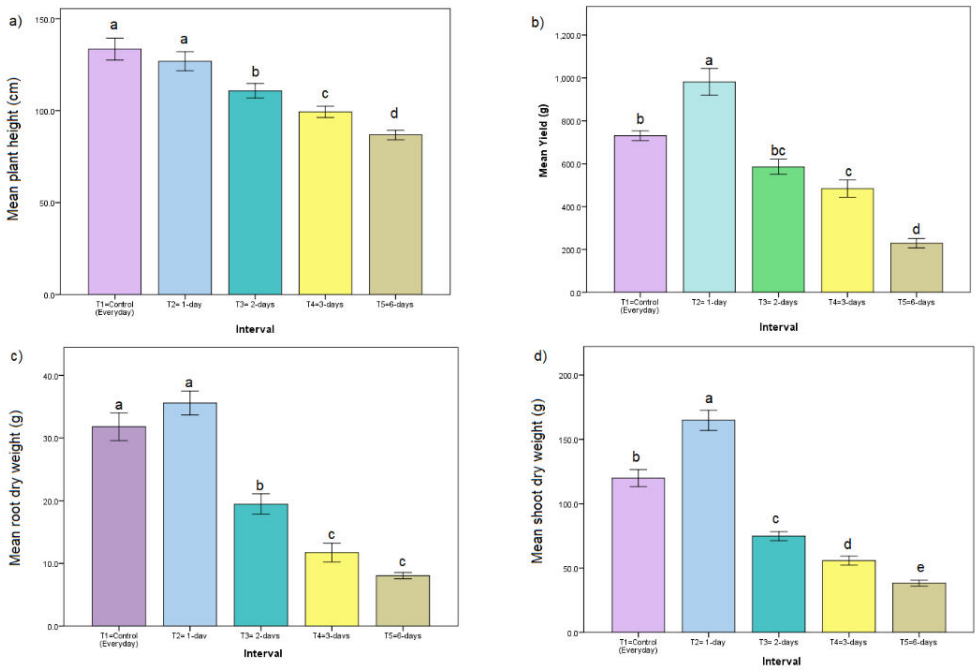

Figure 1: The growth and yield in response to irrigation interval. a) Mean of plant height (cm), b) Mean of yield per plant (g), c) Mean of root dry weight (g), d) Mean of shoot dry weight (g); means with a similar letter are not significantly different by LSD ($p \geq 0.05$).

Cell membrane stability

The more frequent irrigation the plant received (T1, T2, and T3), the higher the cell membrane stability was observed (Figure 2). On the other hand, the plants exposed to a longer irrigation gap revealed a reduction in the cell membrane stability, as shown by T4 and T5. Plants with higher cell membrane stability could perform better in drought environments (Sairam et al., 2002). However, all plants in this study did not show any wilting symptoms. This might be due to the roles of potassium ions (K^+) in the osmotic adjustment of the plants (Hosseinzadeh et al., 2018), thus, enabling the plants to survive prolonged water scarcity (Miranda et al., 2021).

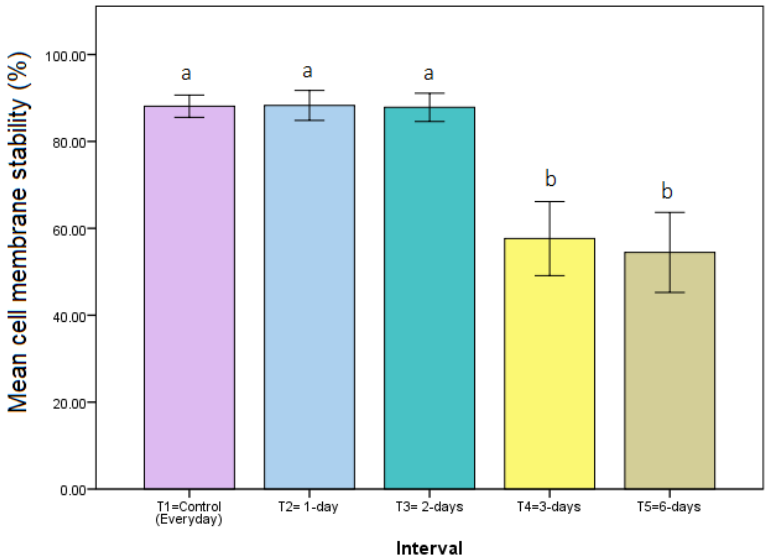

Figure 2: Cell membrane stability in response to the irrigation interval; means with a similar letter are not significantly different by LSD ($P \geq 0.05$).

Conclusions

The application of an amended hydrogel planting medium with one-day irrigation improved growth and yield attributes for the chilli plant. The plant did not show any wilting symptoms throughout the study. Due to the excellent surface area, good biocompatibility, and the ability to attract more moisture, employing hydrogel in the planting medium can help the grower save water and labour while increasing the yield. However, further studies should assess the interaction between hydrogel treatment and the different genotypes. The optimization of the hydrogel application can be carried out to determine the best and economic effect of the hydrogel usage in return.

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Effects of Heat on the Physico-chemical Properties of Black Pepper

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Introduction

Pepper (*Piper nigrum*) is a well-known culinary spice besides being one of the most important spices traded internationally. Pepper contributes significantly to the agri-commodity sector as well as to the economy of Malaysia. Sarawak is the main pepper-producing state accounting for 98% of the country's annual production in 2020. In Malaysia, pepper is predominantly produced by small holders and the final cleaning, grading and packing of the dried pepper is carried out by exporters and government agency – Malaysian Pepper Board.

Sarawak Pepper is the hallmark of quality and has been chosen and commercially used to promote Malaysian peppers, especially those produced in Sarawak (Chennakrishnan, 2012). Sarawak Pepper is now packed for three major user segments comprising the household, food service, and industrial food-processing sectors with full-range packaging developed to accommodate all the major end users (Goh, 2012). This brand name has been registered by the Malaysian Intellectual Property Organization under 'geographical indication' category. It is the first agricultural product from Malaysia that has gained this acknowledgment. Moreover, Sarawak Pepper has received an international recognition from chefs and gourmets as one of their favourite ingredients because of its distinctive flavour and taste. This product development effort is also complemented with state-of-the art pepper cleaning and steam sterilization plants to meet the global quality requirements with laboratory analysis support.

Most processing of Sarawak Pepper at farm level is relatively simple in traditional way. Processing of black peppers varies among farmers. Farmers will incorporate trampling, beating with sticks or by hand to remove berries from spikes. Some farmers will utilize motorised thresher, separator to speed up the post-harvest processes. The berries will be spread on the bamboo mats or raised platform under the sunlight to reduce the moisture until 12 or 13 percent. After drying, the pinheads, light berries, extraneous matters such as stones, sticks, sand and et cetera will be removed by winnowing and hand picking. Most of the pepper exporters will send pepper for cleaning and grading in Malaysian Pepper Board prior to export. Grading is normally done according to the overseas buyer requirement. Peppers are usually stored in warehouse prior to shipment.

The quality of pepper is governed by the organoleptic properties (such as aroma, appearance, colour and other physical characteristics) of the pepper. Black pepper is prepared from whole matured pepper berries. The harvesting is carried out when there are a few green berries turn yellow or red at around 6 to 7 months. Special care should be taken to harvest only the matured spikes. Immature fruits on drying tend to shrivel up and will reduce the quality of the produce. Early harvest of pepper berries before full maturity stage ended up with light berry. Light berry has higher percentage of oil and oleoresin. This produce is mainly catered for food and beverage industry. Statistics shows 70% of the pepper is processed into black pepper and the remainder into white pepper and green pepper in Malaysia.

The idea of this study is to discover the physiological changes in black pepper with different heat treatment at post-harvest stage. *Piper nigrum* L. berries with high flavour profile, chemical composition and nutritional properties are highly demanded for the application in downstream industry. The berries are evaluated in terms of nutrient composition, antioxidant activities and intrinsic chemical properties. In this project, the effect of different processing method with heat treatment is evaluated for the sustainability of pepper industry.

Materials and Methods

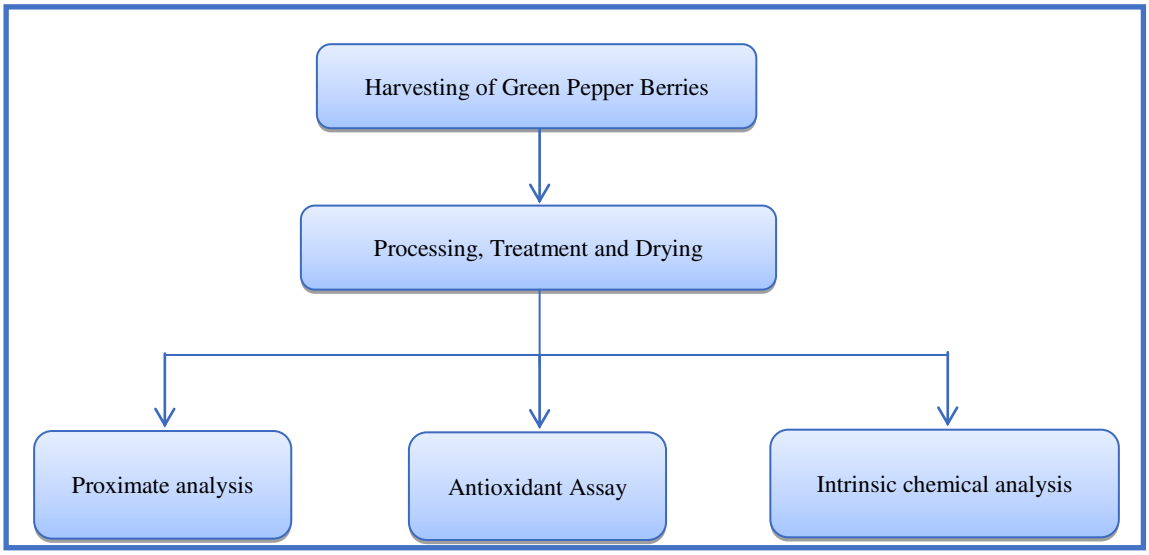

Figure 1: Overview of the project**.**

Sample processing of black and white pepper

Black pepper is prepared from matured green pepper berries. The harvesting is carried out when there is a few green pepper berries turn yellow or red. Green pepper berries are threshed and blanched in the hot water at 80 °C and 100 °C respectively for up to 10 minutes followed by drying under the sun to reduce the moisture to approximately 13 percent (maximum). Green pepper berries content relatively high in moisture and it functions as a raw material to be further processed into black pepper. Its naturally occurred polyphenolic compounds in the pericarps will cause the blackening of green peppers over time.

White pepper is traditionally prepared from the fully ripened reddish berries which are decorticated and dried. Peppers are packed into gunny bag and placed in the slow running water for 10 to 14 days. The bags are removed from the water and trampled to remove any remaining pericarp followed by drying under sun to reduce the moisture to approximately 14 percent (maximum).

Figure 2: Green pepper berries, black pepper and white pepper.

Proximate analysis

Determination of protein, fat, carbohydrate and energy contents of pepper berries are analysed according to the Guide to Nutrition Labelling and Claims (2006) and Springer (2010).

DPPH (2, 2 -diphenyl-1-pycryl-hydrazyl) Free Radical Scavenging Action

According to Mensor et al. (2001), 1 mL from 0.3 mM methanol solution of 2, 2 -diphenyl-1-pycrylhydrazyl (DPPH) is added into 2.5 mL sample or standards. The solution is mixed vigorously and left to stand at room temperature for 30 min in the dark. The mixture is measured spectrophotometrically at 518 nm.

Intrinsic chemical analysis

Moisture, volatile oil, piperine content and non-volatile ether extract (NVEE) are analysed by using Fourier Transform Near Infrared technology in black pepper and white pepper produced from different *Piper nigrum* varieties, namely Semongok Aman and Kuching (ASTA, 1997).

Results and Discussion

The berries are evaluated in terms of nutritional composition, antioxidant activities and intrinsic chemical properties. In this project, the effect of different processing method with heat treatment is evaluated for the sustainability of pepper industry. The nutritional compositions of black pepper and white pepper are evaluated and the results are shown in Table 1.

Table 1: Nutritional compositions of black pepper and white pepper production.

Nutrition	Sample	
	Black Pepper	White Pepper
Energy (kcal/100g)	330	360
Carbohydrate (g/100g)	62.1	71
Protein (g/100g)	12.9	10.8
Fat (g/100g)	3.4	3.6

Results showed that antioxidant activities are significantly affected by the heat. Antioxidant activities are determined with the highest value up to five minutes blanching process at 80 °C (199.64%), followed by boiling at 100 °C (93.14%) in black pepper (Figure 3). Moreover, higher intrinsic chemical compositions such as piperine, volatile oil and NVEE contents are exhibited in white pepper if compared to black pepper (Figure 4(a) and 4(b)). The total ash content in white pepper is lower than black pepper due to the removal of pericarp (outer skin) in white pepper production.

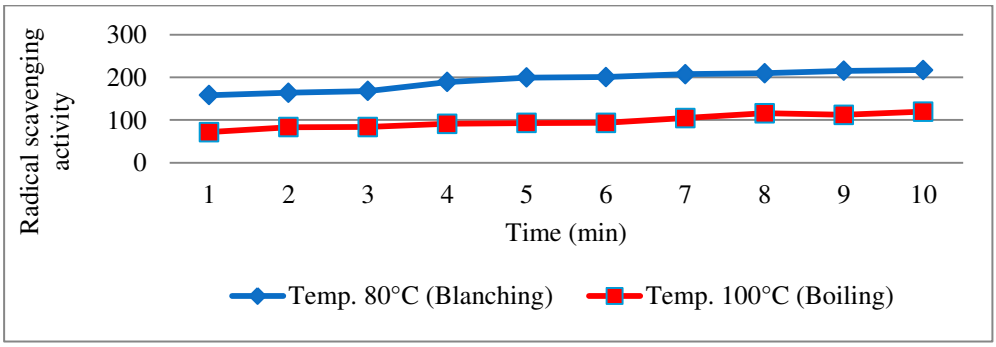

Figure 3: Effect of different temperature and time interaction on DPPH free radical scavenging action of black pepper.

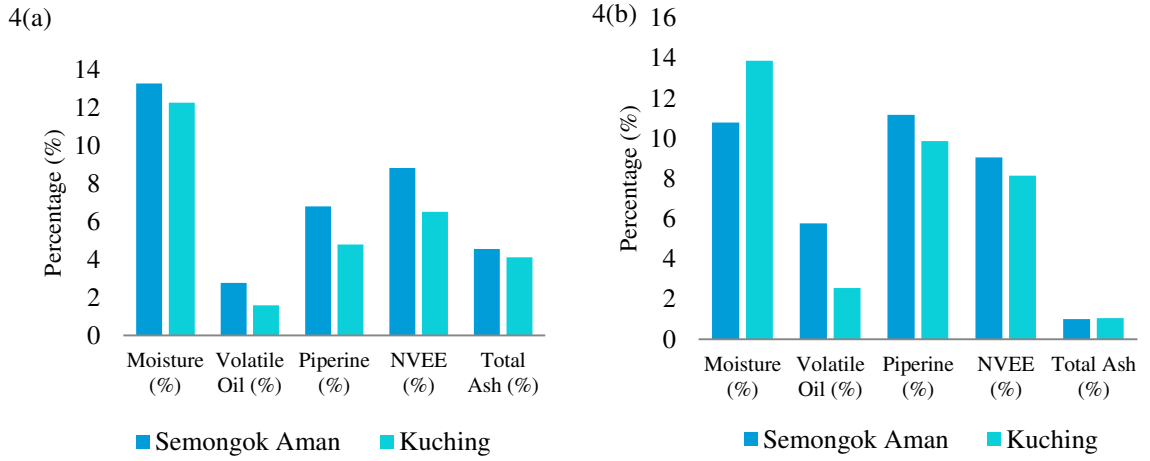

Figure 4 (a-b): Intrinsic chemical analysis by using FT-NIR in different varieties of *Piper nigrum* for black and white pepper production respectively.

Conclusions

In conclusion, blanching method at 80 °C is revealed to retain better colour, aroma, nutritional compositions, antioxidant properties and intrinsic chemical composition of black pepper production. Further study such as loss of chemical compounds and microbiological properties will be pursued for more comprehensive and conclusive findings.

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The Effects of Elevated Temperature on Physiological Performances of Papaya Variety Sekaki During Vegetative Stage under Controlled Environment System

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Introduction

Papaya (*Carica papaya*) belongs in the ten main fruits in Malaysia, with planted area of 2,623.07 ha and an annual production of 61,775.75 Mt in 2020 (DOA, 2020). The optimum temperature range for optimal growth of papaya plant is between 21 °C and 33 °C (Campostrini and Glenn, 2007; Oliveira and Vitória, 2011; Workneh et al., 2012). The exposure to extreme environment causes the morphological, physiological and developmental changes in plants (Jeyakumar et al., 2007). Since papaya is sensitive to the growing environment, any changes in the environmental factors will negatively influence the productivity and quality of fruits. It was reported that at high temperature, there is a tendency for papaya plant to produce male flowers as papaya plant gender expression can be influenced by air temperature (Campostrini and Glenn, 2007). This will eventually affect the fruit yield produced. Besides, the heat stress experienced by the papaya plant due to high temperature will cause the degradation enzymes in the cell wall to be inactivated and leads to a disorder known as "hard lump in pulp" (Oliveira and Vitória, 2011). Therefore, it is important to study the impact of high temperature towards plants as temperature is expected to be continually rising in the future. In this study, the effects of high temperature (38 °C) on physiological performances of papaya plant during the vegetative stage were investigated at weekly intervals for four weeks.

Materials and Methods

Papaya plant variety Sekaki was transplanted at the age of two months in a polybag containing a mixture of soil: peatmoss: perlite: vermiculite at a ratio of 2:1:1:1. The experimental design was a nested design. For the high temperature treatment at 38 °C, four papaya plants, at the age of 60 days after transplanting, were transferred to a phytotron (ThermoStable GC-1000, Daihan Scientific, Korea) (Figure 1). The heat stress treatment was given to the papaya plants at 400 µmol photons/m^2/s from 8:00 to 14:00 (6 h) with a 12-hour day length. Night temperature was set at 25 °C, while relative humidity was maintained at 70%. The control papaya plant was grown under natural conditions at ambient temperature inside a greenhouse. Fertilization, irrigation and pest control were well controlled and done according to standard practice.

The effects of temperature on physiological performances of papaya plant were studied at weekly intervals for four weeks. A portable photosynthesis system (LI6400XT, LICOR Inc. Nebrasca, USA) was used to measure the net photosynthetic rate, transpiration rate and stomatal conductance, while Plant Efficiency Analyzer (PEA) (FMS 2, Hansatech Instruments Ltd, U.K.) was used to measure the Fv/Fm ratio, which determined the leaf chlorophyll fluorescence responses. The data obtained was subjected to statistical analysis using the SAS 9.4 software and the mean between two treatments were compared using Duncan's multiple range test.

Figure 1: The heat treatment was given to the papaya plants inside a phytotron at a temperature of 38 °C daily from 8.00 to 14.00 (6 hours).

Figure 2: Determination of plant physiological performances using plant efficiency analyzer (left) and portable photosynthesis system (right).

Results and Discussion

It can be observed from Table 1 that the effect of heat stress on chlorophyll fluorescence (Fv/Fm ratio) was significantly reduced in week 4 after the high temperature treatment compared to the control plants. This showed that heat stress affected the photosystem II (PSII) as Fv/Fm ratio indicates the maximum quantum efficiency of PSII photochemistry in dark-adapted leaves, where in response to heat stress, it would detect and quantify damage in PSII (Sharmaa et al., 2015). Photosystem II is considered as the most sensitive and heat labile component, which primarily limits photochemistry.

Table 1: Chlorophyll fluorescence (Fv/Fm ratio) and leaf physiological responses as affected by heat treatment.

Weeks after high temperature treatment	Treatment	Chlorophyll fluorescence (Fv/Fm)	Photosynthetic rate (μmol/m^2/s)	Stomatal conductance (mol/m^2/s)	Leaf transpiration rate (mmol/m^2/s)
Week 1	Non-heated (control)	0.75[a]	6.46[a]	0.26[a]	2.91[a]
	Heat-stressed (38 °C)	0.69[a]	2.28[b]	0.05[b]	1.74[b]
Week 4	Non-heated (control)	0.80[a]	18.90[a]	0.21[a]	10.44[a]
	Heat-stressed (38 °C)	0.72[b]	0.22[b]	0.01[b]	0.44[b]

**Means followed by the same letter within column are not significantly different by DMRT at P ≤ 0.05.*

Based on results in Table 1, the net photosynthetic rate, stomatal conductance and leaf transpiration rate of the heat-stressed plants were significantly lower in week 1 and week 4 compared to the control plants. This was consistent with the previous study reported by Jeyakumar et al. (2007) that the leaf net photosynthetic rate of the papaya plants was reduced when the temperature exceeded 35 °C. Stomatal conductance and plant photosynthesis are highly related (Mathur et al., 2014). Rubisco is the enzyme responsible for the carbon dioxide fixation, where heat stress environment decreased the activation state of Rubisco. The rates of the carboxylase and oxygenase activities determine the rate of photosynthesis and photorespiration, respectively, which Rubisco catalyses the first step in those competing pathways. This would further cause the net photosynthetic rate and stomal conductance to be inhibited, thereby leading to structure and membrane composition, causing leakage of ions (Jeyakumar et al., 2007; Mathur et al., 2014).

Conclusions

Elevated temperature affects the physiological performances, directly or indirectly, of papaya plant during vegetative stage. From this study, it was found that high temperature caused heat stress to the papaya plants and significantly reduced the leaf photosynthetic capacity as reflected by the chlorophyll fluorescence (Fv/Fm ratio), the net photosynthetic rate, stomatal conductance and leaf transpiration rate. This could result in morphological and developmental changes in papaya plant, which might lead to reduction of growth rate and yield of papaya.

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Chapter 3

Post-harvest Technology and Quality Control

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Effect of Boron Spray on Banana Characteristics and Glucose Content

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Introduction

Banana cultivation (*Musa acuminata*) in Malaysia has experienced several constraints that have been intensively discussed by local researchers (Mohamad Roff et al., 2012). The main constraints commonly reported were low yield and high production cost. Most of the low yield is attributed to insufficient nutrient application, improper crop management, and lack of exploration of suitable micronutrient concentration, intentionally creating low income and soon making this industry unprofitable (Zewail et al., 2020; Izzah et al., 2022).

Exploration of selected micronutrients positively impacted crop physiology, including metabolic activity. For example, the use of boron (B) in optimum concentration had evident in healthier crop growth due to important roles in cell walls, reproductive structure, and so on. This condition may activate specific crop parts to increase production especially yield. According to Chatterjee and Bandyopadhyay (2017), B had a constructive impact on the high yield of crops due to its function in the reproductive phase. In field conditions, B improves yield and strengthens growing tissues, including the transportation of carbohydrates and sugars (Yamaki, 2010; Davarpanah et al., 2016). In the meantime, the optimum concentration of B and effective application methods remain questionable as it varies according to climatic conditions, soil types, crop cultivar, and so on. Therefore, this study aimed to evaluate the effect of different B concentrations on bunch characteristics and glucose content in the ripe stage on a banana cultivar of Berangan.

Materials and Methods

The field experiment was conducted in UPM Bintulu Sarawak Campus (UPMKB) on the banana cultivar of Berangan (3^{o} 12’ 20.31” N; 113^{o} 4’ 56.90” E) by practising recommendation of banana plantation management as suggested by the Department of Agriculture of Malaysia. Two sword suckers were planted in every hole with a growing distance of 3 m length x 3 m width. Thinning of the poor growth of sword sucker was performed after a month. Seven replications were used for each treatment to come to a total of 28 holes. The treatment was arranged in separate blocks to avoid contamination from different B concentrations and ensure reliable results.

Four treatments were established, with T1 (control) serving as an important reference represented by current practices commonly adopted by farmers and T2 (0.1% B), T3 (0.2% B), and T4 (0.3% B) defining different B concentrations. This experiment started by spraying different treatments on banana flowers twice, with (1) directly after the last hand of flowers opened and (2) precisely 30 days after the first spray. The banana bunch was harvested once the finger skin showed colour changes to yellowish and segregated according to analysis such as agronomic measurement on bunch weight (kg), total fingers (pieces), finger weight (g), and proximate analysis of glucose contents (Chaipai et al., 2018). All the results were analysed according to the analysis of variance (ANOVA) and tested for significant differences using Tukey's studentised range (honest significant difference) at $p = 0.05$ in

SAS Version 9.4 statistical software and graphically presented in bar graphs with a standard error by SigmaPlot Version 14.0.

Results and Discussion

The effect of different B concentrations on selected bunch characteristics and glucose content was presented in Figure 1. The effect of B sprayed (T2, T3, and T4) in Figure 1 (a) had significantly different on heavier bunch weight (kg) compared to control (T1) and followed by heavier finger weight (Figure 1 (c)). It is insignificantly different from the number of fingers recorded in this study (Figure 1 (b)). Glucose content in ripened bananas on different treatments exhibited significantly greater T3 with 0.2% B, while the rest indicated a comparably effect.

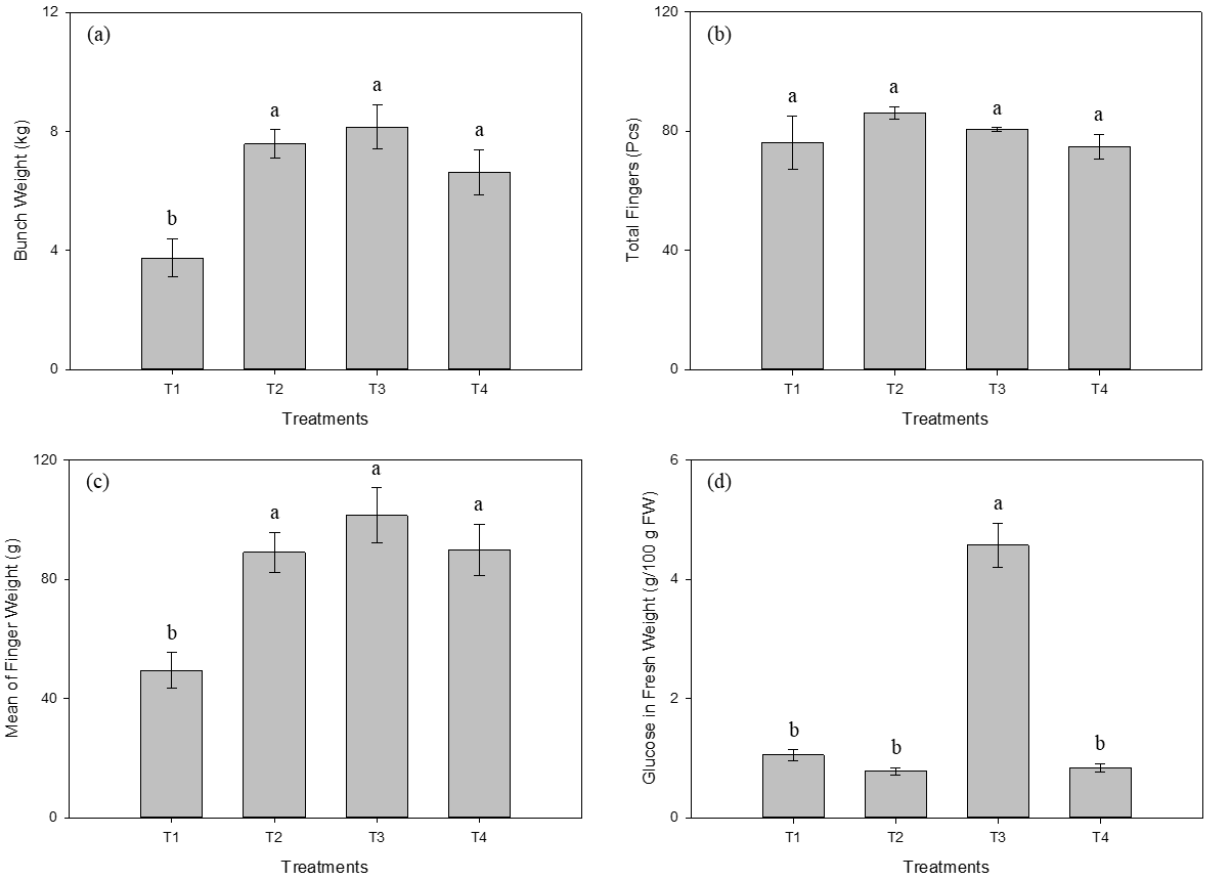

Figure 1: Effect of the different B concentration with T2 (0.1% B), T3 (0.2% B), and T4 (0.3% B) towards T1 (control) on (a) bunch weight (kg), (b) total fingers (pieces), (c) mean of finger weight (g), and (d) glucose content in fresh weight (g/100 g FW). Mean with different alphabets are significantly different at $\rho = 0.05$ using Tukey's studentised range (honest significant difference), while the error bar in all graphs represents the standard error.

The differences in B concentration applied to the banana cultivar of Berangan had a positive effect on bunch characteristics. The heavier bunch (>6.60 kg) in this study, upon completing the second spray, may initiate the reproductive phase for an optimum yield due to the function of B that contributed onset of the reproductive phase, including cell wall formation and strengthening growing tissues (Chatterjee and Bandyopadhyay, 2017). Upon spraying, that activity may boost the yield production compared to T1 (control), which only relies on traditional practices of sufficient NPK fertilisation. Even though our study was a field experiment, we successfully controlled drought season by ensuring sufficient water was supplied to increase B efficiency. This could be true as our results successfully

indicated roles of B compared to a study conducted in drought season, which exhibited low yield closely related to decreasing number of flowers (Cordeiro et al., 2022; Khan et al., 2022).

The total fingers (Finger 1 (b)) in terms of pieces were comparable to all the treatments, but their weight (>88 g) differed significantly. As the bunch weight was heavier, we expect the finger weight (Figure 1 (c)) may correlate with high finger circumference compared to T1. At the same time, ripened banana of T3 (0.2% B) exhibited significantly higher glucose contents (Figure 1 (d)) by 4.57 g, while the rest are comparable with <1.05 g. In the selected study on B effectiveness, most researchers concluded foliar sprays on numerous fruits have resulted in effective carbohydrate and sugar transportation (Yamaki, 2010; Davarpanah et al., 2016). According to Ganie et al. (2013), that transportation was facilitated by artificial lipid bilayer membranes and increased glucose content in our study. However, T2 (0.1% B) and T4 (0.3% B) exhibited differently, whereas each treatment was in the range of lower or higher than T3 (0.2% B). This finding may conclude that the optimum B concentration positively impacts glucose even though those treatments resulted in heavier bunch weight.

Conclusion

Different B concentration on T2 (0.1%), T3 (0.2%), and T4 (0.3%) had positive impact on bunch characteristics compared to T1 (control). Meanwhile, others bunch characteristics such as total fingers by pieces exhibited comparably resulted for all treatments except mean of finger weight (g) which indicates tree sprayed with B has a positive impact on its weight. The effectiveness of B was also tested for glucose content in ripened bananas which revealed T3 (0.2%) has the highest content, significantly different from T1, T2, and T4. Even though B represents a significant improvement in bunch yield, its concentration plays an important role in glucose because optimum concentration should activate the transportation of sugars. Moreover, further analysis of fruit composition in this study was in progress to provide in-depth information on the effect of different B concentrations on the banana variety of Berangan.

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Effect of Different Sound Waves in Delaying Ripening of Berangan Banana (*Musa* AAA Berangan)

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Introduction

Banana is one of the most economically important fruits and widely cultivated around the world. It is also recognized as one of the major staple food crops in most tropical areas, just behind rice, wheat and maize in world trade (Lupatini et al., 2017). Banana has high popularity among consumers due to its deliciousness, multiple uses and health benefits to human. The compound in banana can acts as anti-diarrheal, antimicrobial, antioxidant, anti-ulcer, anti-lithic and wound healing (Qamar and Shaikh, 2018). Based on Food and Agriculture Organisation (FAO) 2020, banana ranked the eight most important crop in the world with total production approximately 114 million tonnes. In Malaysia, banana ranked the second most cultivated fruit crop, covering around 26,210 ha with the production around 313,811 tonnes in 2020 (Department of Agriculture, 2020). The most banana variety in demand by local farmers is Berangan, followed by Rastali, Raja, Awak, Abu, Nangka and Tanduk. Most of the bananas were exported to Singapore, Brunei, Hong Kong and the Middle East (Jain and Swennen, 2014).

Banana, which belongs to Musaceae family is categorized under climacteric natural product, once initiated to mature with ethylene or ethylene-created assets, their shelf life is just about 3-5 days, contingent upon ethylene treatment and holding temperature after treatment (Ding and Darduri, 2009). Classified as a climacteric fruit, banana will continue the ripening even after being harvested. The continuous production of ethylene led the faster deterioration of the bananas thereby shorten postharvest life and increase postharvest losses. Various methods in slowing down ethylene-induced ripening produces including banana have been conducted such as by application of 1-Methylcyclopropene (Sisler et al., 1995), gibberellin (GA_3) (Wan Zaliha et al., 2016), agricultural waste charcoal (Wan Zaliha et., 2014; Siti Amirah et al., 2017) and UV radiation (Nur Izzati Malek et al., 2021). However, the impact of sound waves in delaying bananas is scant. This warrants further investigation. Previously, Kim et al. (2015) claimed that the sound wave treatment delays tomato fruit ripening by altering the expression of important genes in the ethylene biosynthesis and ethylene signaling pathways.

Banana is suitable to be stored at temperature above 13 °C and relative humidity of 85 to 95% for three weeks (Hailu et al., 2013). If stored at below its threshold safe temperature, chilling injury symptoms will occur. However, harvested bananas can withstand only for one to three weeks at ambient temperature depending on maturity stages and cultivars. Hence, there is a need to delay ripening process as farmers and retailers will have the flexibility in marketing their goods. As mentioned earlier, the physical methods such as sound wave, which is cheaper, safe, and easy to apply could be a promising alternative to delay ripening process of Berangan banana. Hence the objective of the study is to determine the effect of different sound waves in delaying the ripening and maintaining quality of Berangan banana.

Materials and Methods

The experiment was conducted at the Postharvest Technology Laboratory, Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu. Berangan banana with similar size, weight, maturity stage and free from damage and bruises were purchased from the local market in Kuala Terengganu. A total of 63 hands of Berangan banana at maturity stage 2 were used in the study. The fruits then were

washed with 200 mg/L of sodium hypochlorite to remove dirt and left to be air dried. The ripening process of the fruits was triggered by soaking in 400 mg/L of ethylene for five minutes. Each hand of banana (13 fingers) then was placed in unclosed plastic boxes and further placed in the closed chamber at 26±2 °C.

The experiment was laid out as according to a complete randomised design (CRD). The treatments were: i) control (no sound, 56 dB), ii) Al-Quran recitation at 80 dB, and iii) heavy-metal songs at 80 dB with three replications. Each treatment was conducted separately since there was limited chambers available. The duration of the experiment was 15 days and the assessments were done at three-day intervals i.e. 0, 3, 6, 9, 12, and 15. The parameters such as starch pattern index (SPI), fruit skin colour, soluble solids concentration (SSC), fruit firmness, titratable acidity (TA), and percentage weight loss (PWL). For SPI, the fruit was cut into half and the surface of the pulp was immersed in iodine solution for a few minutes. The presence of starch on the pulp causing a dark blue colour and the score was given based on Starch Pattern Chart (Kader, 2002). Fruit skin colour attributes such as chromaticity value a*, b*, lightness (L) and hue angle (h°) were measured using Konica Minolta Chroma Meter (Model R200 Trimulus Colour Analyzer, Minolta Camera Co, Ltd, Japan) based on the method of McGuire (1992). Meanwhile, PWL using weighing balance and fruit firmness data were recorded based on Wan Zaliha et al., (2014). For soluble solids concentration (SSC) and TA, the measurement was as following the method of Dadzie and Orchard (1997) and AOAC (1984), respectively.

All data were subjected to the One-Way Analysis of Variance (ANOVA) using GLM (General Linear Models) procedures with SAS 9.1 software package, SAS Institute Inc, Cary, NC, USA. Treatment means were further separated by Tukey test-(HSD) for least significance at $P < 0.05$ (SAS Institute Inc, 1999)

Results and Discussion

Sound waves affect plant growth and fruit ripening has become one of the important aspects in plant physiology. Particularly, in postharvest physiology and technology, much research have been conducted in improving plant growth through sound waves technology such as the application of different frequencies, sound pressure level, exposure periods and distances from the sources of the sound (Reda et al., 2014). Sound waves application has been reported to stimulate growth and yield of many plants such as sweet pepper, cucumber, tomato, spinach (Spinacia oleracea), cotton, rice, and chrysanthemum (Bochu et al., 2004). Recently, sound waves had been proved to delay ripening of tomato fruits by altering the expression of important genes in ethylene biosynthesis (Kim et al., 2015). In the present study, Berangan banana were exposed to 30 juzz of Quranic recitation at 80 dB, heavy-metal songs at 80 dB and normal condition (56 dB). All these sound waves were applied for 15 days at 26±2 °C condition.

The application of these three sounds had similar effect on the external and internal quality of banana except for PWL (Figures 1, 2, 3, 4, 5, 6, 7, 8, 9 and Table 1). Even though no apparent effects were observed on the quality of banana, fruits exposed to 30 juzz of Quranic recitation tends to have lower a* value which indicate the fruits were slightly green after day 9. As illustrated in Figure 2, the effect of different sound waves on the Lightness (L) of banana, the ripening stage begins after day 9. After day 9, all fruits slowly undergo ripening process. Fruits exposed to ethylene ripen within 2 to 3 days. However, bananas that have been soaked in 400 mg/L ethephon and exposed to Quranic recitation (80 dB) and heavy-metal songs (80 dB) ripen 9 days after treatment. Similarly, Kim et al. (2015) claimed that tomato treated with 1 kHz sounds remain green over 14 days experiment as compared to untreated fruits.

During ripening, various chemical and biochemical changes occur such as the surface skin colour from darker green to yellow due to the activity of enzyme chlorophyllase that degrade the chlorophyll pigment (Jacob et al., 1999). In contrast, Kim et al. (2015) claimed that the changing in tomato fruit

skin colouration was delayed with the application of 1 kHz of sound waves. The contradict outcomes might be attributed to the condition of the experiment. Kim et al. (2015) conducted in the sound prove chamber while, in the present study, the bananas were placed in no-sound prove chamber. Sound waves from other sources such as vibration of chamber nearby due to air conditioning equipment possibly affect the current result.

For PWL (Figure 9), the fruits exposed to Quranic recitation (80 dB) had low moisture loss after 15 days of treatment and were still in saleable range. Wills et al. (2007) reported that water loss ranged between 3 to 10% is acceptable for consumer purchasing decision. The possible mechanism involve might be ascribed to the sound enhance the activity of cell wall fluidity and activity of protective enzymes (Reda et al., 2014). Results of other qualities attribute such as SSC, TA and fruit firmness were not correlated with previous studies. However according to Kim et al. (2015), the tomato fruits treated with 1 kHz sound waves were able to maintain their flesh firmness, thus lengthen the shelf-life for 5 days than non-treated fruits.

Table 1: Effect of different sound waves on starch pattern index (SPI) according to Starch Pattern Chart (Kader, 2002) of Berangan banana (*Musa AAA*. Berangan). Number in the box with photo denotes to SPI score of Berangan banana.

Days after treatment	Treatment		
	Control (No Sound : 56 dB)	Treatment 1 (Heavy Metal Song : ±80 dB)	Treatment 2 (Al-Quran Recitation : ±80 dB)
0			
3			
6			
9			
12			
15			

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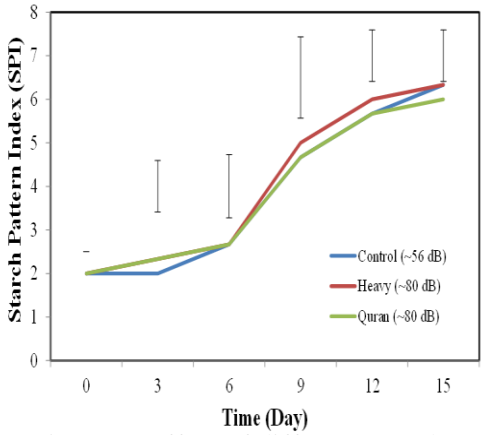

Figure 1: Effect of different sound waves on the Starch pattern Index (SPI) of Berangan banana. Vertical bars represent the HSD value at 5% level.

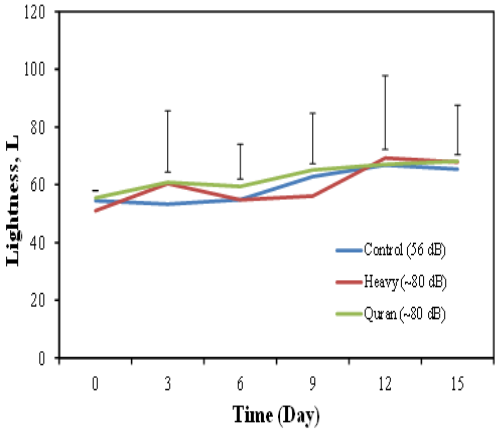

Figure 2: Effect of different sound waves on fruit colour attributes, lightness (L*) of Berangan banana. Vertical bars represent the HSD value at 5% level.

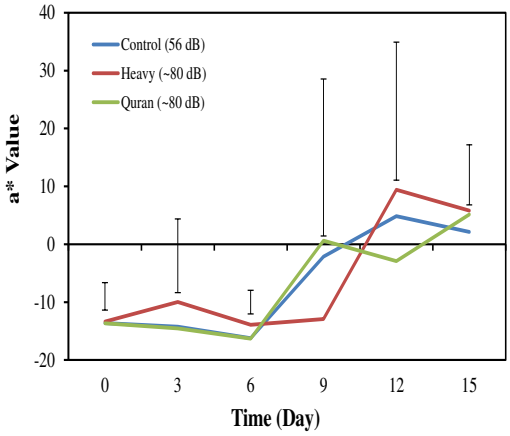

Figure 3: Effect of different sound waves on fruit colour attributes, chromacity a (a*) of Berangan banana. Vertical bars represent the HSD value at 5% level.

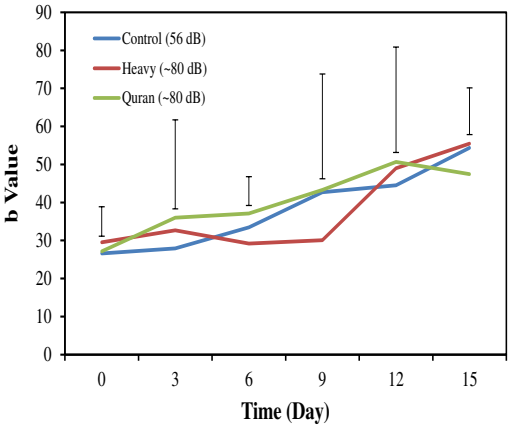

Figure 4: Effect of different sound waves on fruit colour attributes, chromacity b (b*) of Berangan banana. Vertical bars represent the HSD value at 5% level.

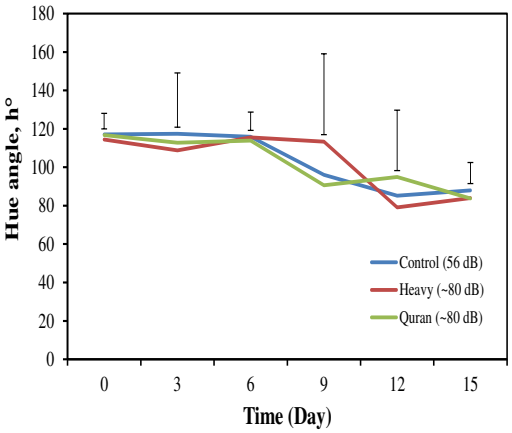

Figure 5: Effect of different sound waves on fruit colour attributes, hue angle (h°) of Berangan banana. Vertical bars represent the HSD value at 5% level.

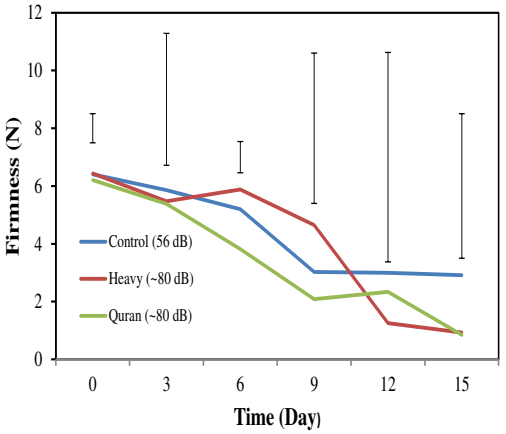

Figure 6: Effect of different sound waves on fruit firmness of Berangan banana. Vertical bars represent the HSD value at 5% level.

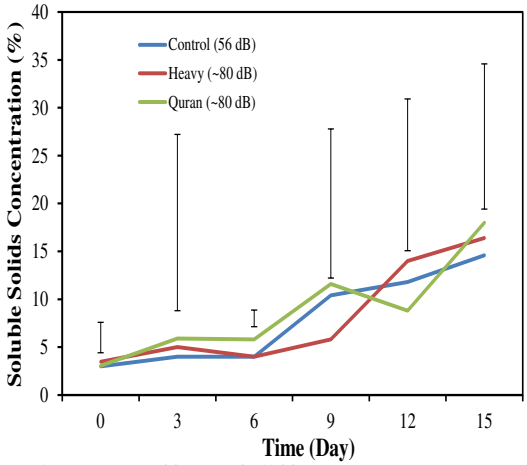

Figure 7: Effect of different sound waves on soluble solids concentration of Berangan banana. Vertical bars represent the HSD value at 5% level.

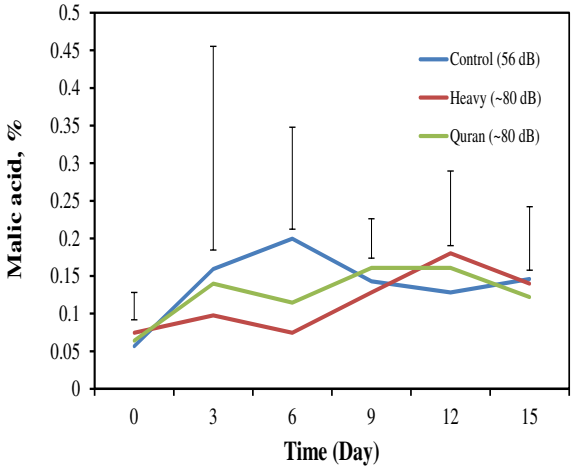

Figure 8: Effect of different sound waves on titratable acidity of Berangan banana. Vertical bars represent the HSD value at 5% level.

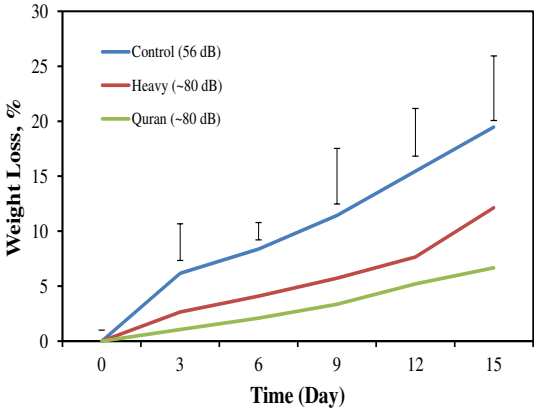

Figure 9: Effect of different sound waves on percentage weight loss of Berangan banana. Vertical bars represent the HSD value at 5% level.

Conclusion

All sound waves treated Berangan bananas had similar quality in terms of firmness, colour, titratable acidity, soluble solids concentration and starch pattern index (SPI) to control bananas except the percentage weight loss (PWL). However, the Quran recitation tends to have fresh yellow-green skin surface without anthracnose disease symptom and acceptable percentage water loss. In addition, the shelf life could be extended for few days as the soluble solids concentrations is still under 21%. For future research, it is recommended that the sound waves treatments should be applied at different exposure time in delaying fruit ripening. In addition, other source of sounds such as fireworks, waterfall and bird chirping can be included.

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Cryogenic Freezing of Whole Mango Fruit at Different Freezing Temperatures

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Introduction

Mango is one of the important crops in Malaysia but even until 2021, Malaysia is still unable to fulfill self-sufficient level of this seasonal fruit. Freezing is one of the preservation techniques used to prolong shelf life of food product for a long time. There are different types of freezing techniques such as direct freezing, air blast freezing and cryogenic freezing (Espinoza Rodezno et al., 2013) where each freezing method has different mechanism to freeze the food. For cryogenic freezing method, foods are frozen using expandable gaseous refrigerants such as nitrogen and carbon dioxide which are sprayed directly onto the surface of the food. These gases are considered as safe and eco-friendly as they are commonly found in abundance in the atmosphere and also due to the ability of this technique to freeze products within a short time. In Malaysia, cryogenic freezing of fruits has been used widely used in durian recently due to the high demands for Malaysian durian such as Musang King in light of the new market access to China in 2018 (Suhana et al., 2021). Hence, this study was conducted to evaluate the potential of other fruits to be cryopreserved to expand the market.

Materials and Methods

Sample preparation

Mangoes variety Susu from Thailand were used in this study. Fruits were obtained from local wholesaler at the ripe stage, and washed and dried followed by storage overnight in the cold room at 12 °C. These fruits were then transported to the cryogenic freezing facility in Bentong, Pahang.

Cryogenic treatment

Fruits were arranged individually on stainless steel trays that could withstand freezing temperature to ensure that freezing was done equally before the trays were stacked on the caged rack fitted into the cryogenic chamber. Temperature probe was then inserted into the center core of the fruit to monitor the temperature until it reached -18 °C. Three freezing temperatures were used in this study which were -40, -60 and -80 °C. These freezing temperatures referred to the temperatures of the chamber and the process was completed when the core of the fruit reached -18 °C. Blast freezing was used as a control to compare the time taken for the core of the fruit to reach -18 °C and the effects of freezing on fruit quality. When the core temperature reached -18 °C, the time was recorded and the machine was stopped. Fruits were then taken out and immediately packed into nylon plastic bags and vacuum-sealed before being stored at -20 °C.

Quality evaluation

Fruits were thawed at room temperature for approximately 45 min before quality evaluation was conducted. Total soluble solid was measured using digital handheld refractometer PAL-α (Atago Co. Ltd, Japan) while pH was taken using the HI2211 pH meter (HANNA, USA). Titratable acidity content was measured by titrating 20 mL extract of the sample with 0.1 M NaOH until pH 8.2 was

reached. Meanwhile for ascorbic acid measurement, 10 mL extract from 10 g sample and 100 mL 3% metaphosphoric acid were titrated with standard dye until the extract turned into faint pink color. Thawed mango skin and flesh texture were measured using a texture analyser (Stable Microsystems, UK).

Statistical analysis

The experiment was designed using Completely Randomized Design (CRD) with three replications. Statistical analysis was performed using ANOVA and difference of means was determined using Duncan Multiple Range Test at 5% level.

Results and Discussion

From the data log of the probe used, it showed that -80 °C treatment was able to lower the temperature of mango core the fastest (45 min) compared to other treatments. This was followed by -60 °C freezing temperature that took 55 min and freezing temperature of -40 °C took the longest time at 120 min, which was the same as blast freezing. However, there was no significant difference in terms of chemical qualities and texture for all parameters between all treatments used (Table 1). Fruits from all treatments showed freezing injury symptoms after thawing (Figure 1) where the skins displayed water-soaked signs and became soft and hard to cut. During the thawing process, fruit juice was observed to ooze out of the fruits indicating that the cells had been compromised during the freezing or thawing as this process can cause physical changes such as cell volume stress, water dislocation, mechanical damage or freeze cracking (Chassagne-Berces et al., 2010). Although the same freezing technique applied on whole durian proved to be efficient (Razali et al., 2022), the results on whole mango showed that it was not suitable due to high water content in both pulp and peel of mango compared to water content in durian (Leguizamon-Delgado et al., 2019). Pulp texture was also observed to become soft with jelly-like texture after thawing and this was in agreement with previous study on different water content of apples. Leila et al. (2016) suggested that the removal of water content upon freezing treatment can help to prevent the negative impacts of freezing on apple fruit firmness. However, this process might be inefficient and hard to be used on whole mango as the peel will refrain water movement from the fruit.

Table 1: Changes in pH, Total Soluble solid (TSS), total titratable acidity (TTA), ascorbic acid content (Vit C), and texture of whole mango (var. susu) treated with different freezing temperatures.

Treatment	pH	TTA (%)	TSS (°Brix)	Vit C (mg/100g)	Texture	
					Skin	Flesh
T1 (Blast Freezer)	3.837[a]	0.167[a]	11.100[a]	4.910[a]	11.813[a]	3.520[a]
T2 (-40 °C)	4.240[a]	0.103[a]	13.700[a]	6.187[a]	11.018[a]	3.309[a]
T3 (-60 °C)	4.283[a]	0.131[a]	14.300[a]	5.950[a]	14.593[a]	2.873[a]
T4 (-80 °C)	4.413[a]	0.094[a]	12.733[a]	4.383[a]	10.911[a]	1.159[a]
F Test	ns	ns	ns	ns	ns	ns

Means separation within columns and main effect by Duncan's Multiple Range test at P ≤ 0.05.
*ns, *, ** Not significant or significant or highly significant at P ≤ 0.05, respectively.*

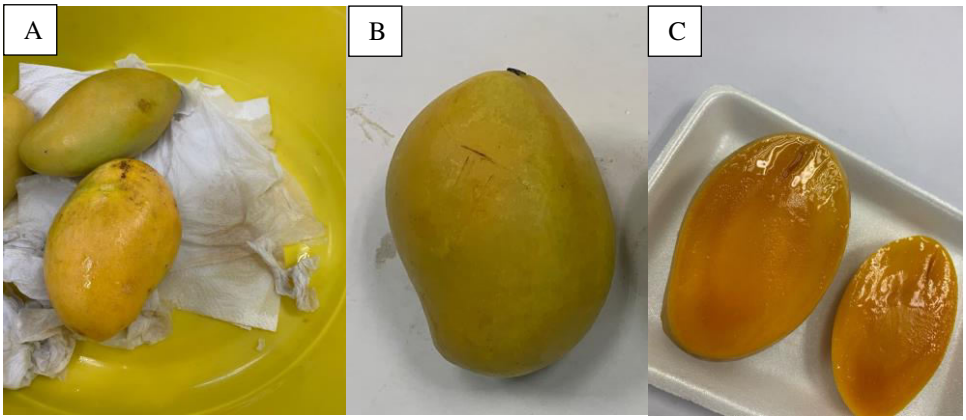

Figure 1: Physical and chemical qualities of mangoes treated with different freezing temperatures. (A) Coloured liquid oozed out of the fruits, (B) Water-soaked skin after thawing, and (C) Cracking on flesh after the fruit was cut in half.

Conclusion

Freezing using cryogenic chamber at -80 °C froze the mango fruits the fastest and did not give different effects compared to other treatments. However, the fruit quality of whole mango is not suitable for fresh eating. Thus, further studies need to be conducted to minimise the effect of freezing on whole mango fruit.

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Effects of Salinity Sources on Fruit Physical Appearances of Grafted Rockmelon (*Cucumis melo* L.)

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Introduction

Salt addition into a nutrient solution is a simple procedure in many plants to improve fruit quality. However, increasing salinity by salt addition may reduce fruit yield and plant growth by interfering with the physiological process due to salt-stress impairment (Zhang et al., 2016). In melon, plants exposed to high salt concentrations in the nutrient solution causes the reduction of overall plant performances except for fruit quality (Rouphael et al., 2012). Therefore, the use of bottle gourd as salt-tolerant rootstock for rockmelon became the viable solution. Fruit physical appearances become one of the important criteria in rockmelon production. It plays an important role in marking the quality level of rockmelon fruit. This covers the size of the fruit, the colour of the skin and the flesh of the fruit. By using salt-tolerant rootstock of bottle gourd, the utilization of additional salt in saline conditions is suspected to sustain or improve the size and the color of the fruit. The response of the plants towards salinity sources is varied accordingly with their types. Chloride is required in small quantities which can help for plant metabolism, photosynthesis, osmosis, and ionic equilibrium within the cell (Padder et al., 2012). Usherwood (1985) referred to KNO_3 as a quality factor in plant production that had a beneficial impact on the quality criteria in fruit such as size, appearance, colour, soluble solids, acidity, vitamin content, flavour, and shelf life. Therefore, this study was conducted with the objectives to evaluate fruit physical appearances of grafted rockmelon under varying sources of salinity.

Materials and Methods

Plant materials and treatments

The experiment was conducted at University's Agriculture Park, University Putra Malaysia (2.98675, 101.70932). The planting materials used in this study are rockmelon (*Cucumis melo* L.) var. Glamour and bottle gourd (*Lagenaria siceraria*). This experiment consisted of four treatments of salinity sources which were arranged in the RCBD in four replications; eight plants per replication totalling to 128 grafted plants. Rockmelon scions were grafted onto a selected salt-tolerant rootstock (Bottle gourd) using Tongue Approach grafting technique as procedure described by Lee and Oda (2003). At 14 days after grafting (DAG), uniform sizes of grafted plants were transplanted into the 12 litres white polyethylene bags filled with 100% cocopeat. The plants were subjected to four types of salinity sources; basic nutrient solution (BNS) (EC=2.5 dS m^{-1}) as control, NaCl (50 mM)+BNS (EC=7.1 dS m^{-1}), KNO_3 (50 mM)+BNS (EC=7.1 dS m^{-1}), and high strength nutrient solution (NS) (EC=7.1 dS m^{-1}). The solution of the treatments was manually drenched every day for 68 days in a sufficient volume with drainage.

Salinity sources applications

The concentration of the BNS used in this study is in accordance to MARDI's formulation that is specifically recommended for rockmelon (Shahid et al., 2009). While, the commercial NaCl salt from groceries and KNO_3 as commercial soluble fertilizer grade (13-0-46) for fruit crops were used in this

study. Each treatment solution was prepared in the 200 L nutrient solution containers which were checked and quantified using EC meter (5061 Pen SHSX). The nutrient solution of the treatments was manually drenched every day for 70 days in a sufficient volume with drainage. The frequency of the nutrient solution given was increased gradually according to the growing stages as employed in commercial rockmelon fertigation system.

Data collections and analysis

At 70 DAT, all the fruits were harvested with careful handling for data collection. The measurements consisted of fruit length and fruit width was taken using an electronic digital solar caliper (Mitutoyo Series No. 500, Japan). Peel and pulp colour index was measured and expressed as lightness (L) using handheld colorimeter (Model CR-400, Konica Minolta®, Japan). All the data taken was computed using statistical analysis software (SAS) version 9.4 (SAS Institute Inc., Cary, NC). GLM procedure was used to do analysis of variance (ANOVA) and mean comparisons were calculated using Tukey's Honest Significant Difference (HSD) at $P \leq 0.05$.

Results and Discussion

Salinity sources applications significantly affected fruit physical components of grafted rockmelon such as fruit length ($P \leq 0.01$) and fruit width ($P \leq 0.05$). Salinity induced by NaCl + BNS, KNO_3 + BNS and high strength NS applications significantly reduced fruit length as compared to control with their respective reduction of 19.45%, 14.00% and 15.75%. In addition, salinity induced by NaCl + BNS and high strength NS applications significantly reduced fruit width as compared to control with their respective reduction of 13.45% and 8.20%. Salinity sources treatments significantly affected lightness (L*) in the peel ($P \leq 0.01$) and pulp ($P \leq 0.05$) of grafted rockmelon. KNO_3 + BNS application significantly increased lightness in peel as compared to control and NaCl + BNS application with the respective increments of 4.96% and 6.93%. In pulp, the lightness was significantly decreased by KNO_3 + BNS application as compared to NaCl + BNS and high strength NS with the respective reductions of 3.27% and 4.82% (Table 1).

Physical characteristics of fruit quality such as fruit length and fruit width were markedly lowered under saline treatments as compared to BNS. Saline treatments may have impacted the length and diameter of the fruit since these properties are directly related to the fresh mass of the fruit, which decreases as the salinity of the nutrient solution increases (Dias et al., 2018). Consistent with the result recorded in this study, Akrami and Arzani (2018) found that fruit physical properties such as fruit length and fruit width were decreased under saline condition. This was also corroborated by Sousa et al. (2016) on different cucurbit species, where irrigation water salinity negatively affected fruit mass, fruit diameter and pulp diameter of mini watermelon (cv. Smile). On the other hand, all saline treatments except for NaCl treated plants exhibited brighter colour in the peel compared to BNS. It is indicated that lightness in the peel was associated with the fruit maturity. Therefore, different maturity levels were attained by the plants. As supported by Kader et al. (1999), base colour is connected to maturity in most fruits. As fruit growth and maturation progress, both chlorophyll breakdown and the synthesis of new pigments such as carotenoids and anthocyanins are detected in the peel. Nevertheless, as the fruit matures and ripens, the chlorophyll concentration decreases, resulting in a decrease of green colour intensity (Rutkowski et al., 2008).

Table 1: Effects of salinity sources on fruit length, fruit width, lightness of peel and pulp of grafted rockmelon.

Factor	Treatments	Fruit length (mm)	Fruit width (mm)	Lightness of peel	Lightness of pulp
Salinity sources	BNS	157.95[a]	151.63[a]	64.35[bc]	59.51[ab]
	NaCl+BNS	127.23[b]	131.23[b]	63.02[c]	60.22[a]
	KNO_3+BNS	135.83[b]	142.21[ab]	67.71[a]	58.25[b]
	High strength NS	133.08[b]	139.19[b]	66.08[ab]	61.20[a]
		F-test (Significant level)			
Salinity sources		**	*	**	*

***Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant*
Means with the different letters in each column within each factor indicate significant differences at P ≤ 0.05% level according to DMRT.
BNS: Basic nutrient solution, NS: Nutrient solution

Conclusions

To sum up, salt addition under fertigation system, had decreased the size of the fruit as a whole in grafted rockmelon. Nevertheless, the appearances of the peel are more attractive by salinity application except for NaCl. All salinity applications are also capable of instigating identical colour appearances in the pulp.

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Effect of Combined Biofertiliser and Chemical Fertiliser on Sweetness of Sweet Potato

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Introduction

Sweet potato (*Ipomoea batatas* L.) is an important staple food crop in several countries including Asia and the Pacific Islands. In Malaysia, sweet potato is the second most important root crop after cassava. Currently, planting sweet potatoes in Malaysia is proving to be a challenge since most agricultural lands are now converted for industrial use. Apart from that, high input cost such as fertiliser, marketing issues, pest and disease outbreak also makes planting this crop difficult (Tan et al., 2005; Loebenstein, 2009). Lembayung purple sweet potato was launched by MARDI in 2021 and it is crowned as a sweet potato variety with high anthocyanins content and can be consumed freshly. Anthocyanins are a group of antioxidants that if regularly consumed, may prevent inflammation and protect against type 2 diabetes, cancer, and heart disease (Datong et al., 2017). Its advantages in the health aspect are expected to increase commercial value and be accepted in the market at a higher price. However, the sweetness of Lembayung is lower than other commercial sweet potato varieties. Phytonutrients, in this case anthocyanin tend to have bitter and acrid taste. This might be the reason why Lembayung tasteless sweet causing consumer aversion. Biofertiliser is a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. They work by fixing, solubilising and transporting nutrients from the soil into the plants. Several functions of biofertilisers include nitrogen fixing, solubilising and mobilising phosphorus and potassium, providing micronutrients and promoting plant growth (Shaista et al., 2021). Sweetness of sweet potatoes can be improved using biofertiliser (Gehan et al., 2013). Plant root and microorganisms work in symbiosis where microbes provide nutrients to the roots and roots provide simple sugars to the microbes as "payment" (Nils et al., 2018). Therefore, this study aims to determine the effects of biofertiliser together with chemical fertiliser application on quality (sweetness) and yield of Lembayung planted in polybag.

Materials and Methods

Experimental design and treatments

The experimental plot was conducted at MARDI Serdang. The soil mixtures used were sand, mineral soil and organic fertiliser at 6:3:1 ratio, prepared in 22" x 22" sized polybags. A factorial experiment with two factors (chemical fertiliser and biofertiliser) was laid out in a Randomized Complete Block Design (RCBD) with three replications. The treatments consisted of four rates of NPK 13:13:21 (chemical fertiliser) at 0, 150, 300 and 450 kg/ha and two types of biofertiliser which were N Biobooster and Bioliquifert. The use of biofertilisers followed the manufacturer's recomendations. The treatments for this experiment are shown in Table 1.

Planting materials

Planting materials used for this experiment was Lembayung variety stem cuttings around 25-30 cm length each. Cuttings were collected from Bachok, Kelantan. Prior to planting, the Lembayung stem

cuttings (30 cm in length) were soaked with carbaryl solution for 15 min. Application of biofertiliser was on the 2nd and 4th week after planting. While chemical fertiliser was applied on the 3rd, 5th, and 8th weeks after planting. The plot was irrigated regularly using a sprinkler system.

Measurements

Data for leaves and vines weight, tuber quality (sweetness) and tubers yield were recorded at harvest after 95 days of planting. The Soluble solid content (SSC) of fruit was measured using a digital refractometer. Sensory evaluation for attributes aroma, texture, taste, sweetness and overall acceptance was done using 7-point hedonic scale ranging from 1 (extremely dislike) to 7 (extremely like). 30 untrained panelists were randomly selected for the sensory evaluation.

Statistical analysis

All data was statistically analysed by Statistical Analysis System (Version 9.4, SAS Institute Inc, North Carolina, USA). The data obtained were analysed using one-way ANOVA, and differences among treatment means were determined using Tukey's Honest Significant Difference (HSD) at $P \leq 0.05$ %.

Table 1: Treatment Biofertiliser and chemical fertiliser.

Treatment	Treatment description
T1	0 kg/ha NPK (13:13:21) + N Biobooster
T2	0 kg/ha NPK (13:13:21) + Bioliquifert
T3	No application of fertiliser
T4	150 kg/ha NPK (13:13:21) + N Biobooster
T5	150 kg/ha NPK (13:13:21) + Bioliquifert
T6	150 kg/ha NPK (13:13:21)
T7	300 kg/ha NPK (13:13:21) + N Biobooster
T8	300 kg/ha NPK (13:13:21) + Bioliquifert
T9	300 kg/ha NPK (13:13:21)
T10	450 kg/ha NPK (13:13:21) + N Biobooster
T11	450 kg/ha NPK (13:13:21) + Bioliquifert
T12	450 kg/ha NPK (13:13:21)

Results and Discussion

Sweet potato yield

The results showed that the weight of leaves and vines per plant were significantly influenced by chemical fertiliser application (Figure 1A). The highest weight was shown in T12 which is 338.8% higher than those in T2 (lowest value). The highest weight of tuber produced per plant (1,107.8g) was obtained from T10, 383.6% higher than control (T3) (Figure 1B). The results from this study are also in agreement with the study done by Naglaa et al. (2020). Tuber inoculation with *Bacillus* sp. significantly affected yield and yield components in both years (Ekin et al., 2009). The application of biofertiliser in combination with chemical fertiliser was proven to enhance the growth and yield of the plant compared to the single use of biofertiliser (Mekki, 2016).

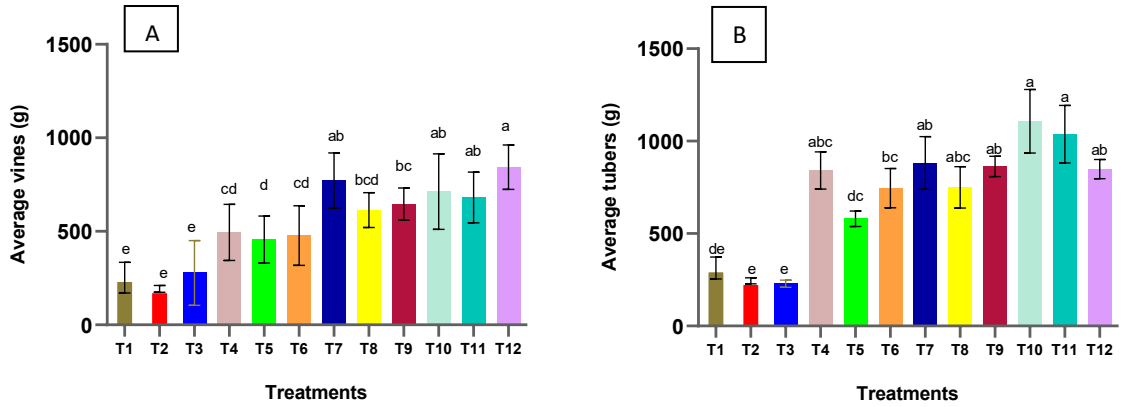

Figure 1: Effects of chemical fertiliser (NPK 13:13:21) rate and biofertilser (N-Biobooster; Bioliquifert) on leaves and vines (A) and tubers (B) weight of Lembayung. Means in each bar chartwith the different letters indicate significant differences at P ≤ 0.05% level according to Tukey's HSD.

Tuber quality (total sugar, soluble solid content (SSC) and sensory test)

Total soluble solid and total sugar has increased significantly with the application of biofertiliser. The application of chemical fertiliser with biofertiliser has significantly affected the total sugar of tubers. The total sugars are important indices for evaluating fruit quality (Wenkai, 2021). The highest total sugar was obtained from T10 (6.51 g/100g) which is 74.5% higher than T3 (control) (Figure 2). A similar study was conducted by Wenkai (2021) on strawberries, resulting in the increase of soluble sugars in the fruit. This reveals that biofertiliser influences not only the plant yield, but also the quality of fruits. As for the Soluble Solid Content (SSC), all fertilisation treatments with biofertiliser and the method of planting in polybag increased the SSC (Brixº) of about 5 to 10 % compared to SSC from actual planting in mineral soil using chemical fertiliser without biofertiliser. The maximum SSC was obtained from T1 (0 kg/ha NPK + N Biobooster) 8.63 Brixº (Figure 3). Results from the sensory test showed that the attribute 'aroma of tubers' of treatments T1, T8, T11 and T12 was most preferred by testers. The attribute 'texture' for treatment T11 was mostly preferred by tester. For taste and sweetness attribute, testers preferred sample T10 the most, followed by T8, T11 and T12. Sample T8, T10, T11 and T12 received high scores in all attributes tested (Table 2).

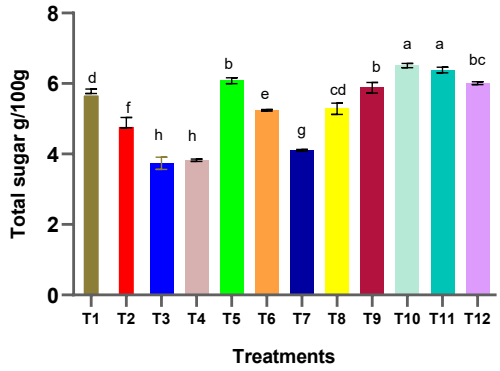

Figure 2: Effects of biofertiliser application on total sugar of Lembayung sweet potato. Means in each bar chart with the different letters indicate significant differences at P ≤ 0.05% level according to Tukey's HSD.

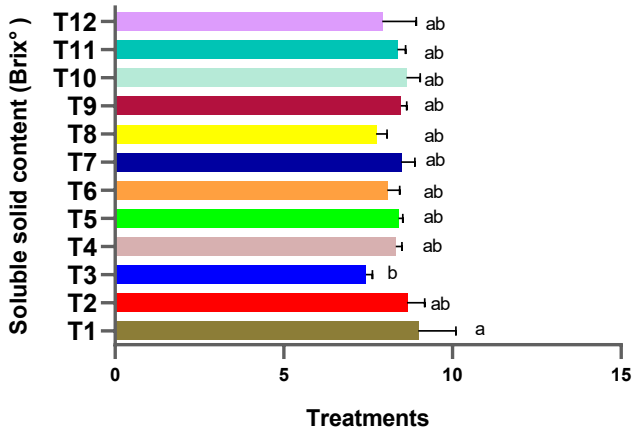

Figure 3: Effects of biofertiliser application on Soluble Solid Content (BRIX °) of Lembayung sweet potato. Means in each bar chart with the different letters indicate significant differences at P ≤ 0.05% level according to Tukey's HSD.

Table 2: Sensory test of Lembayung sweet potato for all treatments.

Treatments	Scores for each sensory attribute				
	Aroma	Texture	Taste	Sweetness	Overall acceptance
T1	5.83^{a}	5.67^{ab}	4.90^{abc}	4.50^{bc}	5.11^{ab}
T2	5.14^{ab}	5.07^{abc}	4.34^{c}	4.20^{c}	4.82^{b}
T3	4.69^{bc}	5.00^{abc}	3.59^{d}	3.43^{d}	3.61^{d}
T4	5.03^{abc}	5.27^{ab}	4.72^{bc}	4.80^{bc}	4.82^{b}
T5	4.83^{bc}	5.10^{abc}	4.66^{bc}	4.37^{c}	4.57^{bc}
T6	5.03^{abc}	5.14^{abc}	4.45^{c}	4.33^{c}	4.75^{bc}
T7	4.86^{bc}	4.93^{bc}	4.59^{bc}	4.69^{bc}	4.68^{bc}
T8	5.72^{a}	5.55^{ab}	5.34^{ab}	5.30^{ab}	5.32^{ab}
T9	4.28^{c}	4.45^{c}	4.20^{c}	4.28^{c}	4.15^{cd}
T10	5.45^{ab}	5.60^{ab}	5.45^{a}	5.43^{a}	5.54^{a}
T11	5.69^{a}	5.73^{a}	5.28^{ab}	5.23^{ab}	5.39^{ab}
T12	5.69^{a}	5.59^{ab}	5.31^{ab}	5.30^{ab}	5.25^{ab}

**Mean in each column with the different letters within the column indicates significant difference at P < 0.05 according to Tukey's HSD.*

Conclusions

To conclude, it is recommended to use chemical fertiliser with the rate of 450 kg/ha (N=58.5 kg, P_20_5= 58.5 kg and K_2O= 94.5 kg) combined with biofertiliser (N Biobooster) as it can improve the sweetness by 10% than that of control and yield by 383% of Lembayung sweet potato planted in polybag. The fertilisation method introduced can be used for other tuber crops planted in polybag/containers as well. The future study will be conducted to investigate the effectiveness of the best treatment at a larger scale field plot.

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Effects of Anti-browning Treatments on Quality of Minimally Processed Tender Jackfruit

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Introduction

Fresh fruits and vegetable products that are freshly cut, washed and packed usually offer consumers nutritious and convenient products. However, they can be perishable and easily proned to chemical and biological changes. One such example is that when young tender jackfruit (*Artocarpus heterophyllus* L.) is harvested, there is a quality-degrading process and this can affect colour and other quality attributes changes. Therefore, some prerequisite processing conditions are required in order to retain its original qualities. Some of these adverse effects include browning on the surface, microbiological spoilage, and loss of nutritious values (Putnik et al., 2016). Other possible good practices such as use of preservatives and processing techniques can also played an important role on the stability of the quality of fresh-cut fruits (Rocculi et al., 2012). However, it was initially discovered that the main challenge to the commercialization of fresh-cut tender jackfruit was due to enzymatic browning with the organoleptic properties of jackfruit being strongly altered by the appearance of brown pigments. This copper-containing enzyme polyphenol oxidase (PPO) causes certain phenolic molecules to oxidise, resulting in enzymatic browning (Paul and Palmer, 1972). This process involves is caused by compartmentalised phenolic substrates reaction and resulting in undesirable browning (Dong and Sugar, 2000; Sapers et al., 2002).

Browning and decaying process in fresh-cut fruits and vegetables can be reduced by the use of natural anti-browning agents such as ascorbic acid and citric acid whom are effective enzymatic browning inhibitors (Gonzalez Aguilar et al., 2000). Langdon (1987) found that immersion of peeled and sliced potatoes in solutions of ascorbic and citric acid followed by vacuum packaging resulted in a product can entend shelf life longer than 14 days. This citric acid has also been widely used in reducing the superficial pH of fresh-cut fruit (Soliva-Fortuny and Martin-Belloso, 2003) and other chemical treatments also found to prolong the shelf-life and reduce colour changes for cut apples (Rocha et al., 1998). For instant, with application of sodium metabisulphite as a preservative, colour and anti-browning agent, the shelf-life for postharvested fruits and vegetables are extended (Sgroppo et al., 2010). Similarly, the minimally processed sweet potato var. '*Colorada correntina*' treated with 2% sodium metabisulphite and with a combination of citric acid can be used to preserve for up to 14 days stored at 5 °C. Therefore, the objective of this study was to investigate the effects of ascorbic acid, citric acid and sodium metabisulphite as an anti-browning agent for fresh-cut tender jackfruit so as to preserve the shelf-life, quality and physicochemical properties at storage temperature.

Materials and Methods

Samples preparation

Freshly picked tender jackfruit of variety J33 was provided by MARDI Jelebu, Negeri Sembilan. The fruits were harvested at maturity age of between eight to ten weeks. All fruits were selected and washed using chlorinated water so to remove dirt, fungicide residue and microbes. After drying, the fruits are immediately stored at 2 °C with relative humidity of 90-95%, in a cold room overnight for precooling processed. All utensils and equipment used for preparing fresh-cut pieces were disinfected with 150 ppm chlorinated water to avoid cross-contamination. Disposable gloves, masks and headwear were used. The fruits were initially processed out in cool conditions at temperature about 16 °C. Jackfruits were manually cut at the base and then skin peeled, followed by dipping into cold water.

The peeled jackfruits were cut into longitudinal cutting styles, cut into cubes size of between 25-30 mm and dipped for one minute. The treatments were:

T1 – without any solution treatment (control)
T2 – treated with ascorbic acid 1%
T3 – treated with ascorbic acid 2%
T4 – treated with citric acid 1%
T5 – treated with citric acid 2%
T6 – treated with sodium metabisulphite 1%
T7 – treated with sodium metabisulphite 2%

Any excess solution was drained and the fruit slices were packed in a low-density 0.04 mm thickness polyethylene plastic bag containing water absorbance and stored at 2 °C. Quality analyses were carried out at three days interval.

Colour

The surface colour of tender jackfruit was measured using a reflectance colorimeter (Minolta Chroma Meter, Model CR-400, Osaka, Japan) with the following parameters: L* (lightness), a* (red-green) and b* (yellow-blue) values as taken values from the side surface of nine pieces per replication. The Chroma (C) and Hue (H°) were calculated based on the formula. Chroma was calculated as $C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$ and hue angle was calculated as *h* degrees = arctan (b*/a*).

Firmness

Measurements were taken randomly based on nine slices using Texture Analyser Meter (Instron Universal Meter Model 1140) and 2 mm diameter probe.

Chemical analysis

Chemical analysis measurements were based on Total Soluble Solids (TSS) by using Digital Refractometer instrument (Atago Co., Ltd, Japan, Model DBX-55). The pH meter used was Hanna Instruments pH 211 Inc. RI-USA, Microprocessor pH Meter and with a Titratable acidity (TTA) reading solution of 20 mL of extraction with 0.1 mol-1 NaOH at pH 8.2 as described by Shaw et al., 1987. Ascorbic acid content was also used by using 10 g of the sample with added 100 mL of 3% metaphosphoric acid. About 10 mL of extraction was then titrated and obtained using a standard dye solution.

Statistical analysis

The experimental design used was Complete Randomized Design with each consisting of four replications and using SAS. The data were analysed using ANOVA and comparisons between means were carried out using Duncan Multiple Range test at $p \leq 0.05$ significant level.

Results and Discussion

Results obtained showed that colour retention was significantly affected using 1% and 2% sodium metabisulphite (SM) when treated in freshly cut jackfruit (Table 1). As such, these treatments can be an effectively applied for preservation purposes. There were less browning as compared to the control and with other treated samples seven days at set temperature of 2 °C. However, samples treated with ascorbic acid and citric acid as compared to the control (T1) showed significantly lower L* values at same storage period indicate continuing browning process. Similarly, the saturation (chroma) and hue color also shown significant changes, indicating a slight shift toward red and yellow and an increase in saturation ($P < 0.001$) (Table 1). As expected, these enzymatic browning inhibitors, sodium

metabisulphite and other sulphites worked to block the enzymatic browning reactions by combining with highly reactive o-quinones so as to produce less reactive diphenols. It was also reported to acts a chelating agent complex with copper in polyphenol oxidase enzyme and can effectively inhibits browning formation (Sapers et al., 2002). As firmness is an important quality criterion for fresh-cut products, sodium metabisulphite treatments also shown to have significant effects on the fruit firmness (Table 1).

Table 1: Effects of different anti-browning agent on flesh colour and texture.

Factor	Flesh Colour			Flesh texture (N)
	L*	C*	Hue	
Treatment				
Control	75.40±12.12^{d}	21.30±7.20^{a}	80.20±10.71^{c}	15.74±1.57^{a}
1% ascorbic acid	80.34±4.70^{bc}	18.92±3.52^{b}	83.13±4.45^{b}	11.56±1.40^{bc}
2% ascorbic acid	82.56±9.02^{b}	19.46±5.38^{b}	83.76±6.59^{b}	10.46±2.78^{c}
1% citric	79.26±7.78^{c}	19.61±4.35^{b}	81.79±7.92^{b}	11.05±2.80^{bc}
2% citric	84.04±4.84^{b}	16.66±4.01^{c}	82.06±6.44^{b}	12.34±1.20^{b}
1% SM	88.85±2.23^{a}	12.59±1.50^{d}	89.06±6.44^{a}	14.65±0.59^{a}
2% SM	89.05±4.80^{a}	12.01±2.51^{d}	88.13±6.59^{a}	14.91±1.73^{a}
F-Test	**	**	**	*

Each value was the mean of four replicates.
**Means within columns and factors followed by the same letter are not significantly different based on Duncan Test at P ≤ 0.05.*
NS,, ** non-significant, significant and highly significant at P < 0.05 and P < 0.001, respectively.*

Sodium metabisulphite was found to have a significant effect (P < 0.05), showing a higher value of total soluble solids compared to ascorbic acid and citric acid treatments (Table 2) as such changes in the soluble solids may be attributed to changes occurring during ripening (Nakasone and Paul, 1999). However, the pH value did not influence any interaction among the treatments. Most tender jackfruit treated with 1% and 2% citric acid and 1% sodium metabisulphite showed high in pH values after storage (Table 2) while those treated with 1% anti-browning and control samples showed significantly (P < 0.05) lower value of titratable acidity as compared to others. However, the titratable acidity value gradually decreased over the duration (data not shown). According to Sgroppo et al. (2010), treatment with 2% sodium metabisulphite, adjusted to pH 2.91 with citric acid resulted in slight changes by almost 20% in pH and titratable acidity using sweet potatoes 'Colorada Correntina' variety during storage at 5 and 10 °C with slight increase in ascorbic acid content. High concentration of anti-browning treatments here shown to exhibit higher ascorbic acid content value compared with the 1% and control sample. Nevertheless, these values did not denote high variations among other treatments.

Table 2: Effects of different anti-browning agents (total soluble solids, pH, titratable acidity and ascorbic acid content) on quality.

Treatment	TSS (°Brix)	pH	TTA (% citric acid)	Ascorbic acid content (mg/100g FW)
Control	3.90±0.40^{b}	5.38±0.12^{b}	0.154±0.06^{c}	2.31±0.33^{bc}
1% ascorbic acid	4.22±0.60^{a}	5.34±0.06^{b}	0.137±0.01^{c}	2.29±0.33^{c}
2% ascorbic acid	3.97±0.51^{ab}	5.17±0.17^{b}	0.171±0.03^{b}	2.50±0.28^{ab}
1% citric	3.93±0.44^{b}	5.43±0.20^{a}	0.133±0.05^{c}	2.16±0.13^{c}
2% citric	3.75±0.49^{b}	5.73±0.24^{a}	0.193±0.04^{b}	2.18±1.20^{a}
1% SM	4.22±0.71^{a}	5.45±0.13^{a}	0.136±0.04^{c}	2.53±0.41^{a}
2% SM	4.06±0.32^{ab}	5.21±0.20^{b}	0.266±0.05^{a}	2.75±1.08^{a}
F-Test	**	*	**	*

*Each value was the mean of four replicates. *Means within columns and factors followed by the same letter are not significantly different based on Duncan Test at P ≤ 0.05. NS,*, ** non-significant, significant and highly significant at P < 0.05 and P < 0.001, respectively.*

Conclusion

In this study, sodium metabisulphite was found to be the most effective browning inhibitor for freshly cut jackfruit and can retain its quality within 7-11 days. However, other treatments using ascorbic acid and citric acid solutions were neither effective nor recommended: both for maintaining flesh colour and as preservatives for quality fresh cut jackfruit. However, further studies are needed by using other effective and safe organic inhibitors.

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Separation of Phytosterols from Orange Juice by Ultrafiltration

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Introduction

Nowadays, natural products from plants are being used in food (Shahidi and Ambigaipalan, 2015), nutraceutical (Sundram et al., 2003), and pharmaceutical (Aiello et al., 2019) industries. Plant sterols, generally known as phytosterols are cholesterol-like compounds that are found in vegetable oils, nuts and fruits (Wang et al., 2018). Phytosterols can lower blood cholesterol levels and thus decrease risk of coronary heart diseases (Ogbe et al., 2015; Meng et al., 2019). Phytosterols are one of the most widely used groups of food additives in food products such as margarine, milk and yogurt drinks (Tolve et al., 2018). Among fruit juices, orange juice has been recognised as one of the important food due to high levels of soluble sugars, pectin, proteins, hemicelluloses and cellulose fibers (Awan et al., 2013). Orange juice is a promising potential source of phytosterols, such as β-sitosterol, stigmasterol, campesterol and other minor sterols (Piironen et al., 2003; Jiménez-Escrig et al., 2006). This paper describes the separation of phytosterols from orange juice via an ultrafiltration process. The objectives of this study are to develop the ultrafiltration process for the separation of phytosterols from orange juice using regenerated cellulose acetate (RCA) membranes and to optimise the phytosterols analysis method. Orange juice as a model solution that has similar bioactive compound with natural rubber serum has been used in this study. Orange juice was found to have similar total phytosterols content (0.2-0.3 mg mL^{-1}) to those present in natural rubber serum (NRS). The pH of orange juice (pH 3.45) (Jiménez-Escrig et al., 2006) was almost similar with pH of NRS (pH 3.56) (Muhammad, 2018). Therefore, the same concept is thought to be applicable and transferable to natural rubber industry.

Materials and Methods

Materials

Orange juice (not from concentrate) was purchased from the local juice's manufacturer (*Cobell*, UK). It was then stored in a cold room at 4° C up to 2 months. The juice was first pre-filtered using a stainless steel 25 μm cartridge filter (*Memtech*, UK) to remove pulp prior to ultrafiltration. Chloroform, methanol, acetic anhydride and sulphuric acid were obtained from *Merck*, UK. Stigmasterol from *Sigma Aldrich*, UK was used as characterisation standard. Protein assay kit was acquired from *Bio-Rad*, UK. All the regenerated cellulose acetate (RCA) membranes used were supplied by *Alfa Laval*, Denmark.

Particle size analysis

The particle size distributions were characterised by the light scattering techniques such as laser diffraction and dynamic light scattering (Williams et al., 2017). In this study, the particle size distribution was analysed by laser diffraction using a Malvern Mastersizer X (*Malvern*, UK) at 20 ^{o}C. Five to 10 mL of orange juice was dispersed in reverse osmosis (RO) water and introduced into the Mastersizer and circulated at 2000 rpm. The reading was recorded by a computer equipped with a Malvern Mastersizer software.

Ultrafiltration experimental setup

The ultrafiltration experiments were carried out by using a cross flow membrane filtration system *LabStak M10* manufactured by *DSS* (now *Alfa Laval*), Denmark. The total filtration area of the membrane was 0.036 m^2. The crossflow velocity (CFV) was measured by a rotameter. The transmembrane pressure (TMP) was measured using a pressure transducer. The ultrafiltration method consists of membrane conditioning, pure water flux (PWF), filtration, rinsing and cleaning steps (Argyle et al., 2015).

Total phytosterols analysis

Total phytosterols content was determined spectrophotometrically by using Liebermann-Buchard (LB) based method (Mbaebie et al., 2012; Sathishkumar and Baskar, 2014). The absorbance was measured at 420 nm using an Ultraviolet-visible (UV-Vis) Spectrophotometer (Cary 100, *Agilent,* USA). The LB reagent was prepared by dissolving sulphuric acid in acetic anhydride in the ratio 1:10. 5 mL chloroform was added to 1 mL sample in a test tube, followed by vortex mixed for 1 minute. A portion of 2 mL extract was taken from the solution and mixed with 2 mL LB reagent. The tubes were incubated for 5 to 20 minutes under dark condition at 20 ^oC. The colour of the solution was found to change from yellow to green after the addition of LB reagent indicating the presence of phytosterol. Standard solutions of stigmasterol were used for calibration. Chloroform was used as the blank. The total phytosterol content (TPC) was calculated using the standard photometric correlation Equation (1) (Kim and Goldberg, 1969; Araújo et al., 2013):

$$\text{TPC} = C_s \times \frac{A_u}{A_s} \quad (1)$$

where C_s is the concentration of stigmasterol in standard solution, A_u is the absorbance of the sample, A_s is the absorbance of the standard solution.

Protein analysis

Protein concentration was quantified by the Bradford method (Kruger, 1994; Cassano et al., 2008). The assay is based on the binding of acidic dye solution Coomassie Brilliant Blue G-250 to protein at maximum absorbance from 465 to 595 nm (Bradford, 1976). A calibration curve was constructed by a serial dilution of bovine serum albumin (BSA) from 0.2 to 1.0 mg mL^{-1}. Five ml of diluted dye reagent was added to 100 µL of standard and sample solutions. The mixed solutions were mixed vigorously and incubated at room temperature for 10 minutes. Absorbance for the protein concentration was measured at 595 nm using UV-Vis Spectrophotometer (Cary 100, Agilent, USA).

Determination of selectivity

The effectiveness of membrane process was described in terms of selectivity (Mulder, 1996). Selectivity was expressed as the rejection ratio (R) and calculated by Equation (2):

$$R = (1-\frac{C_p}{C_f}) \times 100 \quad (2)$$

where C_p was the solute concentration in the permeate and C_f was the solute concentration in the feed.

Results and Discussion

Modification of total phytosterol analysis

Total phytosterols analysis was carried out after UF using UV-spectrophotometer by the LB method. The LB method have been widely used for the qualitative and quantitative determination of steroids

especially cholesterols (Kenny, 1952; Kim and Goldberg, 1969). Phytosterols are cholesterol-like molecules that are present in plants and therefore LB method has been applied in this work (Mbaebie et al., 2012; Araújo et al., 2013; Sathishkumar and Baskar, 2014). The analysis method was modified for the total phytosterols content in orange juice since there is no study reported using this sample. The incubation time for the reaction to take place was modified because other studies used different incubation times between 5 to 30 minutes before the UV analysis. The LB reagent reacts with the chloroform extract to produce a greenish solution that indicates the presence of phytosterol, and the absorbance was measured via UV spectrophotometer. In the existence of the LB reagent, the phytosterols were protonated and dehydrated with loss of H_2O, which produces carbonium ion of 3,5-cholestadiene (Burke et al., 1974). The absorbance for phytosterols detection was observed at two wavelengths i.e. 420 nm (Kenny, 1952; Mbaebie et al., 2012) and/ or 625 nm (Kim and Goldberg, 1969; Araújo et al., 2013) after wavelength scanning from 400 nm to 900 nm. Figures 1 (a) and (b) show the UV spectrums for the LB reaction of standard stigmasterol at concentration 1.0 mg mL^{-1} and 0.5 mg mL^{-1}, respectively. The suitable wavelength for this analysis was at 420 nm, as shown in Figure 1 (c), because the orange juice samples produced the absorbance signal only at wavelength 420 nm. Similar findings have been observed in other studies (Kenny, 1952; Mbaebie et al., 2012). The chemical reaction produced final compound called cholestahexaene sulfonic acid which can be detected at 420 nm. The reaction produced an intermediate compound called pentaenylic cation which detected at 625 nm. The behaviour of the reaction is possibly due to conversion of acetate derivatives of the steroids after the reaction with LB reagent as discussed by Burke et al. (1974). According to the incubation time for the reaction, for both concentrations of standard stigmasterol, maximum absorbance was achieved at 15 min after the addition of LB reagent (Figure 1 (a) and (b)). The absorbance increased from 5 min to 15 min and then decreased after 15 min. Therefore, the incubation time during the analysis of orange juice was carried out for 15 min.

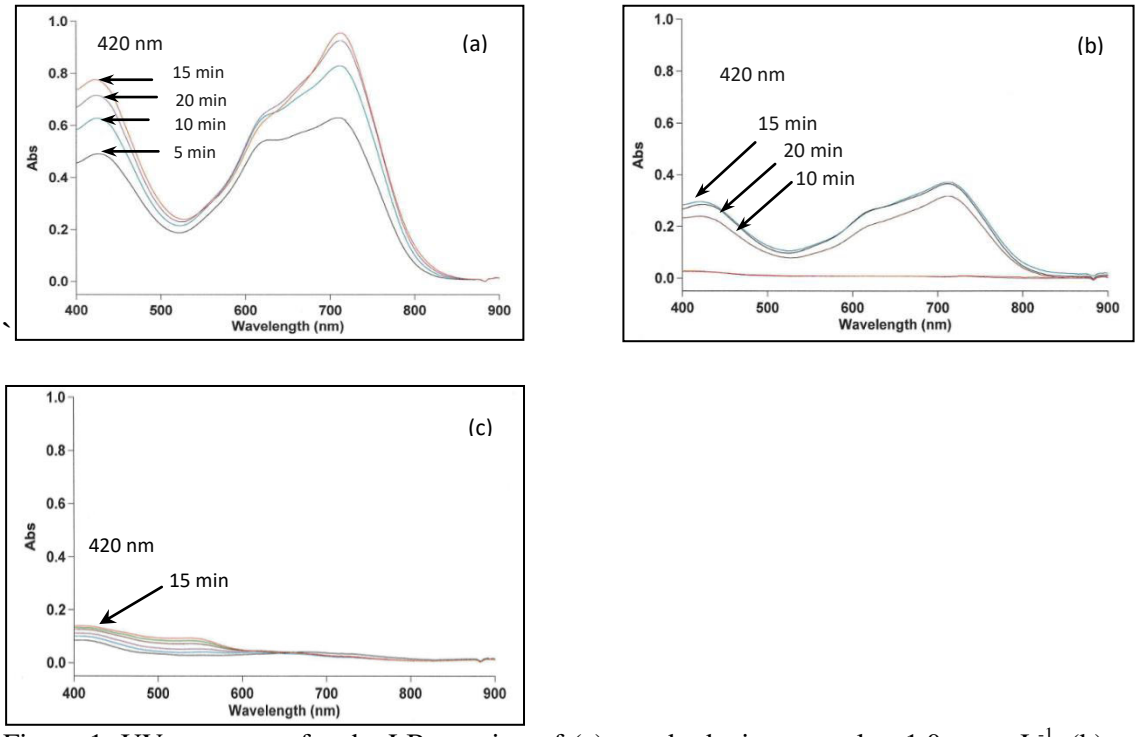

Figure 1: UV spectrums for the LB reaction of (a) standard stigmasterol at 1.0 mg mL^{-1}, (b) standard stigmasterol at 0.5 mg mL^{-1} and (c) orange juice samples at different concentrations. Arrows indicate the absorbance signal at wavelength 420 nm at different incubation time from 5 min to 20 min. Maximum absorbance for stigmasterols standards were achieved at 15 min after the addition of LB reagent ((a) and (b)).

Particle size distribution

Orange juice contains a polydisperse distribution of particle size from pulp trashes to small particles which are less than 2 µm in diameter (Corredig et al., 2001). The pre-filtration step was carried out using a 25 µm cartridge filter. A pre-filtration step was required to remove pulp and particles with diameter > 25 microns prior to UF. The UF process was conducted using RCA membranes with 10 kDa MWCO. To assess the efficiency of the pre-filtration and UF process, particle size distribution of orange juice was investigated. Figure 2 shows the particle size distribution in four orange juice samples namely, Pre-filtration-Feed, Pre-filtration-Permeate, UF-Feed and UF-Permeate. Sample named Pre-filtration-Feed was fresh orange juice before the pre-filtration process and Pre-filtration-Permeate was orange juice collected after pre-filtration using the 25 µm cartridge. Sample labelled as Pre-filtration-Permeate was kept for 24 hrs and used as the feed for the UF (UF-Feed). Meanwhile, UF-Permeate refers to orange juice that was collected after the UF in the permeate. The analysis clearly shows that Pre-filtration-Feed contain particle size more than 100 µm. The particle sizes for Pre-filtration-Permeate and UF-Feed were less than 25 µm. However, the particle size distribution differs between both samples. This confirmed the stability of the feed sample for the UF after 24 hrs of storage, but the samples might be coagulated after 24 hrs storage. The particle sizes of UF-permeate after the ultrafiltration were around 1 nm at 95% composition. To conclude, the UF produced smaller particle size than the pre-filtration and the pre-filtration using 25 µm cartridge filter was efficient in this system.

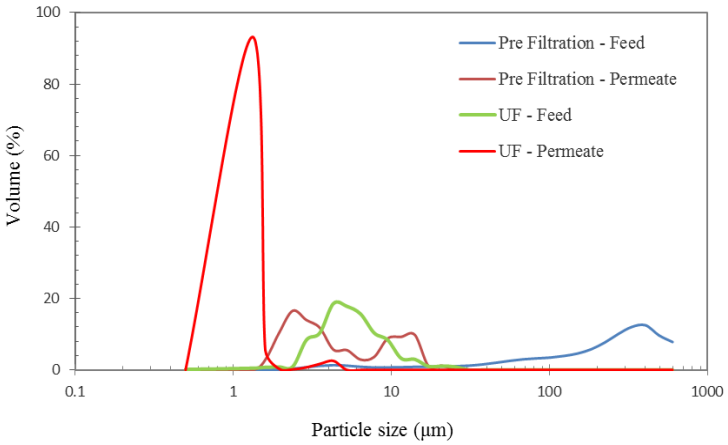

Figure 2: Particle size distribution in different orange juice samples.

Rejection of membranes towards analysed compounds

Figure 3 shows the rejection of total phytosterols and proteins by RCA 10 kDa membranes at three different temperatures. The optimal separation would have a low rejection of phytosterols and a strong rejection of proteins. Figure 3 shows that UF at lower temperature (10 °C) using RCA 10 kDa membrane exhibited different separation efficiency with 56±1% rejection of phytosterols and 95±2% rejection of protein. The results also show that more phytosterols were collected in the permeate at higher temperatures. Therefore, an attempt had been made to run the filtration at a higher temperature which was at 40 °C. As expected, lower rejection of phytosterols (35±5 %) was achieved during the UF at 40 °C using RCA 10 kDa membrane. In general, proteins were highly rejected by RCA 10 kDa membrane at 10 °C and 20 °C. However, only 72±2% rejection of proteins was observed at 40 °C. Soy bean processing at high temperature (40-50 °C) changed the conformation of the protein structure that leads to protein precipitation (Zayas, 1997). Temperature above 40 °C caused denaturation of proteins called pectin methylesterase (PME) in orange juice. The composition of the protein changed when hydrogen bonds were broken and the tertiary protein structure unfolded at 40 °C (Aghajanzadeh et al., 2017). Therefore, it is possible that more proteins have been passed through the RCA 10 kDa membrane and collected in the permeate at 40 °C.

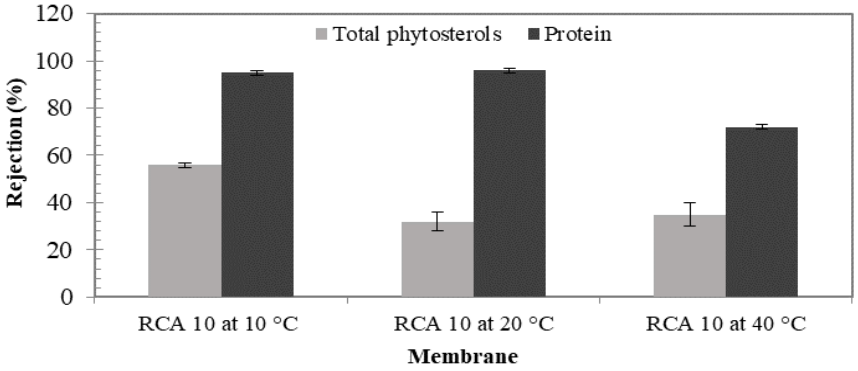

Figure 3: Rejection of phytosterols and proteins by RCA 10 kDa membranes at different temperatures.

Surface roughness measurements

Surface roughness of RCA membranes varied with molecular weight cut-off (MWCO) such that RCA30 > RCA100 > RCA10 (Table 1). All membranes displayed increased roughness values after fouling, indicating that relatively rough surface deposits were present (Jones et al., 2011). Membranes with rougher surfaces displayed a higher fouling capacity than those with smoother surfaces (Gulec et al., 2017). The foulant appears to be more highly entrapped by rougher surfaces. The surface roughness values reduced after cleaning but did not return to the initial roughness values. This could suggest that the surfaces had not been returned to a pristine condition.

Table 1: Surface roughness values.

Membrane	Surface Roughness (nm)		
	Conditioned	Fouled	Cleaned
RCA 10 kDa	3±1	31±2	10±2
RCA 30 kDa	17±1	42±3	20±2
RCA 100 kDa	10±2	39±2	15±1

Surface charge measurements

Figure 4 compares the zeta potential values of different MWCO RCA fouled-once membranes examined at pH 3 to 8. The zeta potential of RCA 100 kDa conditioned membrane was used as a reference. Fouling caused all membranes to have a greater negative charge regardless of the pore size, due to negatively charged species deposited on the membrane surfaces (Jones et al., 2011). The magnitude of the negative charge on the membrane surface fouled-once membranes varied with MWCO such that RCA 30 > RCA 100 > RCA 10. This corresponds to the order of the increased surface roughness. It is likely that some foulants with positive charges were fouled on both membrane surfaces at lower pH. This may suggest that protein foulants with positive charges were deposited on membrane surfaces, since proteins become positively charged at low pH and negatively charged at high pH.

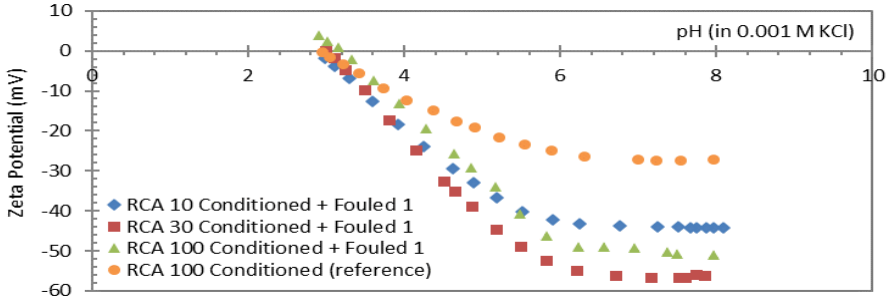

Figure 4: The zeta potential of RCA conditioned and fouled membranes as a function of pH.

Conclusions

The best separation of phytosterols from orange juice (43±2 mg L^{-1}) with the lowest rejection of phytosterols (32±4%) and the highest rejection of proteins (96±1%) with a selectivity factor of 17, was obtained using regenerated cellulose acetate (RCA) 10 kDa membrane at 20 °C. Ultrafiltration at low temperature was found to be more effective in separating phytosterols in orange juice to avoid precipitation of proteins and to reduce membrane fouling. Membrane surface roughness and surface charge varied as a function of MWCO such that RCA30 kDa > RCA100 kDa > RCA10 kDa. It can be concluded that membranes properties were more important than MWCO in determining the performance of ultrafiltration membranes in this system. The best wavelength for total phytosterols analysis of orange juice was found at 420 nm with 15 min incubation time. These findings have important implications for the industrial application of membrane technology to process orange juice and other sterol-rich feeds such as natural rubber serum.

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Different Leaf Maturity Affects the Postharvest Quality of Fresh-cut Curly Kale During Cold Storage

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Introduction

Curly kale (*Brassica oleracea* var. *sabellica*) is a green leafy vegetable belonging to the Brassicaceae family and is mainly cultivated as a salad vegetable. The leaves are usually generally consumed as fresh or cooked and are sold in fresh, canned or frozen forms (Fahey, 2003). Due to its high content of antioxidants, anti-inflammatory properties and dietary fibre (Podsędek, 2007), curly kale is often marketed as a fresh-cut product in the market. The fresh-cut vegetable market is constantly driven by consumers' demand for fresh, convenient and nutritious vegetables as a part of their daily intake either raw or fresh-cut as recommended by the World Health Organization (WHO, 2020) during the rise of the COVID-19 pandemic. The quality of fresh-cut produce is determined by the condition of the raw materials e.g. maturity and ripeness stage at cutting for improved post-harvest handling and storage duration (Barrett et al., 2010). Horticultural maturity for most leafy vegetables such as spinach and Swiss chard, the leaf and petiole length are good maturity indicators to ensure the quality of fresh-cut produce (Gil et al., 2012). For kale, the plant consists of several leaves of different sizes and the leaves are harvested when they reached a horticultural maturity size of 10-15 cm long (Casajus et al., 2021). This study would provide information of leaf maturity since it may influence the quality of raw materials during processing for fresh-cut since expected shelf-life of these products is generally much shorter than whole produce. A high quality raw material is required since fresh-cut processing of vegetables increase their perishability due to the physical damage/wounding caused during preparation (Cantwell and Suslow, 2002). Therefore, the objective of this study was to contribute a better understanding of leaf maturity to the fresh-cut quality of curly kale (*B. oleracea* var. *sabellica*) during cold storage.

Materials and Methods

Harvesting of curly kale

Curly kale used in this experiment was grown using a multilayer self-watering system under a rain shelter located in MARDI, Serdang, Selangor. The optimum harvesting time for curly kale was estimated to be 30-40 days after transplant. After harvest, the plants were placed in plastic trays and transported immediately to Postharvest Complex MARDI on the same day.

Table 1: Leaf maturity of curly kale (*Brassica oleracea* var. *sabellica*) determined by leaf size.

Leaf size	Small	Intermediate	Large
Length (cm)	<20	20-30	>30
Width (cm)	<4	4-5	> 5

Fresh-cut preparation of curly kale

Due to the multiple sizes of leaves of the curly kale in one plant during harvest, the curly kale plants were selected and graded according to leaf maturity (Table 1) adapted from Albornoz and Cantwell (2016) for the kale cultivar 'Lacinato'. After sorting, the leaves were treated with chilled water (2 °C) for 30 seconds. Excess water was shaken off before packing using low-density polyethene (LDPE) bag with a thickness of 0.04 mm. Each LDPE bag contained 8-12 leaves and is equivalent to one replication. All fresh-cut products of curly kale were kept at 5 °C for three weeks.

Determination of fresh-cut curly kale quality during storage

The quality of each leaf maturity of fresh-cut curly kale was visually assessed on a scale of 9 to 1 where: 9 = excellent, fresh appearance, 7 = good, 5 = fair, (limit of marketability), 3 = poor (unusable) and 1 = unusable, severe (Albornoz and Cantwell, 2016). The colour of the leaf was measured using a chromameter (model CR-400 Minolta Corp., Osaka, Japan) and was expressed as lightness (L*), chroma (C*) and hue (h°) value. The leaf texture of the curly kale was measured using a texture analyser (Stable Microsystems, UK). Ascorbic acid (AA) content was determined using the titration method (Ranggana, 1997) and expressed in mg/100g per fresh weight for total phenolic content (TPC) and antioxidant activity, 5 grams of each sample was extracted using 50 % methanol (1: 10 w/v) according to the method described by Leong and Shui (2002) with some modification. The total phenolic content was determined by the spectrophotometric method (Sunita and Dhananjay, 2010) and expressed as milligrams of gallic acid equivalents (GAE) per fresh weight. The free radical scavenging activity of the samples was measured using a 2,2′- diphenyl-1-picrylhydrazyl (DPPH) assay as described earlier (Leong and Shui, 2022) and the scavenging activity was estimated based on the percentage (%) of DPPH radical scavenged. Ferric Reducing Antioxidant Power (FRAP) of curly kale was determined using the method described by (Tachakittirungrod et al., 2007). FRAP values were expressed as ferrous sulphate ($FeSO_4$) equivalents per milligram of fresh weight.

Statistical analysis

The study was conducted using a completely randomized design (CRD) in a factorial arrangement of treatment (three leaf maturity stages x four storage duration with four replicates). The data were analysed using analysis of variance (ANOVA) (SAS Version 9.4, 2021). A mean comparison was performed using Duncan multiple range test (DMRT) at the 5% significance level.

Results and Discussion

Visual appearance and leaf colour are important quality assessments for most leafy vegetables since it had a direct effect on attractiveness for consumers during purchasing. The interaction effect of leaf maturity (LM) and storage duration (SD) on visual appearance was significant ($P < 0.05$) (Table 2). The visual appearance of small and intermediate leaves had a significantly higher score compared to the large leaf of curly kale during storage (Table 2). The visual appearance of curly leaves of small and intermediate size is still acceptable compared to large leaves after three weeks of storage. The loss of visual appearance in larger leaves might be due to a higher rate of transpiration due to a larger surface area compared to small and intermediate leaves. The L*, C* and h° values of curly kale were not affected by LM except for SD (Table 2). Regardless of leaf maturity, the leaf colour of the curly kale had turned significantly (decreased L* and h° values, increased C* value) ($P < 0.05$) after one week of storage at 5 °C. The change of colour especially in a decrease of h° value indicated the leaves were slowly turning yellow (degradation of chlorophyll and carotenoids) due to senescence processes as observed in Asian leafy vegetables (Able et al., 2003).

The leaf texture of curly kale was not significantly affected by LM except for SD (Table 3). The leaves exhibited signs of wilting and became less firm after 14 days of storage. Prolonged storage of fresh-cut curly kale leaves resulted in a loss of turgor and collapse of cells due to senescence and water

loss (Toivonen, 2011). There was a highly significant interaction effect of LM and SD on ascorbic acid (AA) ($P < 0.001$) (Table 3). Larger leaves of curly kale had higher AA content since the larger leaf are found on the outside of the plant and received more light than small and intermediate leaves resulting in better-developed leaves and higher AA content (Lee and Kader, 2000). A decrease in AA content during storage was due to tissue senescence (Gill et al., 1999). There was also a significant interaction effect of LM and SD on total phenolic content (TPC) ($P < 0.05$) (Table 3). Larger leaves of curly kale contained lower TPC compared to small and intermediate size leaves and it decreased slowly during storage. Radical scavenging activity as measured in the percentage of DPPH inhibition was not affected by LM except for SD (Table 3). From the study, a decrease in TPC and DPPH activity during storage (Table 3) is most likely due to tissue senescence (Gil et al., 1999). On the other hand, there was a significant interaction effect of LM and SD on the Ferric Reducing Antioxidant Power (FRAP) activity ($P < 0.05$) which resulted in a significantly higher value of antioxidant properties found in the intermediate leaf followed by smaller and larger leaf (Table 3). The increased FRAP activity during storage differs from the DPPH assay obtained in this study. These differences occurred since antioxidant compounds react differently according to the type of molecule and the oxidizing agent used (Prior et al., 2005).

Table 2: Main and interaction effects of different leaf maturity on colour development (*L*, C** and *h°*) of fresh-cut curly kale (*Brassica oleracea* var. *sabellica*) during storage.

Factor	Visual acceptance	*L**	*C**	*h°*
Leaf maturity (LM)				
Small	7.83[a]	45.38[az]	22.29[a]	125.91[a]
Intermediate	8.00[a]	44.54[a]	22.29[a]	125.55[a]
Large	6.40[c]	44.41[a]	23.56[a]	124.74[a]
F-significant	**	ns	ns	ns
Storage day (SD)				
D0	9.00[a]	46.21[a]	17.58[b]	128.18[a]
D7	8.43[a]	44.54[b]	25.23[a]	124.40[b]
D14	6.20[b]	43.85[b]	24.08[a]	124.40[b]
D21	6.0[b]	43.73[b]	24.38[a]	12416[b]
F-significant	*	*	**	**
Interaction (LM x SD)	*	ns	ns	ns

[z]*Mean values with different letters in the same column indicate statistically different ($p < 0.05$) according to DMRT.*

Table 3: Main and interaction effects of different leaf maturity on firmness, ascorbic acid (AA) content, total phenolic content (TPC) and antioxidant activities of fresh-cut curly kale (*Brassica oleracea* var. *sabellica*) during storage.

Factor	Leaf texture (N)	AA (mg/100 g FW)	TPC (mg GAE Eq/100g)	Antioxidant activity DPPH (% inhibition)	Antioxidant activity FRAP (mg FeSO4 Eq/100g)
Leaf maturity (LM)					
Small	39.16[az]	48.73[b]	22.51[a]	86.07[a]	466.51[b]
Intermediate	37.50[a]	48.87[b]	21.99[a]	86.02[a]	496.70[a]
Large	37.07[a]	55.55[a]	19.64[b]	85.80[a]	399.95[c]
F-significant	ns	**	*	ns	**
Storage day (SD)					
D0	31.80[a]	58.26[a]	27.29a	84.04[a]	323.56[c]
D7	32.24[a]	52.93[b]	25.89a	84.63[a]	532.92[a]
D14	27.17[b]	53.31[b]	21.01b	88.09[b]	508.16[b]
D21	24.22[b]	29.26[c]	22.72b	88.87[b]	533.48[a]
F-significant	*	**	**	**	**
Interaction (LM x SD)	ns	**	*	ns	**

[z]*Mean values with different letters in the same column indicate statistically different ($p < 0.05$) according to DMRT.*

Conclusions

The results of this study suggested that the fresh-cut leaves of the small and intermediate size of curly kale (*B. oleracea* var. *sabellica*) showed better visual appearance and storage potential (21 days) than large-sized leaves (11 days) at 5 °C. However, the larger leaf had more AA content than small and intermediate-sized leaves, therefore more study need to be done to improve the postharvest performance of fresh-cut curly kale with minimal visual deterioration during storage.

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The Effect of Harvesting Age on Erycomanone Content of Tongkat Ali Root

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Introduction

The determination of active content is important as a guideline for herb harvesting. This active content indicates the required quality of the herb. The higher the activity level of the content, the higher the quality and value for money. Tongkat ali (*Eurycoma longifolia*) is a native herb that has been used for aphrodisiacs, antibiotics, appetite stimulants, health supplements, antipyretics, aging, malaria, diabetes, anxiety, aches and constipation, exercise recovery, fever, increased energy, increased strength, leukemia, osteoporosis, stress, and glandular swelling (Ang et al., 2001; Jamal et al., 2006; Rehman et al., 2016). Its medicinal constituents have also demonstrated promising anticancer efficacy, making it a potential adjunct therapy for the treatment of various types of human cancer (Thu et al., 2018). Its bark is mainly used as a vermifuge, whereas its taproots are used to treat hypertension (Mohd Zaki et al., 2015).

Tongkat ali belongs to the Simaroubaceae family and thrives in Malaysian forests under the forest canopy. Previous research indicates that the primary tongkat ali resources are wild forest reserve collections. Excessive harvesting of wild tongkat ali endangers the species' extinction (Jusoh et al., 2015). So far, no additional research involving tongkat ali harvesting at various ages has been reported. There have been reports of harvests at several locations, but the age of the tree has not been specified (Valero and Serrano, 2013). The optimal harvesting age is the stage in the growth process when sufficient quality and highly active compounds are present (Abewoy, 2021).

Understanding plant development and determine an appropriate harvest stage as a guideline for harvesting and processing require the study of different harvest ages (De Willingen and Van Noordwijk, 1987). In this study, tongkat ali was harvested on an existing farm, and the effect of harvesting age on eurycomanone content was investigated. The purpose of this research was to determine the optimal harvesting age for high eurycomanone production. These findings are the primary impetus for research into high-quality plant material production for the global market. In fact, it serves as a guide for farmers to cultivate high-quality tongkat ali to produce healthcare products.

Materials and Methods

Preparation of plant materials and samples

Tongkat ali was harvested at different ages from selected farms, beginning at 5±2, 10±2, 15±2, 20±2, and 25±2 years. Harvesting is done in two ways i.e. manually and with machinery. A total of four samples were collected, with three replications representing the age studied. The weight of tongkat ali parts such as leaves, stems, and roots has been recorded. The harvested samples were then cleaned, shredded, and dried in an oven for sample preparation.

Eurycomanone extraction and analysis techniques

1) Extraction of solid/powder samples

250 mg of sample is weighed into 10 mL of High-Performance Liquid Chromatography (HPLC) grade methanol solvent and ultrasonically processed for 15 min. The sample was then diluted to a concentration of 5 mg/mL after being filtered through a 0.4 m filter.

2) Liquid sample extraction

A liquid-liquid separation technique was used to extract eurycomanone. In a 1:1:1 ratio, methanol:chloroform:water solvents were added to the sample. After that, the mixture was placed in a separator funnel for 10 min. Two layers will form after 10 min. Rotavapor was used to thicken and collect the top layer. The concentrate was then filtered through a 0.45 m filter with 10 mL of HPLC grade methanol solvent. The resulting solution is ready for HPLC analysis.

3) Analysis of HPLC equipment

Standard curves were made with standard eurycomanone solvents at concentrations of 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, 0.2, and 0.1 µg/mL. In 20 L HPLC systems, samples and standard solutions were injected. The following were the HPLC conditions used: Luna C18 particle size 150 mm x 4.6 mm particle size 5 m column.

Acetonitrile (A) and 0.1 % formic acid in water (B) were used as solvents and were released in the gradient order shown below (Table 1).

Table 1: The mobile phase gradient program.

Time/min	Retention time (mL/ min)	% Solvent A	% Solvent B
0	1.0	10	90
13	1.0	25	75
15	1.0	10	90
20	1.0	10	90

Statistical analysis

The sample was subjected to descriptive statistical analysis, which included the minimum (min), maximum (max), median (med), standard deviation (SD), and coefficient of variation (CV) values for tongkat ali root weight (kg), and percentage content of the active content of eurycomanone for various harvesting age groups. The method employed is a tendency measure based on the effects of tree age and key indicator chemistry (compound content). Its goal is to describe or give an idea of the data that has been collected. In quantitative research, descriptive data analysis is used to determine the characteristics of data involving the tongkat ali tree for each of the ages studied, which are 5, 10, 15, 20, and 25 years, with two years to determine changes in physiology and the content of the eurycomanone.

Results and Discussion

The minimum (min), maximum (max), median (med), standard deviation (SD), and coefficient of variation (CV) values for tongkat ali root weight (kg) for various harvesting age groups have been discovered (Table 2). The weight of roots at different ages shows an upward trend with increasing tree age where at the age of five years, the root weight is in the range of 0.7 to 2.9 kg, at the age of ten years, it is 2.3 to 4.5 kg, at the age of fifteen years, it is 2.5 to 5.6 kg, and at the age of twenty years, it showed the highest yield, 9.5 to 13.20 kg, and it began to decline at the oldest age investigated, which is only 5.8 to 7.9 kg.

Table 2: Descriptive analysis of tongkat ali root weight (kg) at various harvesting age groups, including minimum (min), maximum (max), median (med), standard deviation (SD), and coefficient of variation (CV) values.

Harvesting age (Years)	Min	Max	Med	SD	CV
5±2	0.70	2.90	1.95	0.66	0.36
10±2	2.30	4.50	3.05	0.69	0.22
15±2	2.50	5.60	3.75	0.88	0.23
20±2	9.50	13.20	11.05	1.11	0.10
25±2	5.80	7.90	6.85	0.65	0.10

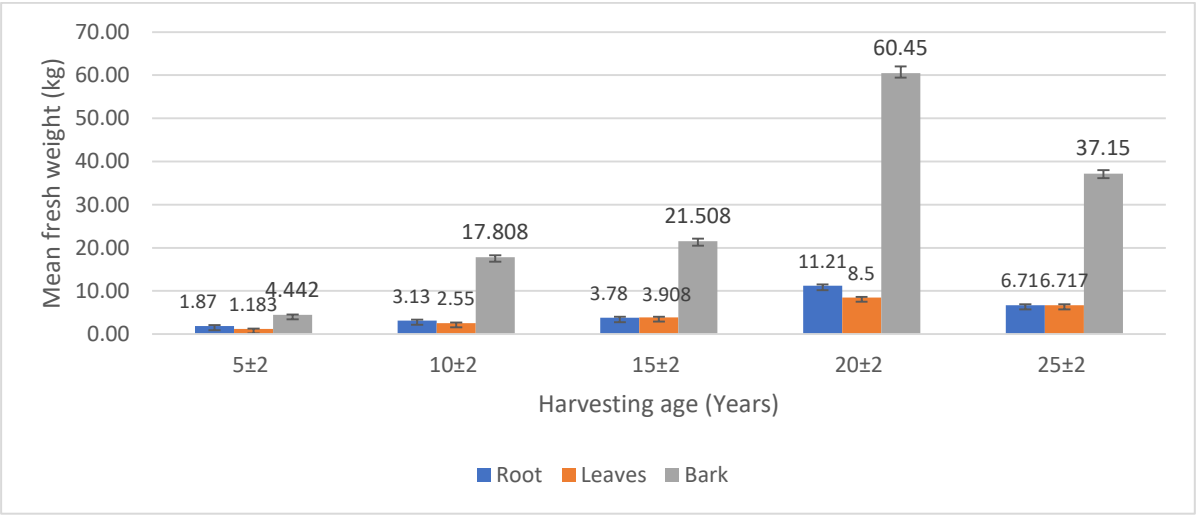

Figure 1: Mean fresh weight (n=12) of tongkat ali root, leaves, and bark at different harvesting age. Bars indicate standard errors.

According to Figure 1, the increase in root weight relates to the increase in the top part of tongkat ali, i.e., leaves and bark. This indicates that the weight of the root at 20±2 years of age is heavier than at earlier ages because the weight of the stem is 60.45 kg and the leaf parts are 8.5 kg. As a result, it's no surprise that the root at the age of 20±2 is the highest. This is due to the importance of the roots in supporting the growth of the tree's top section. Roots absorb water and nutrients, whereas bark distributes and uses them during the growth period. Furthermore, based on morphological balance, it is vital to maximise root growth in order to achieve better growth of the plant's upper half (Bashir et al., 2017).

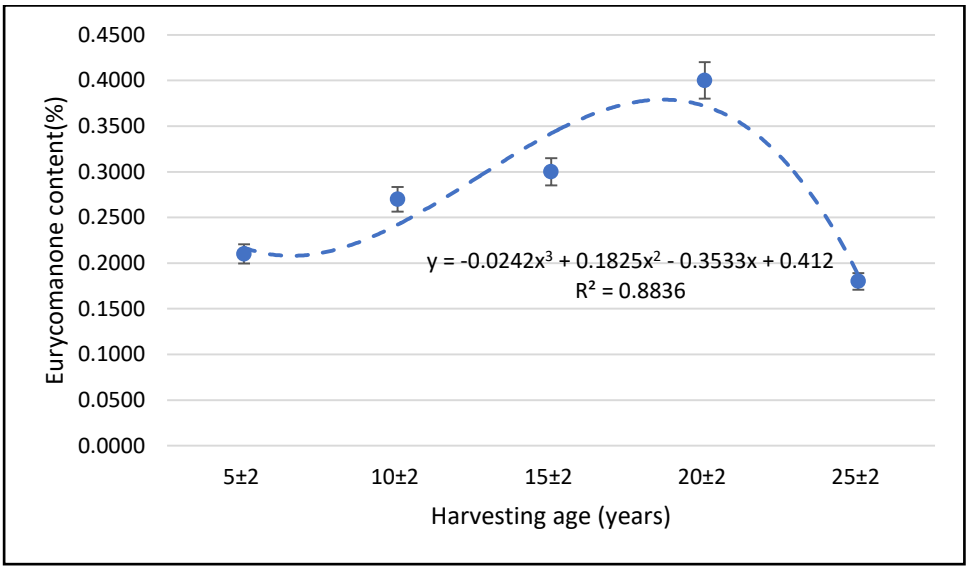

Figure 2: Mean percentage eurycomanone of tongkat ali root, at different harvesting age. Bars indicate standard errors.

The effect of root mean percentage eurycomanone content on tongkat ali age was found to be variable. Figure 2 shows that the mean percentage of eurycomanone content roots increase significantly as the age of the tongkat ali tree increases from 5 to 25±2 years. The content of eurycomanone in the roots of tongkat ali is 0.2% at 5±2 years, 0.27% at 10±2 years, 0.3% at 15±2 years, 0.4% at 20±2 years, and 0.2% at 25±2 years. This finding suggests that the best time to harvest the main indicator chemical compound eurycomanone is around 20±2 years after the tree tongkat ali is planted. This discovery suggests that tongkat ali matured at the age of 20±2, which is five years earlier than previous statements of 25±2 (Mohd Zaki et al., 2015). Tongkat ali is a perennial herb with a long lifespan and the ability to flower and bear fruit repeatedly. These plants are structured and can respond to changes in the environment and temperature. The growth curve is sigmoid-shaped, similar to the data from this study, which is at the zero and constant growth phase at that age.

This stage indicates that the tree has reached maturity and will decline as a result of the ageing process. This trend can be detected in the compound content and chemistry of the main tongkat ali indicators. This suggests that the age of the tree is an important factor in determining quality. This statement is supported by findings from other researchers who conducted studies involving tongkat ali aged 1, 4, and 11 years, showing that the age of the tree has a direct effect on the active content of the compound (Turfan et al., 2018). There are statistically significant differences in the content of chemical compounds related to the age of the tree, and this difference represents changes in metabolites (Song et al., 2019). A similar trend can be seen in other herbs, such as ginseng; the time of harvest and the age of ginseng have been identified as important factors influencing ginseng's effectiveness (Talbott et al., 2013). As a result, many studies on the root of tongkat ali have been conducted, including the development of a method for determining eurycomanone in *Eurycoma longifolia*, tongkat ali aqueous root extract, and commercial products (Low et al., 2013; Khari et al., 2014; Ahmad et al., 2018; Fen et al., 2021, Turck et al., 2021).

Conclusion

According to the study's findings, the yield and percentage of eurycomanone content in tongkat ali vary with age. The eurycomanone content is optimal at the age of 20±2 years. This research can be used to estimate the best age for obtaining the highest eurycomanone content. This discovery provides a basis for further research into the production of high-quality plant material for the global market. In reality, it acts as a guide for farmers growing high-quality tongkat alii for the production of health care

products. To the best of our knowledge, this is the first report on the effect of harvest time on the eurycomanone content of tongkat ali at ages 5 to 25 years.

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Chapter 4

Pest and Disease Management

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Telenomus remus, a Potential Parasitoid for the Biological Control of *Spodoptera frugiperda* in Malaysia

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Introduction

Fall armyworm (FAW) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a new invasive pest in Malaysia. This pest originated from South America but spread to Africa, Asia, and Malaysia in 2019. The fall armyworm, *S. frugiperda*, is a lepidopteran pest that feeds in large numbers on the leaves, stems, and reproductive parts of more than 350 plant species, causing major damage to economically important cultivated grasses such as maize, rice, sorghum, sugarcane, and wheat but also other vegetable crops and cotton. FAW represents a real threat to food security and livelihoods of millions of smallholder farmers native to America, FAW has spread across sub-Saharan Africa, the Near East, and Asia. In 2018, *S. frugiperda* was first reported from the Indian subcontinent in Asia (Ganiger et al., 2018). It has since invaded Bangladesh, Thailand, Myanmar, China, and Sri Lanka. In 2019, FAW reached Malaysia and infested grain and sweet maize. The current control strategy method most practiced by farmers is to use insecticides to manage outbreaks of *S. frugiperda* populations (Morales and Ferguson, 2001). Among the negative effects of using insecticides is the occurrence of insecticide resistance in FAW and environmental pollution (Diez-Rodriguez and Omoto, 2001).

Biocontrol has great potential to control pests, such as insects, mites, weeds, and plant diseases, using other organisms. The main biocontrol of pests especially insects are through predation and parasitism. Biological control for FAW has used a few parasitoids and predators to control the population and the damage it caused. Among the parasitoids used, one of the parasitoids was actually imported from Sarawak and New Guinea. The eggs parasitoid, *Telenomus remus* which originated from Peninsular Malaysia, Sarawak and New Guinea was first described in 1935 (Polaszek and Kimani, 1990; Wengrat et al., 2021). *T. remus* was found to be a potential biocontrol agent in many countries as it attacks various *Spodoptera* species (Kenis et al., 2019). The success of biological control in integrated pest management (IPM) depends on understanding the biology and ecology of insects and their natural adversaries (Cave, 2000). Therefore, the objective of the study is to study the efficacy of *T. remus* as biocontrol agent of *S. frugiperda* in Malaysia.

Materials and Methods

Survey for samples collection

Survey areas were conducted in maize plots in MARDI Serdang and Farmer's plots in Sekinchan, Selangor. The survey was conducted according to the FAO guide on integrated management of the FAW on maize. Plants were thoroughly inspected at each part including leaves, stalk, tassel, fruit, tiller, and leaf sheath according to their stages. This thorough inspection was done to search for FAW egg masses which can be deposited on any part of the maize plant. Any FAW egg masses were collected and kept in a pill container with a net as a cover and brought back to the laboratory to be observed. Unparasitized eggs became neonates and were transferred to another container containing maize kernels for larval food. Larvae are bred to adults to ascertain the species. Any emergence of parasitoids from egg masses was recorded, reared, and identified.

Rearing of parasitoid

Parasitoid emerging from FAW eggs was collected and transferred to a new pill container with a netted cover for aeration. The parasitoid was placed in the laboratory with a temperature of 27±2 °C and 70±10% humidity with an 8-hour light:16-hour dark-light cycle. Pure honey was given and put inside the pill container as a food source for the parasitoid. Fresh and newly deposited FAW egg masses were put in a chiller for 4 hours to stop the egg's development or kill the FAW eggs before it was given to the parasitoid to be parasitized as its next generation. FAW egg masses were replaced every day and kept in a different pill container. New egg masses were put inside a container with parasitoids until all the adult parasitoids are dead.

Life cycle of parasitoid

The newly emerged of parasitoids were collected and sorted into 1 male and 1 female and put in a different pill container. Pure honey was given to the parasitoid pair in the container as a food source. FAW egg masses were put and replaced every day from the container with the parasitized egg masses being put in a new container. The life of the male and female adult parasitoids was observed and recorded until it was deceased.

Results and Discussion

From the survey conducted a total of 10 FAW egg masses were collected from maize crops during sampling. Of the total collected, only 1 FAW egg mass was parasitized by parasitoid in the farmer's plot, Sekinchan. The parasitized egg masses showed to have a blackened colour compared to normal egg masses with brownish green colour as in Figure 1. Egg masses were hard to spot and find in the field as FAW egg masses only took 2-3 days to hatch into neonates. Most of the time, only neonate or larva was found during the survey. One of the low parasitism rates on FAW especially was because it was an invasive alien species which could be new to the area and therefore not many natural enemies or biocontrol agents were available.

Out of 10 FAW egg masses collected, only 1 egg mass was found to be parasitized by parasitoid. The parasitoid was then collected and reared in the laboratory. The parasitoid was identified morphologically as *T. remus* Nixon (Hymenoptera: Platygastridae). Morphological description of adult *T. remus*: body length of *T. remus* measuring 0.55 mm from the tip of the head to the tip of the metasoma.

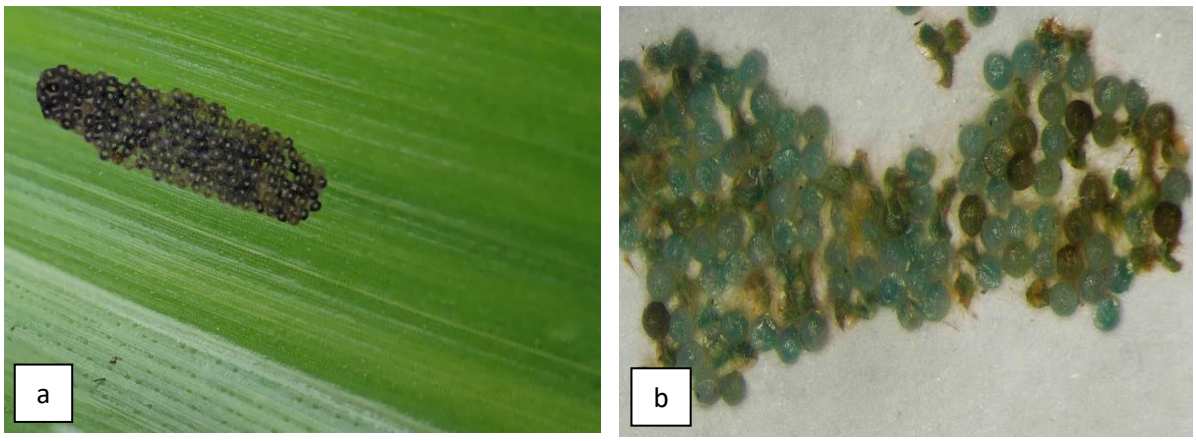

Figure 1: Pasasitized eggs found in maize field (a) and unparasitized eggs (b).

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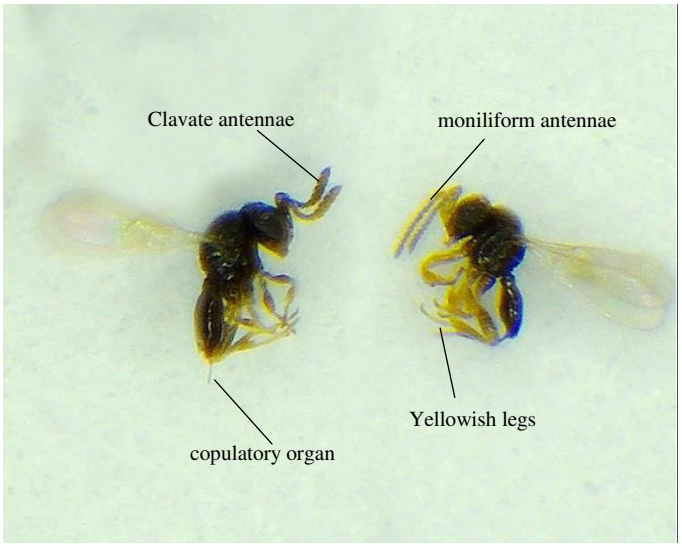

Female Male

Figure 2: Adult female and male *T. remus.*

Female *T. remus* usually have bigger body compared to male *T. remus* (Figure 2). The female usually is black or darker body and legs while the male has yellowish legs. The Female has clavate (club-like) antennae while the male has moniliform antennae (bead-like antennae). The distinct difference between males and females is the copulatory organ of a female that deposits eggs onto host eggs which is absent in a male.

Adult *T. remus* can survive up to 12 days in the laboratory with honey as food source. *T. remus* males emerge from the identical host egg mass faster than females because they have one fewer larval instar than females (Cave, 2000). To ensure that they may mate with females as soon as they emerge, freshly emerging males guard egg masses. Adult males helped to open the eggshells of adult females when it started to emerge from the eggshells. An adult male would fight with other males to mate with a female. Mating happened right after the female emerged from the eggshells. Females search for FAW eggs by using the sensitive clavate antennae. The female *T. remus* drummed on the FAW eggshells to determine whether the eggs are suitable to be parasitized or it already has been parasitized by other parasitoids. Female *T. remus* only parasitized and inserted a single egg onto the single FAW egg to prevent hyper-parasitism. Parasitized FAW eggs became blackened compared to unparasitized eggs which were brownish green. It takes about 10 to 14 days for *T. remus* to develop inside FAW eggs before it emerged into an adult parasitoid (Figure 3).

Figure 3: Lifecycle of *T. remus* which it parasitized and survived on FAW eggs and disrupted its lifecycle.

Conclusions

Fall armyworm, *S. frugiperda* is a new invasive pest and entered Malaysia in 2019. Severe FAW attacks could cripple the grain maize industry and can cause 100% damage if no integrated controls are carried out. Heavy use of chemical insecticides to control FAW may cause injurious to human and environmental health as well as resistance development to FAW. The study on its potential biological control can help to manage the population of FAW in the field and help farmers, especially to reduce the cost of operation of maize production.

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Management of the Fall Armyworm, *Spodoptera frugiperda* on Sweetcorn Through Farmer Participatory Integrated Pest Management (IPM) Approach in Tanjung Karang, Selangor

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Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a destructive pest of various crops globally, dispersing throughout Europe, Africa, Asia, and Australia (Goergen et al., 2016; Early et al., 2018; Kebede and Shimalis, 2019; Hang et al., 2020; Ginting et al., 2020). Originated from the South American continent, this migratory pest can travel long-distance with reported cases of over 100 km in a single night (Johnson, 1987). The *S. frugiperda* was first detected as an invasive species in Africa in 2016 (Goergen et al., 2016). From Africa, the pest has spread across South Asia within a couple of years (Kalleshwaraswamy et al., 2018). It did not take long for the pest to invade South-east Asia. By 2019, the pest has been reported to attack sweetcorn in Vietnam, Thailand, Myanmar, and Indonesia (Ginting et al., 2020; Hang et al., 2020; Yee et al., 2019). In Malaysia, the *S. frugiperda* was first detected in the northern states of Peninsular Malaysia (Kedah and Perlis) in February 2019 (Jamil et al., 2021). By the end of the calendar year, the insect has spread to all states in Malaysia including Sabah and Sarawak (Jamil et al., 2021). The International Food and Agriculture Organization of the United Nation (FAO) has declared the pest as one of the major pests in the world. Furthermore, the FAO also alarmed countries to give considerable attention to the management of *S. frugiperda* as the pest can threaten the food security due to its polyphagous nature (FAO, 2017). Being polyphagous, the larvae of *S. frugiperda* may feed on more than 350 plant species, including several economically important crops such as corn, sugarcane, or rice (Montezano et al., 2018). As the name implies, the presence of large numbers of *S. frugiperda* larvae, causes severe damage to grains and other important horticultural crops. In addition, to ensure their survival, an adult female moth may also lay up to 1,500 eggs throughout its life – complicating the management of this aggressive pest. All these natural features contribute directly to the success of *S. frugiperda* spreading and conquering the globe.

A comprehensive number of studies on different management practices have been recommended so far in America and Africa to reduce the damage and losses caused by the *S. frugiperda* (Midega et al., 2018; Prasanna et al., 2018; Harrison et al., 2019). Most of the recommendations were based on the Integrated Pest Management (IPM) concept. IPM can be defined as a sustainable, ecosystem-based, decision-making process that integrates biological, cultural, mechanical, and chemical approaches to identify, manage and reduce long-term risk from pests and their damage. In IPM, pesticides are only used as a last resort and done after thorough monitoring process with the goal of managing the target organism rather than total eradication. Nonetheless, often, a practice that works well in a location might not be as effective in another location. In addition, due to the recent introduction of this pest in Malaysia, there is limited information about the management of *S. frugiperda* locally. The main objective of this study was to validate the efficacy of a farmer participatory-based Integrated Pest Management (IPM) approach to manage the *S. frugiperda* on sweetcorn. Previously, sweetcorn farmers in Sungai Burong Tanjung Karang reported losses of up to 70% (pers. comm.). Information

gained from this study may directly impact local corn farmers as well as relevant stakeholders to combat and minimize the damage of *S. frugiperda* on sweetcorn in Malaysia.

Materials and Methods

Location

The study was carried out from September 2021 to December 2021 in a farmer-owned maize plot in Jalan Tali Air 2, Sungai Burong, Tanjung Karang, Selangor (3° 30' 34.3116" N, 101° 10' 45.1992" E). The evaluation of the farmer-participatory IPM approach was done in a 1.5-acre land owned by the farmer and was compared to a similarly sized, adjacent land where the farmer practiced conventional methods to cultivate the sweetcorn. The experiment was conducted on a plain agricultural land and sweetcorn were cultivated in three staggered stages (two weeks apart). The study was done in staggered to complement the number of farmers and to facilitate farmers marketing their yield. The average temperature during the experiment was 28.7 °C with a maximum temperature of 42.4 °C and a minimum temperature of 22.8 °C. While the maximum temperature recorded was relatively high (42.4 °C), it was recorded only for one day and did not adversely affect the growth of the plant. Mean relative humidity throughout the experiment was 82.5%. Temperature and relative humidity were taken using a data logger (OM-CP-RHTEMP1000IS-A, OMEGA, Germany).

Farmer-participatory IPM approach

The farmer-participatory IPM approach (f-IPM) module used in the study was developed by the Malaysian Agricultural Research and Development Institute (MARDI) in 2021. The f-IPM strongly promotes the involvement of farmers in every single agronomic activity as well as data observation and data collection. During the experiment, in f-IPM plots, monitoring of the *S. frugiperda* were done daily. Plants were carefully inspected from signs of damage as well as the presence of insects. Upon detection, fall armyworms are physically destroyed by hand. Both, monitoring and mechanical control methods, were conducted regularly throughout the experiment. Spraying of pesticides was only done when necessary (if the number of fall armyworm reached an average of 3 individuals per plant) and was exclusively done by the farmers themselves under the supervision of the researchers.

Farmer's conventional approach

The sweetcorn farmers in the project used synthetic pesticides as the sole method to manage pests upon detection. Generally, the farmers often follow a scheduled-spraying regime, and the rule of thumb was to spray at least once every fortnight as preventative measures. In addition, the number of sprays with synthetic pesticides in farmer's practice ranged from 6 to 9 in different locations depending upon the severity of pests and diseases in different seasons. The farmers did not practice any monitoring measures nor mechanical control throughout the study period.

Sampling and observations

Three subplots of 0.5 acres were chosen in each treatment for recording observations on pests as well as the beneficial insects in the experimental plot. In each subplot, scouting was done by inspecting ten plants per transect. Four transects were established by moving along a W-shape design. Another final transect was established in the middle of each zone (Figure 1). In total, 50 maize plants were sampled per subplot in five transects. Distance between two consecutive plants was determined by the function of field size and shape but was representative of each plot area. Sampling of insects was routinely done from 0800 h to 1130 h on a weekly interval. We also recorded the type of pesticides used for insect managements and their respective frequency of sprays. Prices for each pesticide were recorded (at the time of experiment) to evaluate and compare the cost of pesticides used per acre each season. True to the f-IPM concept, yield data were collected and weighed in by the farmer themselves.

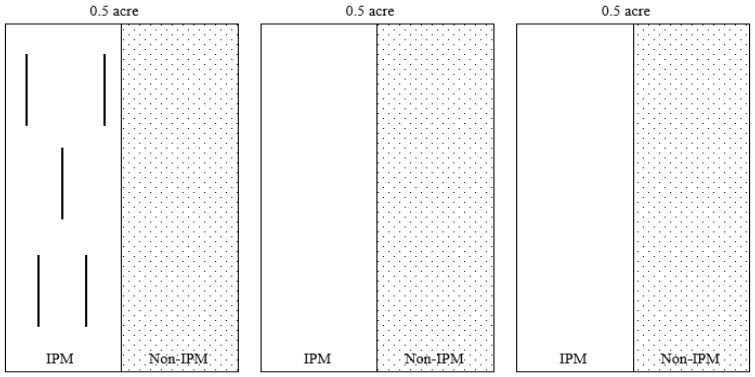

Figure 1: The experimental layout of the study. Line within the plot (left) represents an example of a sampling transects.

Data analysis

The experimental data were subjected to *t*-test for determining the level of significance at p=0.05. The Levene's test for equality of variances was calculated prior to the comparison of means between treatments. A two-sample *t*-test for unequal sample sizes and equal variances was chosen if the two distributions of the pests and beneficial insects' population have similar variances. However, if the variances between the two treatments do not have equal variances, then the Welch's *t*-test (two-sample *t*-test for unequal (or equal) sample sizes and unequal variances) was applied instead. The subplots served as replicates for each treatment. The mean values of pest and beneficial insects for each subplot were done using the statistical software Minitab 20 (Minitab LLC., 2020).

Results and Discussion

The farmers participatory IPM approach was considerably successful from an economic perspective. We reduced the frequency of spraying from 6 times per season per acre (conventional method) to a modest average of 1.67 sprays per season per acre – a reduction of 66.7% sprays per season. Based on spraying frequency, only two sprayings were needed to manage the insect pests throughout the study in the f-IPM plot compared to six sprayings for the conventional plot. Furthermore, based on the type of pesticides used, only two classes of pesticides were used in the f-IPM plot: chlorantraniliprole and emamectin benzoate. In contrast, farmers used four types of pesticides in the conventional plot to control the pests: chlorantraniliprole, emamectin benzoate, lufenuron and acetamiprid. From an economic point of view, due to the smaller number of sprayings, farmers applying the f-IPM approach managed to save RM300 (approximately 76.9%) in pesticides costs (RM190 for the f-IPM vs. RM390 for the conventional). A total number of 4,965 cobs were harvested from f-IPM plot whereas 4,564 cobs were collected from the conventional plot. While not statistically significant, the f-IPM plot did record higher number of cobs in comparison to the conventional plot. The f-IPM treatment recorded a total weight of 2,121 kg in comparison to the conventional treatment which only recorded 1,869 kg (8.4% greater cob count and 12.6% greater weight count in the f-IPM treatment). The average weigh per cob was also higher in the f-IPM plot compared to the conventional plot (0.43 g vs. 0.41 g).

Our experiment recorded a total of 11,865 arthropods (including pests, beneficial insects, and visitors) from 8 major orders (i.e., Hymenoptera, Orthoptera, Lepidoptera, Hemiptera, Coleoptera, Diptera, Blattodea as well as Arachnids). Numerically, we collected more insects in the f-IPM plot in comparison to the conventional treatment (6,090 > 5,775) but statistically there were no significant differences between both treatments ($F(1,18) = 0.14$, $p = 0.715$) (Figure 2). Regarding the number of *S. frugiperda*, there was no significant differences in the population between f-IPM (363 individuals) and conventional plot (382 individuals) ($F(1,18) = 0.08$, $p = 0.779$) (Figure 3). Similarly, the total number of pests between treatment was not significant too ($F(1, 18) = 0.82$, $p = 0.378$). The f-IPM plot recorded a total of 2,854 pests compared to 3,352 pests collected in conventional plot,

respectively (Figure 4). It is important to note that numerical wise, however, there are more pests in the conventional plot compared to f-IPM plot. Common pests in both treatments include the plant hopper *Peregrinus* spp., grasshopper *Melanoplus* spp., fall armyworm *Spodoptera frugiperda*, and corn earworm, *Helicoverpa armigera* (Figure 6). Interestingly, the number of beneficial insects is significantly higher in f-IPM plots compared to conventional plots ($F(1,18) = 5.81$, $p = 0.027$) (Figure 5). A total of 2,808 individuals of beneficial arthropods were collected in the f-IPM treatment in contrast with the conventional treatment, in which only 1,965 individuals were collected throughout the experiment. Common beneficial insects in the f-IPM include the fire-ants *Solenopsis* spp., ladybug *Cheilomenes sexmaculata*, rove beetle *Paederus* sp., and spiders such as the *Oxyopes* spp. (Figure 7).

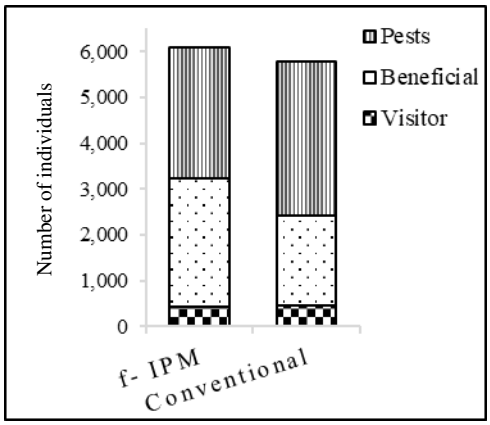

Figure 2: Total number of arthropods between f-IPM vs. conventional plot.

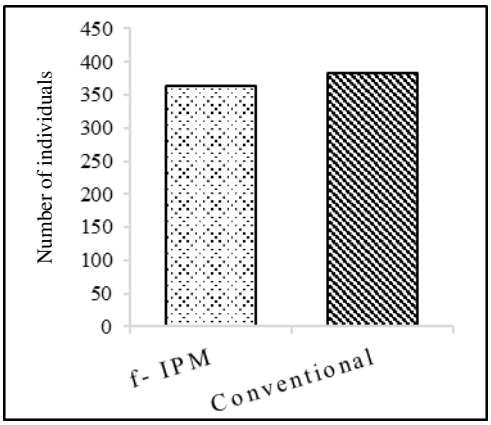

Figure 3: Number of *Spodoptera frugiperda* between f-IPM vs. conventional plot.

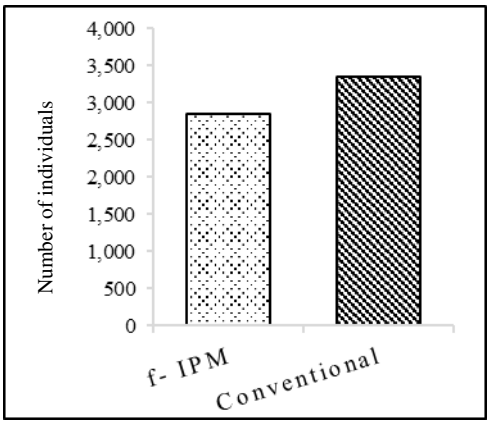

Figure 4: Total number of pests between f-IPM vs. conventional plot.

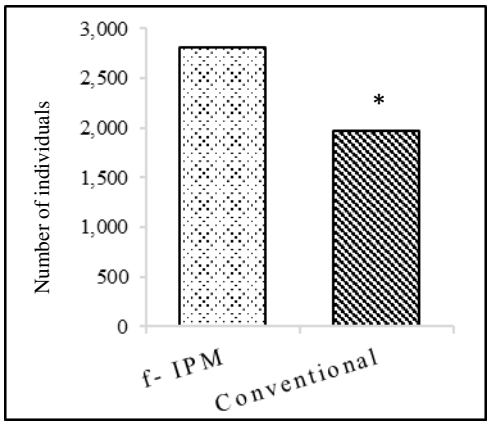

Figure 5: Total number of beneficial insects between f-IPM vs. conventional plot.
(significantly different at $p < 0.05$)*

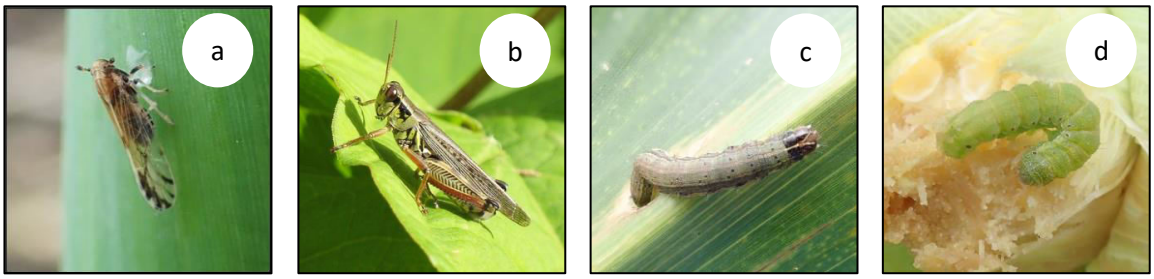

Figure 6: Major pests of sweetcorn in Tanjung Karang, Selangor. (a) *Peregrinus* spp., (b) *Melanoplus* spp., (c) *Spodoptera frugiperda* and (d) *Helicoverpa armigera.*

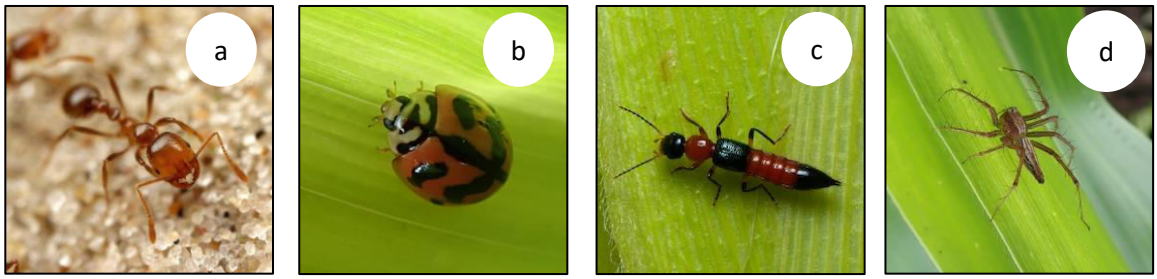

Figure 7: Major beneficial arthropods of sweetcorn in Tanjung Karang, Selangor. (a) *Solenopsis* spp., (b) *Cheilomenes sexmaculata,* (c) *Paederus* sp. and (d) *Oxyopes* spp.

A primary observation of this experiment was the eagerness of farmers to participate and learn about the management of *S. frugiperda* through farmers participatory IPM approach. Younger generation farmers aged between 21-30 years old were more willing to be involved in all steps of the study including data collection and monitoring. There were two main feedbacks received from the farmers: i) they no longer make decision to spray pesticide based on calendar or scheduled spraying or upon sight of a pests and, ii) they now have the basic knowledge to differentiate pests and beneficial insects. These feedbacks indirectly addressed some of the issues related to IPM implementation. A recent survey highlighted some of the issues of IPM implementation locally and discovered that Malaysian fruit growers, in general, showed poor knowledge on different modes of action of pesticides and the ability to identify insect pests (Chang et al., 2021). Similarly, our observations on the eagerness of the farmers to learn about IPM were the same with the results from the same survey. Both gave a positive attitude towards the cost effectiveness of IPM and showed a high willingness to learn and adopt IPM practices in the field. Hence, frequent monitoring and support from the agriculture extension officers as well as researchers are vital to support farmers who are positive towards the implementation of IPM in their farms.

The ecosystem services provided by beneficial insects are important in natural-based pest control such as the f-IPM approach. The use of more pesticides (in frequency and type) has had detrimental effects to natural enemies in the study. Pesticides may affect the number of beneficial insects by i) reducing the survival of a range of life cycle, ii) reducing their reproductive capacity, iii) changing the suitability of hosts for parasitizing or predation, iv) reducing the emergence of parasitoids from sprayed host eggs and, v) cause direct mortality. In our study, less pesticide used was translated to more beneficial insects in the f-IPM plot. Our results are in confirmation with the findings of Puvvala et al. (2020) who reported more beneficial insect population in IPM grown systems compared to conventional systems in okra cultivation. More pesticides used does not necessarily mean better pests' control. In our study, we found that there was no significance in the number of pests, including *S. frugiperda*, between the conventional method (which used 66.7% more pesticide – based on frequency of spray) and the f-IPM method. Resistance towards pesticides is a popular notion on why pesticides are no longer effective against pests (Grassman, 2021). However, there is no evidence from our study to conclude that the pests collected in both plots showed sign of resistance. The information regarding the resistance of pests against common pesticides in maize is lacking, thus more effort should be focused on this matter locally. Our results were consistent with the findings from Chitti et al. (2021) where conventional chemical management approach showed inconsistent results and was unsatisfactory to control the pest in maize.

Conclusions

The farmers participatory IPM approach (f-IPM) is a practical approach that can be adopted by sweetcorn farmers in Malaysia. The f-IPM approach may lessen a farmer management costs by reducing the spraying frequency while simultaneously provide better yield quality and quantity. While no differences were recorded for the number of pests between the treatments, there were more beneficial insects in the f-IPM plot. In general, this study demonstrated the potential of farmer

participatory-based IPM as a cost-effective measure in managing the fall armyworm on sweetcorn in Malaysia.

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Biocontrol Potential of Rice-associated Rhizobacteria Isolated from Endau Rice Field Against Bacterial Panicle Blight of Rice

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Introduction

Rice is an essential staple food crop for more than 50% of the world's population, including Malaysia. It is grown in many parts in the world such as Asia, Africa, and South and Central America. However, rice crop productivity has been affected by various factors, such as harsh weather conditions in the form of floods, droughts and the outbreak of diseases and pests (Lamichhane et al., 2015). A great number of pathogens, like fungi, bacteria, viruses, and nematodes, infect various parts of rice plants and are responsible for cutting down yields throughout the year (Wende and Wang 2016). *Burkholderia glumae*, the causative agent of bacterial panicle blight (BPB), is one of the key pathogens. Acute infection with this pathogen can cause a 75% yield loss (Ham et al., 2011).

In Malaysia, BPB was first discovered in a rice field in Sungai Ache, Penang, in 2017 and later on in Kg. Banir, Kelantan, in 2018. The obvious symptoms of BPB are specifically panicles that tend to remain upright instead of bending over due to a deficit of grain weight, florets with a darker basal part of the glumes, and a reddish–brown border beyond the florets. These symptoms were comparable to BPB symptoms formerly reported on other continents. The BPB disease was declared to have affected rice granaries in up to half of the states in Peninsular Malaysia (Ramachandran et al., 2020). *B. glumae* likes warm nights and high humidity conditions, which usually occur during the rice-growing season. BPB appears during the rice heading phase, when there are high night temperatures and consistent rainfalls, which are critical environmental conditions for disease outbreaks in rice (Cha et al., 2001). The advancement of this disease happened in Malaysia due to beneficial weather conditions for *B. glumae.*

BPB emerges as a severe rice disease as a result of environmental conditions and the lack of effective management approaches. Oxolinic acid is the only effective chemical treatment against *B. glumae*. However, it's banned in certain countries, and the main issue is related to the occurrence of resistant strains. It is relevant to identify an effective method to manage the pathogen, and one of the strategies is to exploit plant-growth-promoting rhizobacteria (PGPR) (Peñaloza Atuesta et al., 2020). The rhizosphere is a thin layer of soil directly enveloping plant roots. This is a highly significant and active space for root activity and metabolism. An extensive number of microorganisms such as bacteria, fungi, protozoa, and algae coexist in the rhizosphere, with bacteria being the most abundant among them (Mjawita et al., 2013). PGPR can incidentally enhance plant growth by inhibiting or deterring the destructive effects caused by pathogens. This response on soil-borne pathogens may occur through a variety of PGPR–pathogen interactions such as, interference in biofilm formation, pathogenicity factors degradation or antagonistic activity. Antagonistic activity is mediated either by the secretion of hydrolytic enzymes or by small molecules with bactericidal activity (Osman et al., 2017).

Previously, we have successfully isolated PGPRs from rice fields of The Northern Region of Peninsular Malaysia (Rashid et al., 2022). We wanted to expand our PGPRs screening to the south area, and Endau rice fields fit the situation. Endau is a little town in Mersing district, Johor. Endau has 6,000 acres of active rice fields along Kampung Semaloi, as well as the Bukit Pasir and Labong settlements.

The objective of this study is to screen for potential antagonists of *B. glumae* in rice-associated rhizobacteria isolated from Endau rice fields. Antagonism of the Endau rice field isolates against *B. glumae* B35 was tested using the well diffusion method at 30 °C.

Materials and Methods

Bacteria

The strain of local isolate *B. glumae* (BG35) was acquired from MARDI Seberang Perai. The rice-associated rhizobacteria isolates were isolated from rice rhizosphere soil from the Endau rice field. Both strains were cultured on LB agar at 30 °C for 48 hours.

Antagonistic activity

Antagonistic activities were performed through the well diffusion method. A single colony of *B. glumae* strain BG35 and rice-associated rhizobacteria were cultured in 5 mL Luria-Bertani broth at 30 °C with 150 rpm shaking for 24 h simultaneously. Subsequently, 100 µL of a 24 hours *B. glumae* strain 35 culture was streaked on LB agar and incubated at 37 °C for 24 h. Using a cork borer, a 5 mm well was made in the middle of the *B. glumae* strain BG35 LB agar plate. The well was then pipetted with 50 L of a 24 h single colony of rice-associated rhizobacteria. The mixture was incubated at 37 °C. Evaluation of the antagonistic activity of rice-associated rhizobacteria against *B. glumae* strain BG35 was done by measuring the diameter (mm) of inhibition zone, that represent antagonistic activity. For each test assay, three replications were performed.

Results and Discussion

The antagonistic activities of 39 PGPRs isolated from Endau rice fields against *B. glumae* strain BG35 are summarized in Table 1. Table 1 shows the diameter of the inhibition zone after 24 hours of incubation at 30 °C. A total of 33 PGPRs showed inhibition against *B. glumae* strain BG35 growth, with a diameter of inhibition zones ranging from 4 to 18 mm while 6 isolates showed no inhibition (Table 1). The highest inhibition zone of 18 mm was represented by bacterial culture of B1-1-27, that showed a moderate antagonistic activity against *B. glumae* strain BG35. It was followed by inhibition zone of 17 mm (B2-C-2-8), inhibition zone of 16 mm (B1-1-7 and B2-C-2-7), inhibition zone of 15 mm (B1-2-6, B2-3-4, B1-3-10, and B2-D2-6), inhibition zone of 14 mm (B1-1-11, B1-2-1-2, B1-3-11, and B2-D2-4), inhibition zone of 13 mm (B1-B2), inhibition zone of 11 mm (B1-1-12, B1-1-15, B1-2-11, and B1-2-4). Meanwhile, 16 isolates showed a weak antagonistic activity below 10 mm inhibition zone, i.e. inhibition zone of 9 mm (B2-D2-3), inhibition zone of 8 mm (B2-C-2-5), inhibition zone of 7 mm (B2-C-2-2, B2-C-2-3, B2-C-2-4, B2-C-2-6, B2-D2-5, and B2-D2-8), inhibition zone of 5 mm, (B2-C-2-1, B2-C-2-9, B2-C-2-10, B2-D2-2, B2-D2-7, B2-D2-9, and B2-D2-10), inhibition zone of 4 mm (B2-D2-1). In the meantime, six other isolates showed no inhibition (B2-5-9, B1-C-2, B2-5-9, B1-3-4, B2-2-3, and B1-4-1) at all.

PGPRs were successfully isolated from the Endau rice field, and 39 PGPRs were tested against a local isolate of *B. glumae* strain BG35. This study can be used as a guide for the isolation of rice-associated rhizobacteria from other rice fields and selecting PGPRs to inhibit pathogenic bacteria. The data obtained in the present experiment suggest that four PGPRs (B1-1-27, B1-1-7, B2-C-2-7, and B2-C-2-8) were able to moderately inhibit *B. glumae* strain 35 with the highest inhibition zones ranging from 16-18 mm (Figure 1). These isolates will be chosen for further investigation. However, more analyses are needed, particularly to identify the isolates and their inhibition mechanisms.

According to Peñaloza Atuesta et al. (2020), a number of plant-growth-promoting bacteria with antagonistic activity against pathogenic microbes have been isolated from the rice crop rhizosphere. The characteristics of a rice plant make it a suitable habitat for the growth of a diverse range of microorganisms (Mjawita et al., 2013). A few examples of bacteria isolated from plant crop

rhizosphere and leaves that were able to inhibit *B. glumae* growth are *Bacillus oryzicola* (YC7007-YC7010), isolated from the rice root in rice fields in Korea, A20 Streptomyces strain isolated from rice rhizosphere in rice fields in Tolima (Colombia) and *Bacillus spp*., isolated from the leaves of rice plants in Louisiana (Magbanua et al., 2014; Suárez-Moreno et al., 2019; Shrestha et al., 2019). This is consistent with our findings, as early screening discovered promising isolates from the Endau rice field with antagonism activities against the pathogenic *B. glumae*.

Table 1: Antagonistic activity of PGPRs against *B. glumae* strain BG35 using agar well diffusion method.

PGPRs code name	Diameter of inhibition zone (mm)
B1-1-27	18
B2-C-2-8	17
B1-1-7	16
B2-C-2-7	16
B1-2-6	15
B2-3-4	15
B1-3-10	15
B2-D2-6	15
B1-1-11	14
B1-3-11	14
B1-2-1-2	14
B2-D2-4	14
B1-B2	13
B1-1-12	11
B1-1-15	11
B1-2-11	11
B2-D2-3	9
B2-C-2-5	8
B2-C-2-2	7
B2-C-2-3	7
B2-C-2-4	7
B2-C-2-6	7
B2-D2-5	7
B2-D2-8	7
B2-C-2-1	5
B2-C-2-9	5
B2-C-2-10	5
B2-D2-2	5
B2-D2-7	5
B2-D2-9	5
B2-D2-10	5
B2-D2-1	4
B1-1-13	No inhibition
B2-5-9	No inhibition
B1-3-4	No inhibition
B1-4-1	No inhibition
B1-2-4	No inhibition
B1-C-2	No inhibition

** Values are the mean diameter of the inhibitory zone (mm) of 3 replicates.*

(a) (b) (c) (d)

Figure 1: Inhibition diameter zones obtained by well diffusion method for 4 PGPRs with the highest inhibition zones; (a) B1-1-27 (18 cm), (b) B2-C-2-8 (17 cm), (c) B1-1-7 (16 cm), and (d) B2-C-2-7 (16 cm).

Conclusions

Rice-associated rhizobacteria isolated from the Endau rice field were able to inhibit the growth of a local isolate of *B. glumae* strain 35. These PGPR isolates exhibit antagonistic activities and possess biocontrol potential against bacterial panicle blight in rice. However, more tests need to be done, especially the identification of the isolates and their inhibition mechanisms.

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Photosynthetic Performance of Healthy and Virus Infected Sweet Potato Plant at MARDI Serdang

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Introduction

Sweet potato (*Ipomoea batatas* L.) is one of the main and healthy food sources for Malaysian. Originating from the Convolvulaceae family, sweet potatoes are valuable as a source of calcium, protein, vitamins, minerals, dietary fiber and antioxidants (Razean Haireen et al., 2021). One of the popular sweet potato varieties in Malaysia is the Lembayung. It is a new variety of purple sweet potato with high yield and rich in nutrition. It can be eaten fresh or processed into a product such as flour, paste, chips, bread and others.

In Malaysia, sweet potatoes are grown using conventional methods and soilless cultivation system by some commercial vegetable producers. During the growth process especially in the field, sweet potato plants can also be infected by pests and diseases. This can cause loss of yield and yield quality. The infected plants become unhealthy which contributes to a decrease in tuber yield which is also affected by the rate of photosynthesis of the plant. During this study, virus was observed as the main disease in sweet potato. Therefore, this study aimed to evaluate on the effect of healthy and virus infected plant on physiological response of Lembayung sweet potato (*I. batatas).*

Materials and Methods

Planting materials

This experiment was conducted at open field condition at Malaysian Agricultural Research and Development Institute (MARDI) Serdang. Sweet potato cuttings from Lembayung variety were used in this study and were obtained from MARDI Bachok, Kelantan. The cuttings were planted in polybags contained standard soil mixture and irrigated using sprinkler. Treatments consisted of 12 different combination of fertilizers; T1 (NPK 0 kg/ha+ Biofertilizer 1); T2 (NPK 0 kg/ha + Biofertilizer 2); T3 (NPK 0 kg/ha); T4 (NPK 150 kg/ha + Biofertilizer 1); T5 (NPK 150 kg/ha+ Biofertilizer 2); T6 (NPK 150 kg/ha); T7 (NPK 300 kg/ha + Biofertilizer 1; T8 (NPK 300 kg/ha+ Biofertilizer 2); T9 (NPK 300 kg/ha); T10 (NPK 450 kg/ha+ Biofertilizer 1); T11 (NPK 450 kg/ha+ Biofertilizer 2); T12 (NPK 450 kg/ha) were used and arranged in Randomized Complete Block Design with three replications.

Leaf gas exchange

Sweet potato leaf physiological measurements were made at 90 days after planting. Photosynthetic rate, stomatal conductance and transpiration rate parameters were measured using a Portable Photosynthesis System (LI-COR 6400XT, Inc., Lincoln, NE, U.S.A.). Photosynthetically active radiation (PAR) of the leaf sampling chamber was set at 1200 µmol $m^{-2}s^{-1}$. The CO2 flow concentration was 400 ppm (µmol mol^{-1}) and the chamber temperature was maintained at 30 ℃. The humidity flow rate was fixed at 500 µmol s^{-1}, controlled between 50-70%. The measurements were done on mature, fully expanded leaves, between 8.30 to 10.30 am, which was presumed photosynthetic rates would be maximal (DiCristina and Germino, 2006; Izyani, 2018). The leaf gas exchange for net photosynthesis rates (*A*) (µmol $m^{-2}s^{-1}$), stomatal conductance (mmol $m^{-2}s^{-1}$) and transpiration rate (mmol $m^{-2}s^{-1}$) of plants were recorded and statistically analysed.

SPAD value

The SPAD (Relative chlorophyll content) was measured on the same leaves as photosynthetic rate measurement on young fully expanded leaves from each sample between 9.30-10.30 am by using SPAD-502 Chlorophyll Meter (SPAD 502, Minolta-Camera Co., Osaka, Japan). SPAD value data points were recorded at four locations of leaf blade and averaged as a single value. 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).

Results and Discussion

The physiological effect between healthy and virus infected sweet potato plant with different fertilizer combination were observed throughout the experiment. There were significant differences between the fertilizers and healthy or virus infected plant on photosynthetic rate, stomata conductance, transpiration rate and relative chlorophyll content of sweet potato (Figure 1, Figure 2, Figure 3 and Figure 4). Treatment T3 contributed to the highest significant photosynthetic rate among the healthy sweet potato while virus infected plant of T3 were significant lowest (Figure 1). Results for stomata conductance and transpiration rate also showed the same trend on most for all the treatment that healthy plant gave the higher stomata conductance and transpiration rate when compared to the infected plants.

Based on the finding of this study, photosynthetic rate of healthy sweet potato crops for all treatment was found to be significantly higher when compared to plant that have been infected by virus. The results obtained in this study are the same as the study by Razean Haireen et al. (2021) that found that photosynthetic rate was significantly lower in virus infected leaves when compared to the virus free plants. Study by Zanini et al. (2020) on cassava crops also found that photosynthetic alteration that related with changes in chloroplast ultra-structure and carbohydrate metabolism that explained the virus infection effect with yield losses.

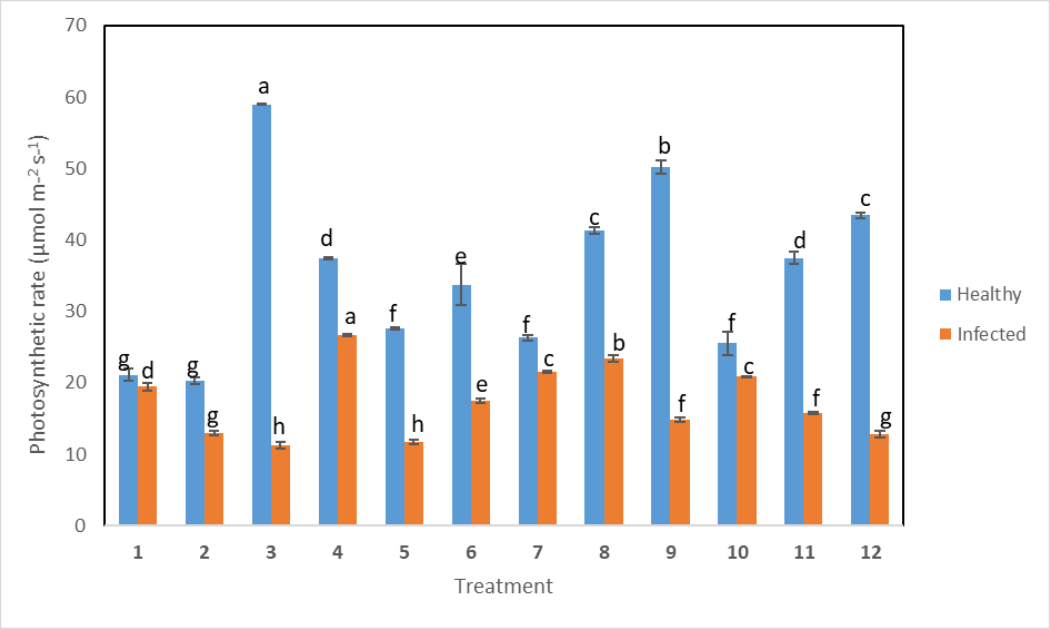

Figure 1: Relationship between fertilizer treatments and photosynthetic rate of healthy and virus infected sweet potato.

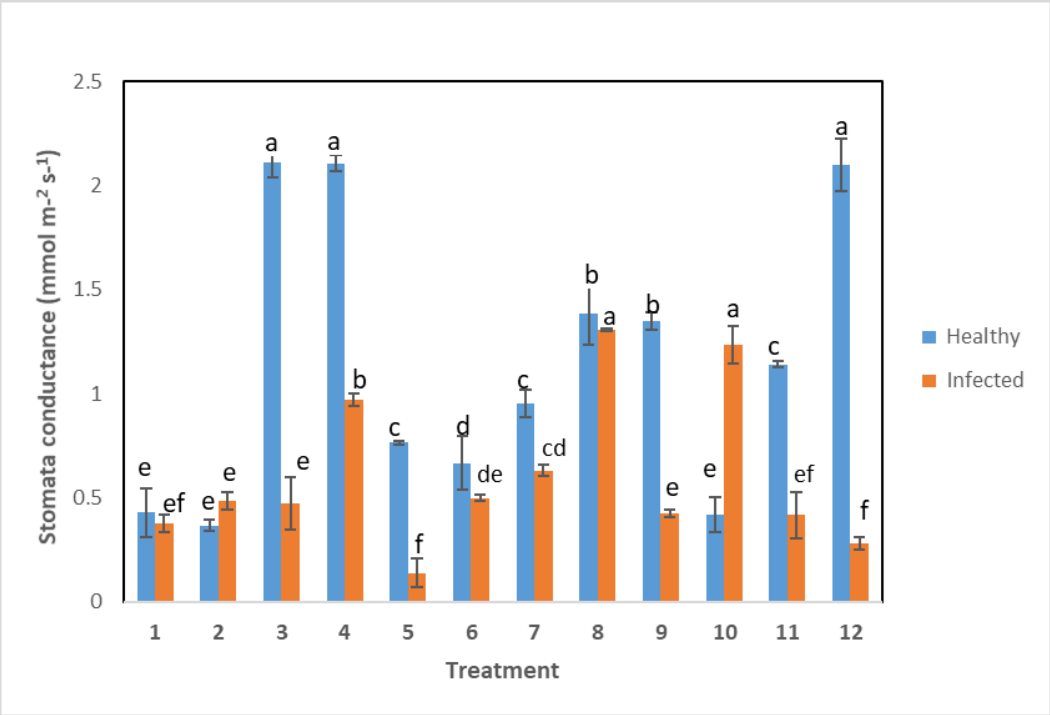

Figure 2: Relationship between fertilizer treatments and stomata conductance of healthy and virus infected sweet potato.

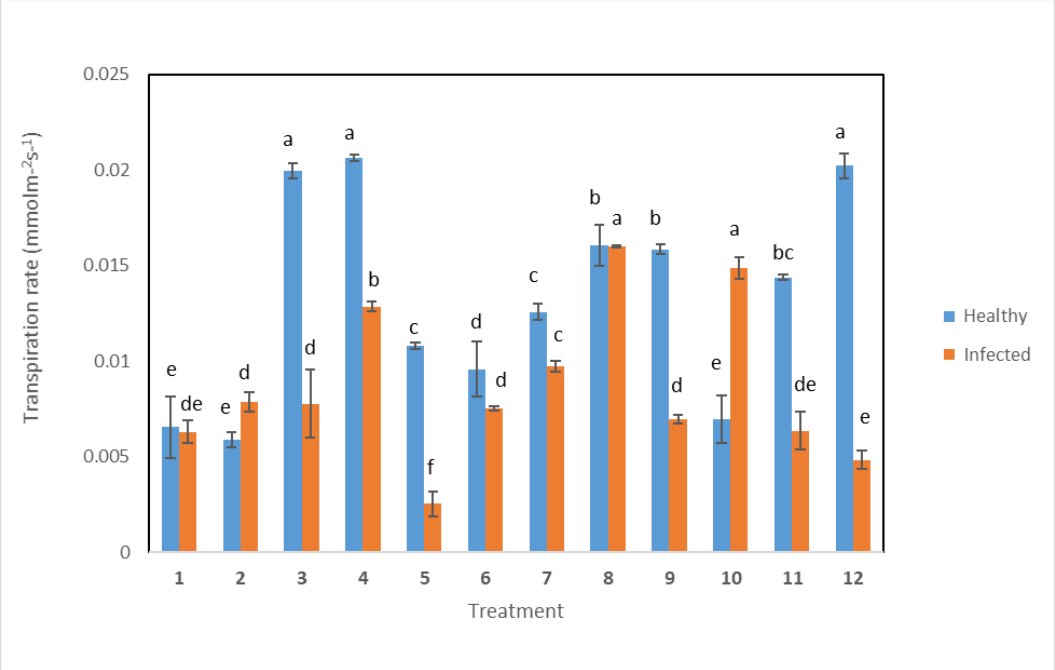

Figure 3: Relationship between fertilizer treatments and transpiration rate of healthy and virus infected sweet potato.

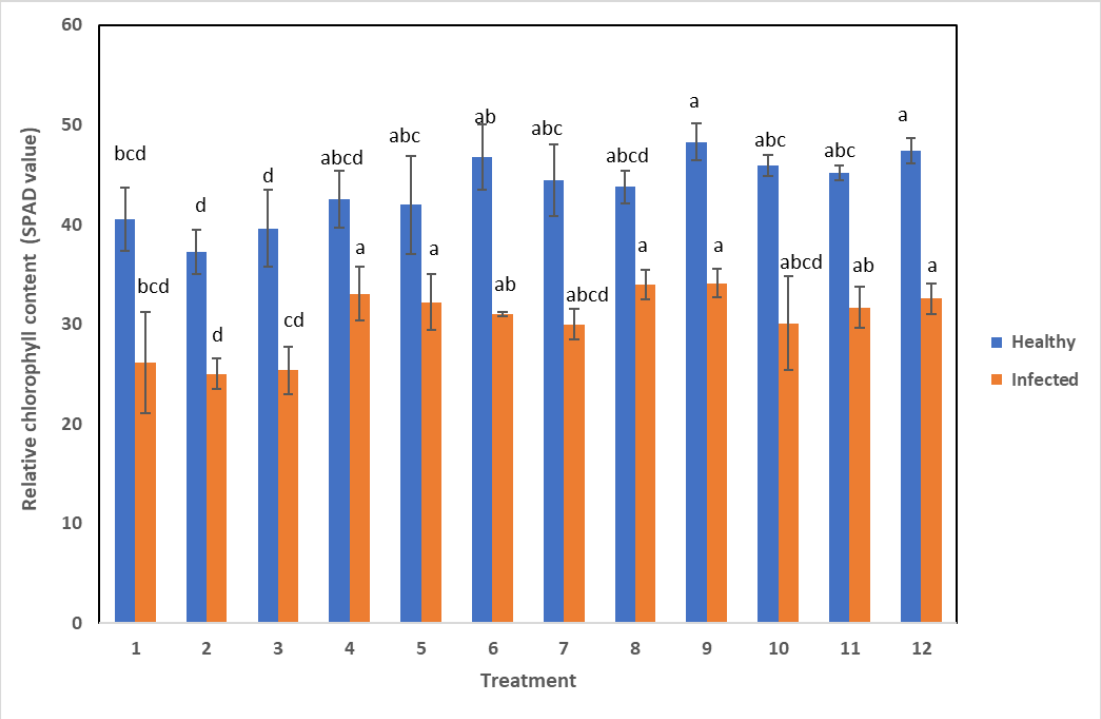

Figure 4: Relationship between fertilizer treatments and relative chlorophyll content of healthy and virus infected sweet potato.

Relative chlorophyll content was significantly different between the healthy and virus infected sweet potato crops. Healthy sweet potato for all treatments showed higher relative chlorophyll content (SPAD meter reading) ranged between 37.3 to 48.3 SPAD value while the infected crop only ranged between 25.0 to 34.1. In this study, reduction in the number of chlorophylls may be due to the chlorophyll biosynthesis and inhibition of chloroplast development, or from the destruction of chloroplasts in plant virus areas (Robledo et al., 1994).

Conclusions

In conclusions, this study found that healthy crops give the highest results for leaf physiological characteristics of sweet potato production for all treatments. All highest significant reading was shown by healthy Lembayung sweet potato for all physiological parameters at 90 days after planting. Further correlation analysis between physiological data and yield or above ground biomass should be done. Therefore, more precise data can be gathered on physiological characteristic between healthy or virus infected sweet potato for understanding the growth and yield of sweet potato.

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Occurrence of Seed-borne Fungi in Different Soybean (*Glycine max* L.) Accessions

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Introduction

Soybean (*Glycine max* (L.) Merrill) is a member of the legume family, Fabaceae. It is an important crop in many countries worldwide as a source of food, oils (both culinary and industrial) and animal feed (Ahmed et al., 2016). Worldwide, soybean is cultivated over an area of 108.51 million ha, with a production of 269.11 million tonnes and productivity of 2330 kg/ha (FAO, 2020). However, several biotic and abiotic stress factors throughout all growing seasons impact soybean production. Diseases are among the most important biotic factors affecting soybean growth and yield (Üstün et al., 2021) especially seed-borne diseases.

Seed-borne diseases can infect soybean seeds before or after planting and can spread rapidly in the fields, leading to significant crop losses (Pérez-Pizá et al., 2019). These diseases are caused by pathogens, such as fungi, bacteria, viruses, and nematodes, which infect the seed and can cause seed rot, seedling blight, and reduced seed germination. Fungi are important as they can cause yield loss and affect the quality of seeds. Several pathogenic fungi from the genera *Rhizopus, Alternaria, Culvularia, Diaporthe, Mucor, Corynespora, Cercospora, Colletotrichum, Phoma, Phomopsis*, *Pythium, Fusarium, Aspergillus, and Cladosporium* have been isolated from soybean seeds (Saylendra and Fatmawaty 2010; Kinnikar et al. 2015; Escamilla et al. 2019).

Disease-free seed production in soybean is important to sustain productivity and maintain the quality of the crop. Therefore, in production systems of high-quality seeds, identifying seed-borne fungal diseases associated with soybeans is vital for managing and developing resistant varieties. Thus, this study was carried out to detect and identify seed-borne fungi that occurred in several soybean accessions.

Materials and Methods

Seed sample

Seed samples of 10 accessions of soybean (A5, A7, A19, A21, A22, A27, A28, A29, A30 and A31) were collected from the glasshouse in December 2021. The seeds were brought to the laboratory, dried to a moisture content <10% (fresh weight basis), and stored in a cold room in an airtight bottle before seed health testing.

Detection and isolation of fungal pathogens from the seeds

Seeds samples were analysed for the detection of seed-borne fungi by the standard blotter paper method (SBM) and potato dextrose agar plate method (APM) following International Rule for Seeds Health Testing (ISTA, 2015) with some modifications. Ten non-sterilised seeds were evenly placed in plastic Petri dishes on three layers of moistened 9 cm diameter filter paper (Whatman No.1). Another

10 seeds were placed on the potato dextrose agar plate after sterilisation by immersion in 1% NaOCl2 solution (3 minutes) and washed three times with sterilised distilled water. A total of 100 seeds were used for each sample. The plates were incubated at 25±2 °C under alternating cycles of 12 hours of light and darkness for seven days. Under a stereo microscope, the growth of the fungal colony was examined. The seed-borne fungi incidence was recorded as a percentage of the total population for each soybean cultivar. Fungi incidence (%) = (Number of seeds infected)/(Number of total seeds) × 100%.

Identification of the fungal isolates

Fungi isolated were identified based on macroscopic (fungal morphology on PDA - form, size, and colour), microscopic (shape, size and colour of conidia) and molecular biology. Fungal species were identified using keys and manuals (Malone and Muskett, 1964; Watanabe, 2002; Mathur and Kongsdal, 2003). Using the formula below (Marasas et al., 1988), the percentage of samples that contained each fungal isolate was calculated and represented as a percentage: Isolation frequency (%) = (No. of samples of occurrence of fungi species/Total no. of samples) X 100.

Experimental design and statistical analysis

The experiments were conducted in a completely randomised design with five replications. Data were analysed by using SAS version 9.4 software. Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure. Treatment means were compared using Tukey's HSD test with a 5% significant level.

Results and Discussion

Incidence (%) of fungi on seeds of different soybean accessions

The blotter and agar plate test methods showed that all 10 soybean accessions were found to be colonised with fungi. Overall, the percentage of fungal incidence in the SBM was higher than in the APM for all soybean accessions (Figure 1). In SBM, A5 (100%) has a significantly higher fungi incidence than A21 (52%), A22 (74%), A27 (40%), A28 (42%), A29 (44%), and A31 (24%), but it was not statistically different from A7 (96%), A19 (84%) and A30 (80%). In APM, A5 (2%) and A28 (4%) had significantly lower fungal incidence than A7 (18%), A19 (22%), A21 (40%), A22 (24%), A27 (38%), A29 (12%), A30 (38%), and A31 (14%). When the two isolation procedures were compared, it was discovered that the fungi incidence was higher in SBM than in APM. This is to be expected since the seeds that were plated on PDA had been subjected to surface sterilization.

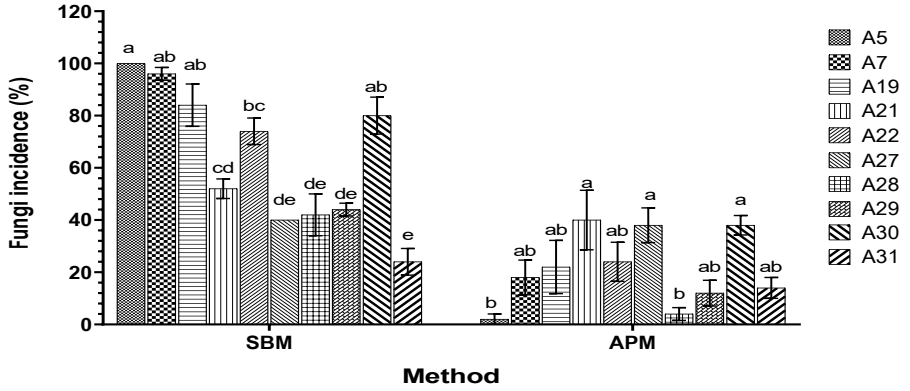

Figure 1: Fungal incidence (%) of soybean seeds in standard blotter method (SBM) and agar plate method (APM). Means of bars followed by different letters indicate significant differences at $p \leq 0.05$ level according to the Tukey test.

Seed-borne fungal pathogens isolated and identified from standard blotter and agar plate methods

A total of 25 fungal species were isolated from soybean seeds; namely, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus* sp., *Cercospora capsici*, *Cercospora cf flaggelaris*, *Chaetomium* sp., *Colletotrichum gleosporiodies*, *Colletotrichum siamense*, *Colletotrichum* sp., *Colletotrichum truncatum*, *Curvularia* sp., *Diaporthe* sp., *D. ueckerae*, *Epicoccum sorghinum*, *Fusarium equiseti*, *Fusarium* sp., *Hypoxylon monticulosum*, *Lasiodiplodia theobromae*, *Phomopsis* sp., *Rhizopus microsporus*, *Talaromyces muroii*, *Trichoderma asperellum, Trichoderma* sp. and including two unidentified fungi. The fungi species were identified based on the morphology of their colonies (Figure 2).

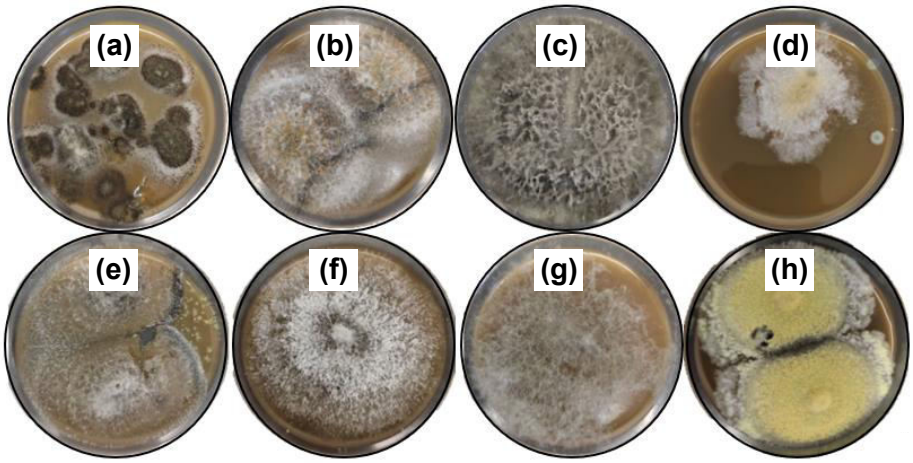

Figure 2: Seed-borne fungal cultural morphology on PDA; (a) *Aspergillus niger*, (b) *Colletotrichum gleosporiodies*, (c) *Lasiodiplodia theobromae*, (d) *Fusarium equiseti*, (e) *Phomopsis* sp., (f) *Diaporthe phaseolorum*, (g) *Rhizopus microsporus*, and (h) *Talaromyces muroii*.

The findings in Table 1 show that 16 fungal species were isolated in blotter media and species in the genera of *Aspergillus*, *Lasiodiplodia,* and *Fusarium* were the most frequently detected in soybean seeds (Table 1). In agar media, 20 fungi species were isolated in agar media (Table 2), of which *Aspergillus* spp. and *Colletotrichum* spp. were the most frequently detected in soybean seeds. However, seed-borne fungi, namely *Chaetomium* sp., *Colletotrichum truncatum*, *Phomopsis* sp., *Rhizopus microsporus* and *Trichoderma asperellum* were not detected in the agar media. It could be because when seeds were surface sterilized with sodium hypochlorite, the fungus that was externally on the seed waseradicated. In blotter media, *Cercospora capsici*, *Cercospora cf flaggelaris*,

Colletotrichum siamense, *Colletotrichum* sp., *Epicoccum sorghinum*, *Trichoderma* sp. and two unidentified species (SS22Aa and SS31Aa) were not detected. Comparing the two methods of isolation, it was shown that APM yielded more fungi isolates than SBM. This may be because the agar medium provides a nutrient-rich substrate for mycelial development and sporulation of fungi pathogens on seed, especially for slow-growing fungi (Mohmed et al., 2019; Zanjare et al., 2020).

The present study has shown that seeds of a few soybean accessions infected with several pathogenic fungi, such as *Phomopsis* sp., *Diaporthe* spp., *Fusarium* spp. and *Cercospora* spp., which can cause serious diseases in the field. *Phomopsis* sp. was detected in A21, A27, A28 and A29 seeds. It was the causal agent of Phomopsis seed decay (PSD) in soybean and had become one of the most economically important seed diseases in soybean (Li and Chen, 2013). PSD severely affects soybean seed quality due to reduced seed viability and oil content, alteration of seed composition, and increased frequencies of mouldy and/or split beans. *Diaporthe* spp. can also cause seed decay, and it was detected in A19, A22, A27 and A28 seeds. Besides *Phomopsis* spp., *Fusarium* spp. has been reported to deteriorate the seed quality and cause root rot, seed decay, and pod and seedling blight. *Fusarium* spp. was detected in A7, A19, A22, A27 and A30 seeds. *Cercospora* spp. which causes purple seed stain disease was detected in A7 seeds, however, it was not as severe as *Phomopsis* sp.

Table 1: Mean percentage incidence of seed-borne fungi identified using blotter test of ten soybean accessions.

Fungi	Fungi incidence (%)									
	A5	A7	A19	A21	A22	A27	A28	A29	A30	A31
Aspergillus fumigatus	0	0	0	0	0	0	0	0	4±4.0	0
Aspergillus niger	64±10.8	20±5.5	6±4.0	0	8±8.0	2±2.0	0	22±7.3	0	0
Aspergillus sp.	0	0	0	0	0	16±5.1	8±4.9	0	0	0
Chaetomium sp.	0	0	0	28±8.6	0	0	0	0	0	0
Colletotrichum gleosporiodies	0	6±4.0	0	0	0	0	0	0	2±2.0	0
Colletotrichum truncatum	0	0	0	0	26±4.0	0	0	0	0	0
Curvularia sp.	0	8±4.9	6±6.0	0	0	0	0	0	4±4.0	0
Diaporthe sp.	0	0	0	0	0	10±4.5	10±10.0	0	0	0
Diaporthe ueckerae	0	0	46±16.3	0	0	0	0	0	0	0
Fusarium equiseti	0	22±6.6	12±8.0	0	14±7.5	0	0	0	0	0
Fusarium sp.	0	0	0	0	26±4.0	0	0	0	0	0
Hypoxylon monticulosum	0	0	0	0	0	4±4.0	0	0	0	0
Lasiodiplodia theobromae	36±10.8	40±8.9	16±6.8	0	0	0	0	0	70±12.2	24±5.1
Phomopsis sp.	0	0	0	2±2.0	0	8±3.7	2±2.0	2±2.0	0	0
Rhizopus microsporus	0	0	0	12±5.8	0	0	22±15.6	20±6.3	0	0
Trichoderma asperellum	0	0	0	10±6.3	0	0	0	0	0	0

Means followed by the same letter in the same column are not significantly different at P ≤ 0.05, according to Tukey's test.

Table 2: Mean percentage incidence of seed-borne fungi identified using agar test of ten soybean accessions.

Fungi	Fungi incidence (%)									
	A5	A7	A19	A21	A22	A27	A28	A29	A30	A31
Aspergillus fumigatus	0	0	0	0	0	0	0	2±2.0	0	0
Aspergillus niger	0	4±2.5	0	0	0	0	0	0	0	0
Aspergillus sp.	0	6±6.0	0	0	0	0	0	0	0	0
Cercospora capsici	0	6±4.0	0	0	0	0	0	0	0	0
Cercospora cf flaggelaris	0	2±2.0	0	0	0	0	0	0	0	0
Colletotrichum gleosporiodies	0	0	22±10.2	0	0	0	0	4±4.0	0	0
Colletotrichum siamense	2±2.0	0	0	18±8.6	0	0	0	0	0	0
Colletotrichum sp.	0	0	0	18±15.6	0	0	0	0	0	0
Curvularia sp.	0	0	0	4±2.5	0	8±2.5	0	2±2.0	24±8.7	0
Diaporthe sp.	0	0	0	0	2±2.0	0	0	0	0	0
Diaporthe ueckerae	0	0	0	0	22±8.6	0	0	0	0	0
Epicoccum sorghinum	0	0	0	0	0	8±3.7	0	0	0	0
Fusarium equiseti	0	0	0	0	0	6±4.0	0	0	0	0
Fusarium sp.	0	0	0	0	0	0	0	0	14±7.5	0
Hypoxylon monticulosum	0	0	0	0	0	2±2.0	0	0	0	0
Lasiodiplodia theobromae	0	0	0	0	0	14±9.8	0	0	0	0
Talaromyces muroii	0	0	0	0	0	0	4±2.5	0	0	0
Trichoderma sp.	0	0	0	0	0	0	0	2±2.0	0	0
SS22Aa (unidentified)	0	0	0	0	0	0	0	2±2.0	0	0
SS31Aa (unidentified)	0	0	0	0	0	0	0	0	0	14±4.0

Means followed by the same letter in the same column are not significantly different at P ≤ 0.05, according to Tukey's test.

Conclusions

All soybean accessions were infected with seed-borne fungi, but A31 was the least infected with fungi. The most prevalent fungi species found in soybean seeds were *Aspergillus* spp., *Colletotrichum* spp., *Fusarium* spp., *Lasiodiplodia theobromae*, *Phomopsis* sp. and *Diaporthe* spp. The important seed-borne fungal pathogens detected in soybean seeds were *Cercospora* spp., *Diaporthe* spp., *Phomopsis* sp. and *Fusarium* spp. The agar plate method was effective in detecting seed-borne fungi of soybean. Thus, these findings will provide valuable references for the effective management of seed-borne fungal diseases and soybean resistance breeding.

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Chapter 5

Plant Production

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Selection of High Yielding Palm for FGV Clonal Production

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Introduction

The oil palm (*Elaeis guineensis* Jacq.) is an African tropical plantation crop cultivated for its source of two types of commercial vegetable oil: palm oil and kernel oil. Palm oil extracted from the fruit pulp called mesocarp (Reeves et al., 1979) is used in the production of soaps, cosmetics, and biofuel while kernel oil extracted from the kernel is used in manufacturing edible products such as margarine and pharmaceutical products (Nair, 2010). Oil palm is the most efficient vegetable oil-producing crop in the world due to the ability to produce much more oil in tonnes per hectare compared to other crop (Barcelos et al., 2015). Because of this, the largest portion of global imports is palm oil (63%) followed by soybean oil (14%), sunflower oil (11%), rapeseed oil (6%), and others (11%) (Sarmidi et al., 2022). The high demand for palm oil requires stable high yielding planting materials to ensure market demand is fulfilled.

The *tenera* or known as D × P planting materials is preferred by oil palm plantations as it produces 30% more oil compared to *dura* because of the optimum percentage of shell thickness and mesocarp ratio (Babu et al., 2017). Oil palm is propagated *via* sexual reproduction by pollinating a female inflorescence *dura* with *pisifera* pollen to produce D × P planting materials. However, the variability in performance of D × P planting materials is high, reflected by differences in the fresh fruit bunch (FFB) production and oil extraction rate (OER) due to genetic segregation. Through tissue culture or known as cloning, the clonal performance is uniformed and true-to-type of elite palm with additional desirable traits such as *Ganoderma* and drought tolerant (Mutert et al., 1999). Mass reproduction of elite *tenera* palm provides a great advantage over hybrid seeds in increasing oil yield in the current cultivated area (Weckx et al., 2019).

The ortet by definition is an individual plant that gives rise to a clonal plant (Potier et al., 2006) (as known as ramets) through vegetative propagation. In FGV, oil palm cloning conducted using the young leaf/cabbage as starting materials. Selected palms will undergo a thorough and meticulous ortet selection screening process of progeny and individual ortet performances followed by phenotypic observation in the field which strictly on superior progenies from breeding trials. The selected ortet must possess elite performances for the FFB and oil yield (OY) along with other important bunch quality components and possess an ideal height increment. The most important selection criteria are bunch number (BNO) and oil to bunch (O/B), as these traits are highly heritable and less affected by the environment (Alwee et al., 2010). Thus, a high BNO with an average bunch size is preferable compared to a bigger bunch with a lower bunch number.

Oil palm tissue culture in Malaysia was started in the early 1980s (Alwee et al., 2018). The big plantation players in Malaysia like FELDA (later known as FGV), IOI Plantation Sdn. Bhd, Applied Agricultural Resources Sdn. Bhd (AAR) and Sime Darby Plantation Berhad have shown great interest in oil palm clonal planting materials by establishing tissue culture laboratories to cater to their clonal planting materials needs (Kamil et al., 2020). FGV started its cloning journey in 1983 at Anjung Felda,

Kuala Lumpur in collaboration with French Agricultural Research Centre for International Development (CIRAD). First clonal planting materials were planted at Pusat Penyelidikan Pertanian Tun Razak, Pahang on 1987 while at Felda Plantation in 2000. FGV has successfully increased its annual production to 250,000 ramets after moving to Tissue Culture Lab, FGV Innovation Centre (Biotechnology), Bandar Enstek, Negeri Sembilan in 2006 then increased to one million since 2010. As of now, more than 500,000 clonal planting materials have been planted at the FGVs' plantations. This study was conducted with the objective to compare the performances of FGV clones and D × P materials.

Materials and Methods

Treatments and experimental design

The trial laid in randomized complete block design (RCBD) with six replications comprised of five clones with different Yangambi backgrounds along with one D × P progeny as control (Table 1). The performance measured based on the yield and bunch analysis of 96 palms per progeny. Breeding trial C26 is located at FGV Agri Services Plantation 1B1, Kota Gelanggi 5, Pahang planted in November 1995 (3˚57'39.5994"N, 102˚35'31.1994"E).

Table 1: List of progenies and their pedigrees.

Clone/Progeny code	Material	Ortet	Parentage		Lineage
			Female	Male	
FC343	Clone	CDJ48	D3D	L238T	IRHO × Yangambi
FC446	Clone	CDQ44	L409D	L519P	IRHO × Yangambi
FC458	Clone	FED32	AMB39	ML161	Ulu Remis-Yangambi × Yangambi
FC460	Clone	FDK3	CCH12	ML161	Banting × Yangambi
FC464	Clone	FDK32	CCH12	ML161	Banting × Yangambi
AN69	D×P	-	RD15/79	ML69	Ulu Remis × Yangambi

Yield recording

The yield performance recorded for all materials included FFB, BNO, and ABW. Yield recording was conducted throughout the 13 years, consecutively (1997-2009), starting from two years after planting. Bunch census conducted quarterly as the quality control for the yield data.

Bunch analysis

One ripe bunch for every individual palm in each progeny harvested for bunch analysis (BA). A total of 96 samples per progeny was sent to BA Lab, Plant Breeding Department at Pusat Penyelidikan Pertanian Tun Razak, Pahang. Harvested bunch was processed following the method developed by Rao et al. (1983). Traits that were closely monitored were oil to bunch (O/B), shell to fruit (S/F), kernel to fruit (K/F), mesocarp to fruit (M/F), and oil to wet pericarp (O/WP).

Statistical analysis

SAS software (version 9.4) was used to compute the Analysis of Variance (ANOVA) followed by the Duncan Multiple Range Test with a 0.05 significant value.

Results and Discussion

Bunch yield

The overall FFB yield for 13 years recording (Table 2) shows clonal produced 190.05 kg/palm/yr (25.85 t/ha), 9.34% higher than D × P planting materials at 172.30 kg/palm/yr (23.43 t/ha). The mean

FFB recorded by this trial was 187.09 kg/palm/yr (25.55 t/ha), making the four clones producing higher yield compared to the mean trial which were clones FC446, FC458, FC460 and FC464. Over the years, clonal material consistently showed higher FFB yield compared to D × P. Improvement in FFB yield productivity is an advantage to plantations in gaining additional income. A revenue of RM 10,696/ha is gained from clones if the crude palm oil (CPO) is priced at RM 4420/tonne (mean CPO price 2021). The overall mean BNO were 11.71 bunches/palm/yr while ABW were 16.03 kg/palm/yr. The highest BNO was achieved by FC446 at 13.68 bunches/palm/yr while D × P AN69 only produced 10.39 bunches/palm/yr. FGV ortet selection strictly on high bunch numbers has contributed to superior yield achieved by clones. The major determinant of oil palm productivity is FFB yield, which is calculated by multiplying BNO with ABW (Constantin et al., 2017).

Table 2: Mean performance of clones & D×P for bunch yield (1997-2009).

Progeny	Lineage	Bunch yield (1997-2009)		
		FFB (kg/palm/yr)	BNO (bunches/palm/yr)	ABW (kg/palm/yr)
FC343	IRHO × Yangambi	168.57^{d}	10.47^{d}	16.13^{b}
FC446	IRHO × Yangambi	206.13^{a}	13.68^{a}	15.07^{c}
FC458	Ulu Remis-Yangambi × Yangambi	187.38^{c}	11.73^{c}	15.98^{b}
FC460	Banting × Yangambi	192.56^{bc}	11.71^{c}	16.45^{a}
FC464	Banting × Yangambi	195.58^{b}	12.26^{b}	15.96^{b}
	Group Mean	190.05	11.97	15.92
AN69	Ulu Remis × Yangambi	172.30^{d}	10.39^{d}	16.59^{a}
	Mean	187.09	11.71	16.03

**FFB = fresh fruit bunch; BNO = bunch number; ABW = average bunch weight.*
Mean with the same letter in the same column are not statistically significant by the Duncan Multiple Range test.

Bunch analysis

The overall mean for F/B, M/F, S/F, K/B and O/B were 67.77%, 85.83%, 8.43%, 3.89% and 32.47%, respectively (Table 3). In this trial, clonal material outperformed D×P by 20.86% higher in oil yield production at 8.58 t/ha and 6.79 t/ha, respectively. Oil yield value is determined by the combined performance of FFB and O/B. Since FFB yield is influenced environmentally, clonal material contributes to improved oil yield since the ortet selection criteria are focusing on a higher percentage of O/B quality components, which is directly involved in oil yield production capability. Clonal material as a group had achieved an O/B of 33.18% compared to D × P AN69 only at 28.97%. Based on the bunch analysis data, it was found that FC458 is the top performer compared to other clones because of the high percentage of F/B, M/F, O/B and low percentage of S/F at 70.12%, 88.37%, 33.37% and 6.88%, respectively. Clones with a high percentage of M/F and a low percentage of S/F are preferred since they produce more oil. Clones as a group recorded 7.95% of S/F while D × P AN69 recorded higher at 10.86%. Clones maintain their S/F percentage below 10% because it is one of the selection criteria for ortet. Clones as a group also produced a higher percentage of M/F at 86.66% compared to D × P at only 81.67%. A high percentage of M/F indicates that the mesocarp flesh in the oil palm fruit is thick producing more crude palm oil. Materials with a high percentage of K/B are suitable for planting materials for collecting kernel palm oil.

Table 3: Mean performance of clones & D × P for bunch analysis.

Progeny	Lineage	Bunch Analysis				
		F/B (%)	M/F (%)	S/F (%)	K/B (%)	O/B (%)
FC343	IRHO × Yangambi	69.74^{a}	84.32^{c}	9.07^{b}	4.63^{a}	34.61^{a}
FC446	IRHO × Yangambi	66.14^{b}	87.90^{a}	7.59^{cd}	2.98^{c}	31.44^{c}
FC458	Ulu Remis-Yangambi × Yangambi	70.12^{a}	88.37^{a}	6.88^{d}	3.32^{bc}	33.37^{ab}
FC460	Banting × Yangambi	65.98^{b}	86.90^{ab}	7.83^{cd}	3.48^{bc}	33.86^{ab}
FC464	Banting × Yangambi	66.09^{b}	85.81^{bc}	8.37^{bc}	3.81^{b}	32.60^{bc}
	Group Mean	67.61	86.66	7.95	3.64	33.18
AN69	Ulu Remis × Yangambi	68.57^{a}	81.67^{d}	10.86^{a}	5.12^{a}	28.97^{d}
	Mean	67.77	85.83	8.43	3.89	32.47

** F/B = Fruit to bunch; M/F = mesocarp to fruit; S/F = shell to fruit; K/B = kernel to bunch; O/B = oil to bunch.*
Mean with the same letter in the same column are not statistically significant by the Duncan Multiple Range test.

Conclusion

Cloning is one of the ways to expedite the propagation of high yielding oil palm at commercial plantations. The ramets' performance is uniform and true-to-type as the selected elite ortet. The ability of clonal materials to surpass the performances of D × P planting materials in terms of yield and bunch analysis performances indicates that good ortet selection process plays a major role for achievement.

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Effects of Different Planting Media on the Growth and Yield Performance of *Persicaria minor* Accessions

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Introduction

Kesum or *Persicaria minor* is an aromatic herb that originates from Southeast Asia (Malaysia, Indonesia, Vietnam, Thailand) and it thrives in moist and watery areas. Kesum is classified as a shrub that is approximately 30-45 cm tall, has reddish, cylindrical, and short segmented stems, and has purple flowers. The leaves are green, small, long (5-7 cm) and pointed. There are two types of kesum, which are erect and decumbent (Christapher et al., 2015). Both types of kesum have a fragrant aroma. The leaves serve as the main ingredient in various Malay cuisines due to their appetizing aroma and ability to evoke the delicious taste of food. Kesum can also be used as a decorative tree in the yard as well as a source of aromatherapy. Kesum is a natural source of aliphatic aldehyde, thus has big potential in food additive and perfume industries (Bunawan et al., 2011). Kesum leaves are used as medicine to overcome health problems, and it has traditionally been used to treat skin fungal infections, stomach ailments, dandruff control, and as a post-natal tonic (Ong and Nordiana, 1999). Kesum is also rich in micronutrients, total phenolic content (TPC) and natural antioxidants. This active ingredient provides medicinal properties in terms of antioxidant, anti-inflammatory, anti-aging, improves memory and promotes the body's immune system (Maizura et al., 2011).

The growing media play a critical role in the propagation of seedlings in nurseries and greenhouses. Growing media used in the production of seedlings in nurseries include organic materials such as peat, tree bark, compost, coconut fiber, vermicompost, rice husk ash or inorganic materials such as perlite or vermiculite (Grunert et al., 2008; Nair et al., 2011; Vaughn et al., 2011). These growing substrates consist of either a single component or mixtures that support plants by providing water, air, and nutrients (Bilderback et al., 2005; Yilmaz et al., 2014; Oagile et al., 2016; Olaria et al., 2016). However, different growing media vary greatly in composition, particle size, pH, aeration and ability to hold water and nutrients (Oagile et al., 2016). Selecting a suitable growing medium is one of the most important considerations when raising containerized seedlings in a nursery (Jacobs et al., 2009). The medium should be lightweight for easy handling and filling, and able to provide a condition that is not offered by the soil (Bilderback et al., 2005).

Previously, water immersion was used for the propagation of kesum, but the percentage of survival during transplant is low (Abdul Rahman, 2001). Several planting media, however, can be used to boost kesum growth and production. Therefore, this study was carried out to evaluate the effectiveness of different planting media on the growth and yield performance of two types of kesum accessions for optimal biomass production in the field. This finding is important for identifying kesum accession with improved productivity and quality of kesum in order to increase farmers' income.

Materials and Methods

Planting materials

The experiment was conducted in the nursery of the Industrial Crop Research Centre, MARDI Serdang, Selangor. The best accession from two types of kesum which are erect and decumbent was obtained from the breeder's germplasm. The cuttings were planted in seed trays with eight different planting media, which includes T1: peat moss, T2: coco peat, T3: peat moss and perlite (1:1), T4: peat moss and vermiculite (1:1), T5: coco peat and burnt husk (1:1), T6: coco peat and peat moss (1:1), T7:

peat block and T8: water immersion (control). Cuttings were manually cut at the same length at approximately 10-12 cm, which contained about 7-8 nodes. The cuttings were dipped in the rooting hormone (Seradix) before being inserted into eight types of planting media in seed trays. They were fertilized with foliar fertilizer Bayfolan (11-8-6) 2 weeks after planting. The plants were grown under 50% shade in a greenhouse and maintenance was carried out according to the normally recommended practices. Irrigation was applied twice every day. After 4 weeks of planting, the plant was transferred to the field for growth evaluation. Plants were fertilized with NPK (15:15:15) and urea after 1 month and 2 months of transplanting, respectively.

Figure 1: Two types of kesum (a) erect and (b) decumbent accessions.

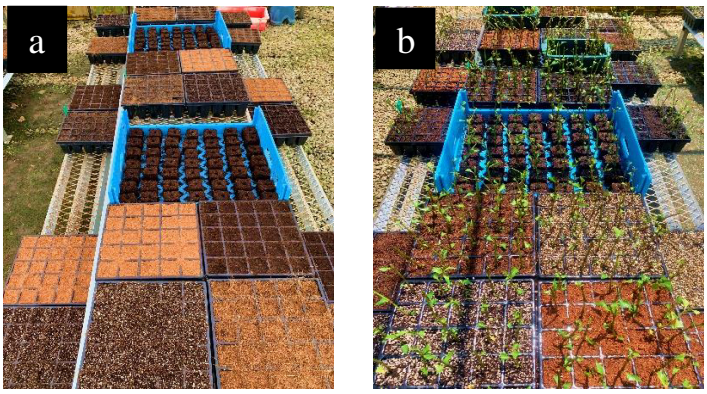

Figure 2: (a) Preparation of eight planting media. (b) Cuttings of kesum accessions inserted into eight types of planting media in seed trays.

Growth measurements

Kesum plants were sampled randomly from each treatment. Growth parameters such as plant height, shoot height, canopy diameter, number of leaves, and cumulative leaf area, as well as yield parameters, which include stem, root, and leaf fresh and dry weights, were recorded 12 weeks after transplanting. The number of fully expanded leaves was manually counted and used for determination of total leaf area. The leaf area was measured using a leaf area meter (LI-3100; LI- COR, Inc., Lincoln, Nebraska, USA). Plant samples from each treatment were harvested and divided to leaf, stem and root to determine the fresh weights of individual plant parts. Leaf, stem and root fresh weights were measured before drying and their dry weights were weighed using a digital balance (QC 35EDE-S Sartorius, Germany) after drying at 75 °C for 72 h.

Experimental design and data analysis

This experiment was conducted in a randomised complete block design (RCBD) with eight different planting media and two types of kesum accessions with four replications; 20 plants per replication.

The data obtained were subjected to analysis of variance (ANOVA) using the SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA). The differences between treatments means were compared using Least Significant Difference (LSD) at $p \leq 0.05$.

Results and Discussion

Growth measurements

Table 1 shows the effect of eight types of media on plant height, shoot height, canopy diameter, stem diameter, number of leaves, and total leaf area of kesum accessions. Overall, kesum accessions had a significant interaction with all parameters except for stem diameter. However, the decumbent accession showed significantly greater values than the erect accession. This can be seen from the shoot height, number of leaves, and leaf area, which were 21%, 17%, and 24% greater than those of the erect accession, respectively.

Interaction between types of accessions and planting media did not show significant differences for all parameters. Additionally, there was no significant difference between types of planting media for kesum growth except for the number of leaves and total leaf area. Combination planting media between coco peat and burnt rice husk (1:1) (T5) showed significantly higher values for all parameters, while water immersion media showed the lowest values for all parameters. The T5 media recorded 30% higher compared to water immersion (T8) in terms of shoot height for both accessions. Number of leaves and total leaf area using coco peat and burnt rice husk (1:1) (T5) were 58% and 56% higher than the water immersion (T8), respectively.

Table 1: Effects of different types of media (T1: peat moss, T2: coco peat, T3: peat moss and perlite (1:1), T4: peat moss and vermiculite (1:1), T5: coco peat and burnt husk (1:1), T6: coco peat and peat moss (1:1), T7: peat block, and T8: water immersion (control)) on plant height, shoot height, canopy diameter, stem diameter, number of leaves and total leaf area of kesum accessions.

	Plant height (cm)	Shoot height (cm)	Canopy diameter (cm)	Stem diameter (mm)	Number of leaves	Total leaf area (cm^2)
Accession						
Decumbent (J)	78.81^{a}	71.40^{a}	113.10^{a}	7.68^{a}	2031.7^{a}	6044.7^{a}
Erect (G)	64.46^{b}	56.46^{b}	89.96^{b}	7.28^{a}	1686.2^{b}	4598.9^{b}
Types of Media						
1	70.94^{ab}	63.78^{abc}	98.50^{ab}	7.63^{ab}	1947.4^{b}	5276^{abc}
2	69.61^{ab}	61.61^{abc}	95.44^{ab}	7.43^{ab}	1657.2^{bc}	4680^{bc}
3	71.06^{ab}	65.06^{ab}	102.78^{ab}	7.96^{a}	2116.7^{ab}	5150^{abc}
4	68.50^{ab}	60.50^{bc}	95.95^{ab}	6.46^{b}	1287.6^{bc}	4202^{bc}
5	76.72^{a}	71.11^{a}	111.502^{a}	7.96^{a}	2632.9^{a}	7213^{a}
6	75.22^{a}	66.50^{ab}	108.39^{a}	7.64^{ab}	2002.0^{ab}	6948^{a}
7	77.78^{a}	68.22^{ab}	110.78^{a}	8.29^{a}	2114.3^{ab}	5928^{ab}
8	63.22^{b}	54.67^{c}	88.89^{b}	6.50^{b}	1113.5^{c}	3176^{c}
Accession	**	**	**	ns	*	*
Media	ns	ns	ns	ns	**	*
Accession X Media	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 difference letters within each parameter indicates significant difference at P ≤ 0.05% level according to LSD.*

Table 2 shows the effect of eight types of planting media on stem, root, and leaf fresh and dry weights. The decumbent accession showed significantly higher values for stem fresh weight, leaf fresh weight, and stem dry weight compared to the erect accession. There was no significant difference among both accessions for leaf dry weight. In terms of root fresh weight and root dry weight, there was no significant difference among accessions and planting media. Decumbent accession showed 30% and

35% higher stem fresh weight and stem dry weight than erect accession, respectively. Meanwhile, the leaf fresh weight of the decumbent accession was 21% higher than the erect accession. Under various planting media, there was a significant difference in terms of stem fresh weight, leaf fresh weight, stem dry weight, and leaf dry weight, but not for root fresh and dry weights. Overall, coco peat and burnt rice husk (1:1) (T5) showed significantly higher values for all the evaluated parameters compared to the control, water immersion media (T8).

Prior studies showed that coco peat is being used as an environmentally friendly substitute for peat in soilless growing media for containerized plants (Noguera et al., 2000; Kumarasinghe et al., 2015). Coco peat is considered a good growing medium component with acceptable pH, electrical conductivity and other chemical attributes (Awang et al., 2009). As a growing medium, it can be used singly or as a component of medium to raise different plant species with acceptable quality (Yahya et al., 1997 and 1999; Blom, 1999; De Kreij and Leeuven, 2001; Treder, 2008). However, coco peat has been recognized as having relatively low levels of mineral nitrogen (N) and micronutrients such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) (Abad et al., 2002). In addition to high water holding capacity, which causes a poor air-water relationship, low aeration within the medium also affects oxygen diffusion to the root (Yahya et al., 2009). The combination of coco peat and burnt rice husk was considered a fine-textured substrate compared to other treatments. The ability of media to hold water is high, and this may create a high moisture condition when the air space between the substrate particles is replaced by water.

Table 2: Effects of eight types of media (T1: peat moss, T2: coco peat, T3: peat moss and perlite (1:1), T4: peat moss and vermiculite (1:1), T5: coco peat and burnt rice husk (1:1), T6: coco peat and peat moss (1:1), T7: peat block and T8: water immersion (control)) on kesum stem, root and leaf fresh and dry weights.

	Stem fresh weight (g)	Root fresh weight (g)	Leaf fresh weight (g)	Stem dry weight (g)	Root dry weight (g)	Leaf dry weight (g)
Accession						
Decumbent (J)	230.54^{a}	12.78^{a}	128.33^{a}	59.92^{a}	3.47^{a}	35.18^{a}
Erect (G)	161.56^{b}	22.13^{a}	101.96^{b}	39.13^{b}	4.38^{a}	30.40^{a}
Types of Media						
1	202.39^{abc}	14.61^{a}	123.28^{abc}	47.22^{ab}	4.06^{ab}	33.00^{ab}
2	141.05^{cd}	39.11^{a}	88.00^{bc}	39.22^{bc}	4.67^{ab}	30.33^{bc}
3	219.22^{abc}	10.89^{a}	115.17^{abc}	52.17^{ab}	3.28^{ab}	34.88^{ab}
4	159.17^{bcd}	11.61^{a}	97.72^{bc}	36.56^{bc}	2.77^{b}	26.50^{bc}
5	281.06^{a}	19.06^{a}	156.06^{a}	65.89^{a}	5.45^{a}	44.00^{a}
6	240.89^{ab}	15.89^{a}	137.33^{ab}	50.78^{ab}	4.06^{ab}	37.39^{ab}
7	219.67^{abc}	16.72^{a}	131.61^{ab}	53.61^{ab}	4.28^{ab}	36.79^{ab}
8	104.95^{d}	9.72^{a}	72.00^{c}	22.72^{c}	2.83^{b}	19.45^{c}
Accession	**	ns	*	**	ns	ns
Media	*	ns	*	**	ns	*
Accession X Media	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 difference letters within each parameter indicates significant difference at P ≤ 0.05% level according to LSD.*

Conclusions

Based on the results, we can conclude that decumbent accession showed promising results in growth and yield performance as compared to the erect accession. In general, combination of coco peat and burnt husk (1:1) (T5) can increase the growth of kesum. Therefore, this planting medium is recommended in the field for optimal biomass production of both erect and decumbent kesum accessions.

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Evaluation of Growth, Yield and Fruit Quality of Harumanis Mango under Greenhouse and Open Field Conditions

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Introduction

The mango variety, Harumanis, is classified as one of the most popular mango varieties in Malaysia with high economic value. As recorded in 2021, there were about 1,575 hectares of Harumanis mango cultivation in Malaysia with 2,527 metric tons of production (Department of Agriculture, 2021). The demand for Harumanis mango is increasing yearly due to the exquisite taste and aroma of the fruit. However, the plant is only cultivated in the northern region of Peninsular Malaysia, such as in Perlis and some parts of Kedah (Muhamad Hafiz et al., 2019). These areas are located in Zone 1 of Malaysia agro-climatic zones, which is characterized by significant drought from January to March, followed by vagaries rainfall in the interim months and heavy rain from September to December (Mohd Aziz et al., 2019). Furthermore, mango tree is categorized as a seasonal plant where flowering takes place from January to February and the fruit production is only limited for once a year around May to June (Farook et al., 2012; Jaafar et al., 2014). Thus, the fruit production does not fulfil the local market demand, and the price increases.

Currently, the open field cultivation of Harumanis mango is affected by the climate change, especially the inconsistent rainfall and short dry season. Heavy rainfall can cause mango flowering to fail and disease incidence to increase (Akinaga and Hasbullah, 2002; Farook et al., 2012; Shaharil et al., 2014). To solve these issues, the greenhouse cultivation concept can be implemented for Harumanis mango. The cultivation of Harumanis mango under a greenhouse production system has become one of the farmers' alternative options in Malaysia. The initiative of mango production in a greenhouse has been successfully utilized in Japan (Akinaga and Hasbullah, 2002; Shiroma et al., 2019), Spain (Medina et al., 2009) and Korea (Lim et al., 2016). By growing the crop in a greenhouse, the flowering of the mango can be controlled and will be protected from heavy rainfall, pests, and diseases (Akinaga and Hasbullah, 2002; Pawlowski et al., 2009; Shaharil et al., 2014). Furthermore, continuous or off-season production of fruits can be obtained from a particular greenhouse if the production is properly planned (Shaharil et al., 2014). Therefore, the objective of this study was to evaluate the growth, yield, and fruit quality of Harumanis trees under the greenhouse and compare them with those under open field conditions.

Materials and Methods

Study site and experimental design

The experiment was carried out in a greenhouse and open field at MARDI Sintok, Kedah, that were planted with Harumanis mango trees in November 2017 using proper and standard agronomic practices. The size of the greenhouse is approximately 714 square meters with a planting distance of 3 m x 3 m. The greenhouse is a three-bay structure made of galvanised steel that includes a ventilator and an automatic irrigation system. The side of the greenhouse was covered with a nylon net of 32 meshes (insect proof), and the roof was covered with a 200-micron plastic sheet with an ultraviolet blocker. Forty-eight (48) Harumanis mango trees at 30 months old were chosen for the experiment under greenhouse and open field conditions based on growth uniformity. All selected trees were divided and arranged in a Completely Randomized Design (CRD) layout with six replications, and

each replication consisted of eight plants. The application of flowering induction to Harumanis trees in the greenhouse and the open field was conducted using Paclobutrazol (PBZ) at a concentration of 2 mL/L of water by the drenching method on the first week of September 2020. The irrigation was stopped one week after the application of PBZ. A portable weather station (WatchDog 2000 series) was installed in the greenhouse and open field to record and monitor daily climatic data such as temperature (°C) and relative humidity (%) starting from the vegetative stage until the reproductive stage. In addition, total rainfall data was also recorded on open fields during the vegetative stage until the reproductive stage.

Measurement of vegetative and reproductive growths

The vegetative growth of the plant was measured at 30 days after PBZ application. Five parameters were measured, including plant height (m), stem diameter (m), canopy size (m), internode length (mm), number of leaves, chlorophyll content, and number of shoots under a greenhouse and in an open field. The 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 the stem using electronic digital caliper (Model SCM DIGV-6), while canopy size and internode length were measured using a measuring tape (Model Stanley Power Lock STHT33428-8). The number of shoots and leaves was manually counted based on fully expanded shoots and leaves. The chlorophyll content of leaves was measured using a SPAD meter (Konica Minolta SPAD-502 Plus), and measurements were taken at four points per leaf located at the halfway point between the leaf tip and the collar, which is about the middle point between the leaf midrib and margin. The measurement within one tree was grouped as a single sample. The reproductive growth was measured beginning with the flowering stage and continued until the fruiting stage. The data collected were the percentage of inflorescence trees (%), first time to produce inflorescence (days), inflorescence period, number of panicles per tree, length of panicle (mm), and number of fruits set per panicle (16-18 cm).

Measurement of fruit yield and quality

The fruits were harvested after 12 weeks of fruit set for yield and quality assessment. Twenty fruits (20) harvested from the greenhouse and open field were selected and labelled for quality assessment. The harvested fruits were washed using water and soaked for 10 minutes in 0.2% Benomyl fungicide to prevent postharvest diseases. Then, it was dried at ambient temperature, and all the fruits were ripened using calcium carbide for 3 nights before being transferred to the laboratory for quality assessment. Evaluation of fruit parameters consists of fruit weight (g), fruit length (mm), fruit width (mm), and pulp weight (g). Fruit weight (g) and pulp weight (g) were measured using an analytical balance (AND ER 180A) while length (mm) and width of the fruit (mm) were measured using a digital caliper (MITUTOYO CD67). Total soluble solid (TSS) was determined using a digital handheld refractometer (ATAGO CO. LTD PAL-α), while the pH was measured using a pH meter (HANNA Instrument HI2211). Total titratable acidity (TTA) content was measured by titrating 20 mL extract from sample with 0.1 M NaOH until it reached pH 8.2, while for ascorbic acid (vitamin C), 10 mL extract from 10 g and 100 mL of 3% metaphosphoric acid were titrated with standard dye until the extract turned faint pink.

Statistical analysis

The data obtained was analyzed 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 $p \leq 0.05\%$.

Results and Discussion

Measurement of climatic data

Figure 1 shows the temperature readings in the greenhouse and the open field, which showed that when the temperature in the open field increased, the temperature in the greenhouse also increased. It showed that the open field temperature had an influence on the temperature in the greenhouse. The temperature in the greenhouse was higher than the temperature in the open field (2-3 °C difference in temperature). In this study, the average maximum temperature in the greenhouse was recorded at 37.31 °C, while the open field temperature was at 36.30 °C. The average minimum temperature in the greenhouse was recorded at 22.80 °C, while the open field temperature was at 21.89 °C. According to Akinaga and Hasbullah (2002), the optimum temperature for growing mangoes is between 24 °C and 27 °C; temperatures below 18 °C will reduce the growth rate, while temperatures over 37 °C will cause injury to the mango. However, the relative humidity in the greenhouse was lower than that in the open field (difference of 8-10%) (Figure 2). These extreme conditions will affect the vegetative and reproductive growth of the mango trees in the greenhouse. For average total rainfall, the highest total rainfall was recorded at 275.54 mm during the vegetative stage, while the lowest total rainfall was at 13.84 mm during the reproductive stage in the open field (Figure 3).

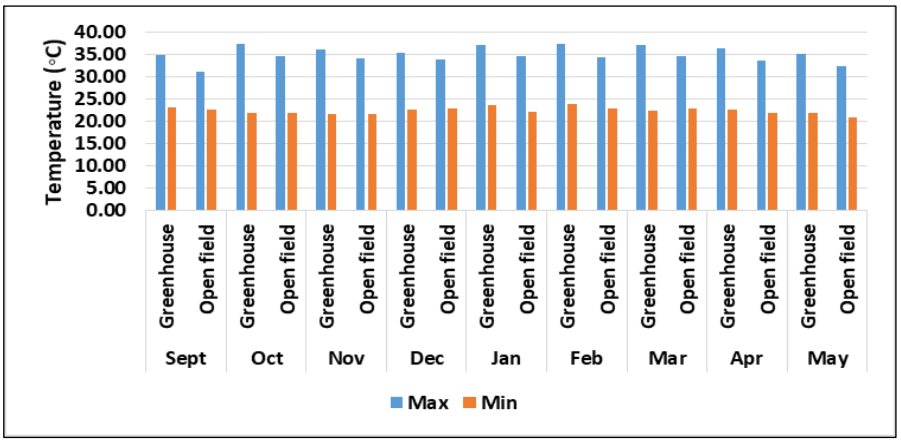

Figure 1: Average temperatures under the greenhouse and open field (September 2020-May 2021).

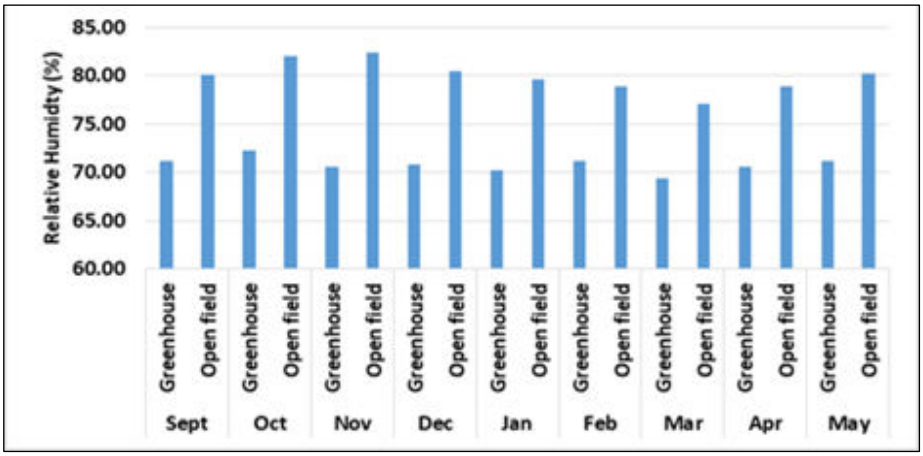

Figure 2: Average relative humidity under the greenhouse and open field (September 2020-May 2021).

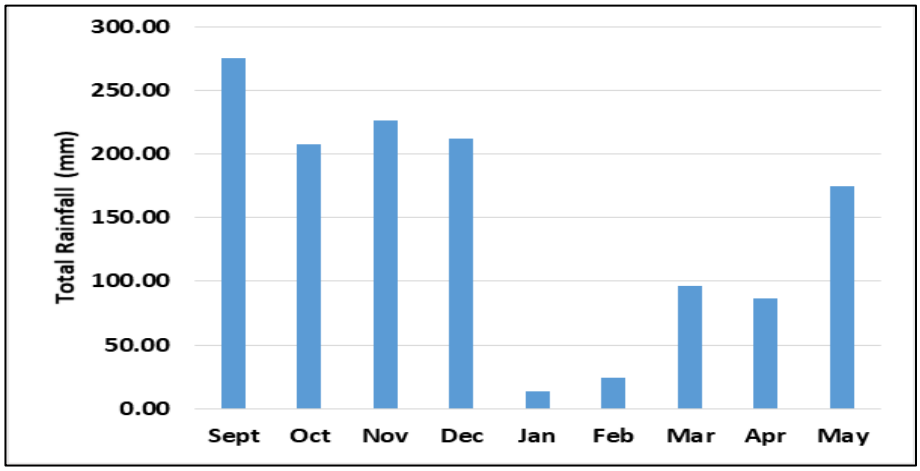

Figure 3: Average total rainfall at open field (September 2020-May 2021).

Measurement of vegetative and reproductive growths

Vegetative growth was different between the Harumanis mango trees grown under the greenhouse and in the open field (Table 1). Based on the parameters measured, such as height, internode length, number of leaves, and shoot number, the growth of the Harumanis mango trees grown under the greenhouse was significantly reduced compared to the open field. The high temperature and low humidity in the greenhouse may affect the vegetative growth of mangoes. The vegetative growth in the open field was higher than that in the greenhouse due to high humidity and soil water content during the vegetative stage. No significant differences were recorded between Harumanis mango trees in the greenhouse and open field for parameters of stem diameter, canopy size, and chlorophyll content.

Table 1: Vegetative growth parameters of Harumanis mango under greenhouse and open field conditions.

Location	Plant height (m)	Stem diameter (mm)	Canopy size (m)	Internode length (cm)	Chlorophyll content	Number of leaves	Shoot number
Greenhouse	1.33^{a}	47.66^{a}	1.78^{a}	23.69^{a}	49.14^{a}	76.83^{a}	78.54^{a}
Open field	2.34^{b}	45.21^{a}	1.88^{a}	41.56^{b}	53.46^{a}	123.30^{b}	93.14^{b}

**Means in each column with the different letters within the column indicate significant differences at $P \leq 0.05\%$ level according to DMRT.*

In terms of reproductive growth measurements, Harumanis mango trees in the greenhouse produced the highest percentage of inflorescence, which was significantly different compared to the open field (Table 2). Based on the first time to produce inflorescence, Harumanis mango trees in the greenhouse produced the earliest and had the fastest response to flowering compared to Harumanis mango trees in the open field. The less optimal conditions in the greenhouse might have promoted the flowering of the mango trees (Farook et al., 2012). The flowering of Harumanis mango in the greenhouse was staggered from the beginning of the 4th week of October 2020 until the 2nd week of December 2020, while the flowering of Harumanis mango in the open field started late and was the shortest during the dry period, beginning from the first week of January 2021 until the 3rd weeks of February 2021. However, there were no significant differences between Harumanis mango trees in the greenhouse and in the open field in terms of number and length of panicles. In the greenhouse, low fruit set formation is caused by the lack of pollinating agents given the closed environment within the greenhouse during the flowering stages compared to the open field. Similar findings on the cultivation of the Irwin mango were made by Sasaki et al. (1998) and Mizuno et al. (2007), who found that mangoes grown in greenhouses without pollination produced fewer fruit sets than those cultivated in greenhouses with pollination from insects like honeybees. According to Yamashita (2000), the presence of pollinators such as honeybees could be a key factor in obtaining better fruit set for Irwin mango under greenhouse

conditions. These showed that greenhouse mango cultivation requires insect pollinators to increase mango fruit set.

Table 2: Reproductive growth parameters of Harumanis mango under greenhouse and open field conditions.

Location	Percentage of inflorescence (%)	First time to inflorescence (Days)	Inflorescence period	No. of panicles	Length of panicle (mm)	No. of fruits set per panicle (16-18 cm)
Greenhouse	80.00[a]	60.00[a]	4th week Oct 2020 - 2nd week Dec 2020	8.62[a]	312.00[a]	3.00[a]
Open field	65.16[b]	125.00[b]	1st week Jan 2021 - 3rd week Feb 2021	9.21[a]	324.15[a]	8.54[b]

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

Measurement of yield and fruit quality

In terms of fruit yield, Harumanis mangoes in the greenhouse were harvested 2 months earlier before the main season beginning from February 2021 until May 2021 compared to those in the open field, which began in April 2021 until May 2021 (Table 3). However, the number of fruits produced under greenhouse cultivation was lower compared to the open field. The low fruit yield is related to the low fruit set formation in the greenhouse. For fruit quality assessment, there were no significant differences recorded in this study for parameters of fruit weight, fruit length, pulp weight, TSS, TTA, pH, and ascorbic acid in the greenhouse and the open field (Table 4). Only the parameter for fruit width showed a significant difference for Harumanis mango in the greenhouse and the open field. These findings revealed that the fruit quality of Harumanis mango grown in greenhouses was nearly identical to that of Harumanis mango grown in open fields.

Table 3: The number of fruits produced under greenhouse and open field conditions (February 2021-May 2021).

Location	February-2021	March-2021	April-2021	May-2021	Total
Greenhouse	5	20	12	8	45
Open field	0	0	55	120	175

Table 4: Evaluation of fruit quality of Harumanis mango under greenhouse and open field conditions.

Location	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Pulp weight (g)	Total soluble solid (TSS) ^{o}Brix	Total titratable acidity (TTA) (g/L)	pH	Ascorbic acid (mg/100g)
Greenhouse	316.00[a]	125.00[a]	84.00[a]	224.00[a]	18.93[a]	1.40[a]	5.04[a]	0.11[a]
Open field	321.32[a]	145.16[a]	60.07[b]	253.89[a]	18.44[a]	1.53[a]	6.28[a]	0.14[a]

**Means in each column with the different letters within the column indicate significant differences at p ≤ 0.05% level according to DMRT.*

Conclusions

Based on the results, cultivation of Harumanis under the greenhouse had the potential for early flowering and fruiting. In terms of fruit quality, Harumanis mangoes produced in the greenhouse are almost similar to those produced in the open field cultivation. However, further study is recommended in the future by using pollination agents and internet of thing (IoT) system for the development of higher yields and high-quality mango production under the greenhouse.

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Effects of Biocontrol Rhizobacteria Applications on Growth and Yield of Tomato and Potato Plants Grown under Controlled Conditions

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Introduction

Potato and tomato are the world's top two most important vegetables, with an annual production of more than 350 million and 170 million tonnes, respectively (FAO, 2021). The high quality and yield of production for these crops are nutrient demanding. Therefore, improving the nutrient and water uptake may reduce production costs and minimise nutrient losses. In addition, these crops are susceptible to early and late blight diseases caused by the pathogens, *Alternaria solani* and *Phytophthora infestans.* The latter could cause a 47 to 100% yield reduction in potato (Ojiambo et al., 2000). The use of pesticides is the most common method to manage fungal infections. However, the overuse of agrochemicals harms human health, the environment and the ecosystem. Therefore, a shift to sustainable agriculture is a way to ensure food security and a healthy food system.

Apart from organic farming and integrated pest management (IPM), incorporating plant growth promoting rhizobacteria (PGPR) is a promising approach to promote sustainable agriculture. PGPR could directly promote plant growth through the production of siderophores, phytohormones, volatile organic compounds (VOCs), extracellular polysaccharide substances (EPS), nitrogen fixation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and mineral solubilisation (Glick, 2012). Our preliminary laboratory studies showed that the rhizobacteria, *Pseudomonas fluorescens* 121, *Serratia rubidaea* 119, 268, and *S. plymuthica* S412, minimised the growth of *A. solani* and *P. infestans* in dual culture tests. Moreover, these isolates produce indole-3-acetic acid and siderophores, fix nitrogen, and solubilise phosphate. Furthermore, from our field trial in a late blight hot spot area, these isolates promoted potato plant height compared to the control treatment (Hanifah, 2022). Recognising their potential as plant growth promoting rhizobacteria (PGPR), an experiment in a controlled environment was conducted to determine the effects of these biocontrol rhizobacteria on plant growth and yield of tomato and potato plants. The findings may provide new insights into their ability to form an association and exert beneficial effects on different crop types.

Materials and Methods

Planting material and experimental design

Tomato seeds var *Moneymaker* and potato seed tubers var *Désirée* obtained from the Department of Plant Protection Biology, SLU Alnarp, Sweden, were used as test plants. Ten biological replicates per treatment were used for each crop. They were arranged in a completely randomized design (CRD) in two separate growth chambers in 16 h light/8 h dark photoperiods supplemented with 250 µmol m^{-2} s^{-1} light, at 20 to 23 °C, and 60% humidity for 60 days. The soils were moistened by watering the pots every 2 days with a similar amount of water in every pot. To minimise positional bias, the plant positions were randomly rearranged at least twice a week.

Bacterial treatment

Firstly, tomato seeds were surface sterilised and sown in a 105-holes seed tray filled with potting soils for growing vegetables (Gröna linje, SW Horto, Hammenhög, Sweden). The growing media were kept

moistened by watering daily. At 25 days after sowing, uniform four-leaf stage seedlings were transplanted in 1 L pot containing 325 g of nursery soil (1 seedling/pot). Thirty-five milliliters of *S. rubidaea* 119 and 268, *S. plymuthica* S412 and *P. fluorescens* 121, bacterial suspensions at OD_{600} of 0.2 prepared with 1X PBS or only 1X PBS (control treatment) were added to the roots of tomato seedlings before they were covered with soils. For potato, all seed tubers were first cleaned with water, treated with 100 ppm gibberellic acid, and dried for 8 days to promote bud initiation. Prior to planting in 2 L pots (containing 650 g nursery soil), the seed tubers were soaked in 2 L bacterial suspension at OD_{600} of 0.2 prepared with 1X PBS or with only 1X PBS (control treatment) for 15 min. The inoculation was repeated 10 and 30 days after planting (DAP) with 35 mL bacterial suspension (OD_{600} of 0.2) for each plant in both crops to ensure the growth of bacteria in the soil. The liquid was poured at 2 to 3 cm from the base of each plant and at a depth of 5 cm in the soil.

Plant growth measurement

The effects of selected rhizobacteria treatments on tomato and potato plant height, total leaf number, total plant dry weight, relative chlorophyll content and yield were determined at 60 DAP. Plant height was measured from the soil surface to the tip of the plant. The yields of tomato and potato were measured by total fresh weight. Firstly, fruits or tubers were harvested and weighed. Then, the shoot and root systems were separated. Leaves were separated from the plant by cutting the leaf petiole, and the number was counted. The intact roots were shaken to remove any soil, briefly washed with water, and gently blotted dry using paper towels. Then, immediately the shoot and root systems were separately placed in paper bags and oven-dried at 65 °C until constant weight. Finally, the dry weight was measured using a precision scale and recorded.

Chlorophyll content index measurement

Chlorophyll content of tomato and potato plants grown in the growth chamber were measured at harvest using the MC-100 Chlorophyll Concentration Meter from Apogee Instruments Inc. (Logan, UT, U.S.A.). The MC-100 was calibrated to measure chlorophyll concentration with units of chlorophyll content index (CCI) and zeroed before commencing measurement according to the manufacturer's instructions. The generic equation was used to measure the relative chlorophyll content of both crops. For tomato plants, the measurements were made by clipping the sensor onto the second terminal leaf on the fifth fully expanded leaf from the top of each plant (Matsuda et al., 2014). Meanwhile, the measurements were made for potato plants on the top point of the top leaflet of the fourth compound leaf (Li et al., 2012). Ten measurements per plant were made on the top side of a leaf, and the average value was calculated and recorded.

Soil sampling and analysis

To evaluate the effects of rhizobacteria applications on soil nutrient content, soils were collected and pooled from all pots of each bacterial treatment for analysis. For the control treatment, soils were collected randomly from eight non-inoculated plants. The soil samples were sent to the LMI AB testing laboratory (Helsingborg, Sweden) for analysis. The soils sampled at harvest were analysed for pH, soil organic matter, electrical conductivity, total nitrogen, available phosphorus (P) and available potassium (K).

Statistical analysis

The results from tomato and potato plants grown in the growth chambers were analysed independently using ANOVA in the SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, U.S.A.), and treatment means were compared using the Least Significant Difference's (LSD) test.

Results and Discussion

An experiment in a controlled environment was conducted to evaluate the effects of selected biocontrol rhizobacteria on tomato and potato plant growth and yield. All parameters tested on both crops were significantly affected by the application of biocontrol rhizobacteria except potato leaf number (Figure 1 and Table 1). The inoculation of *P. fluorescens* 121 significantly increased tomato plant height (Figure 1), total leaf number, total dry weight, relative chlorophyll content and yield (Table 1) by 13.3%, 40.1%, 34.5%, 75.3%, and 183.9%, respectively, compared to non-inoculated plants. Meanwhile, *S. rubidaea* 268-treated plants were recorded with 12.3% greater total dry weight than non-inoculated plants (Table 1). On the other hand, inoculation with *S. rubidaea* 119 and *S. plymuthica* S412 on tomato had no significant effect on all parameters tested compared to the control treatment (Table 1). Meanwhile, the inoculation of *P. fluorescens* 121 and *S. rubidaea* 119 significantly increased potato plant height (Figure 1), total plant dry weight, relative chlorophyll content, and yield at $P < 0.05$ (Table 1). Inoculation of *S. rubidaea* 268 increased total plant dry weight but not plant height, and the reverse was observed for plants treated with *S. plymuthica* S412. Nevertheless, all inoculated plants had significantly higher yields compared to the controls. The highest increase in yield was recorded by *S. rubidaea* 119- (15%), followed by *P. fluorescens* 121-, *S. rubidaea* 268- and *S. plymuthica* S412-treated plants with 13.7%, 11.3%, and 10.7%, respectively (Table 1). Besides morphological parameters, the application of *P. fluorescens* 121 significantly improved the relative chlorophyll content of potato (P=0.0287). Meanwhile, the relative chlorophyll content of *S. rubidaea* 119-, *S. rubidaea* 268- and *S. plymuthica* S412-treated plants were not significantly different from the control treatment (Table 1).

Table 2 shows the soil nutrient status at harvest amended with biocontrol rhizobacteria inoculations on tomato and potato plants. The result was obtained from an analysis of a soil sample that was pooled from 8 pots in each treatment. Due to the lack of replication for soil samples, the significant difference between treatments could not be determined. Nevertheless, rhizobacteria applications amended the soil nutrient content of tomato, especially the total nitrogen (TN) (Table 2). At harvest, the inoculation of *P. fluorescens* 121 resulted in 3.4% higher TN in the soil. Meanwhile, the inoculation of *S. rubidaea* 119, *S. rubidaea* 268 and *S. plymuthica* S412 resulted in 10.7%, 7.7% and 24.3% lower TN in the soil, respectively, when compared to the control. The amount of available P, K, electrical conductivity (EC) and soil organic matter (SOM) were not significantly altered among all treatments. In contrast, all rhizobacteria increased available K over the control by 22.2 to 66.7% in soils planted with potato. Meanwhile, TN was decreased in a small range by *P. fluorescens* 121, *S. rubidaea S. rubidaea* 119 and *S. rubidaea* 268 treatments (2.1 to 6.2%) and slightly increased by *S. plymuthica* S412 treatment (0.5%). The available P, EC, and SOM at harvest were relatively unaffected by rhizobacteria treatments. In addition, the application of these four rhizobacteria did not alter soil pH of both crops either.

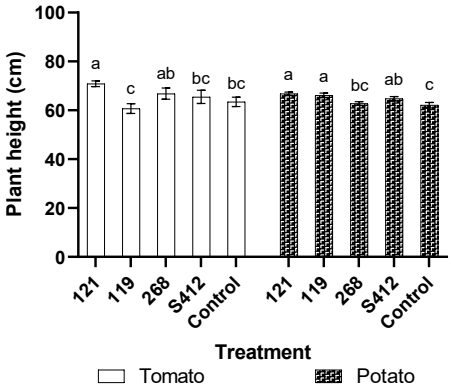

Figure 1: Plant height of tomato and potato plants under *P. fluorescens* 121, *S. rubidaea* 119, *S. rubidaea* 268, *S. plymuthica* S412 and control treatments at harvest.

Table 1: Total dry weight, relative chlorophyll content and yield of tomato and potato plant subjected to inoculations with *P. fluorescens* 121, *S. rubidaea* 119, *S. rubidaea* 268 and *S. plymuthica* S412 inoculations, and control treatment (no inoculation). DAT: days after transplanting.

	Tomato				Potato			
Treatment / Parameter	Total leaf number	Total plant dry weight (g)	Relative chlorophyll content (CCI)	Yield/plant (g)	Total leaf number	Total plant dry weight (g)	Relative chlorophyll content (CCI)	Yield/plant (g)
P. fluorescens 121	19.22^{a}	44.8^{a}	27.30^{a}	73.3^{a}	63.0^{ab}	48.5^{a}	14.20^{a}	149.0^{a}
S. rubidaea 119	14.34^{b}	34.6^{c}	18.77^{b}	32.0^{b}	50.9^{b}	47.8^{a}	13.90^{a}	150.7^{a}
S. rubidaea 268	15.56^{b}	37.3^{b}	18.20^{b}	30.7^{b}	64.1^{ab}	47.2^{a}	10.48^{b}	145.8^{a}
S. plymuthica S412	15.00^{b}	35.2^{bc}	18.57^{b}	30.3^{b}	60.1^{ab}	46.4^{ab}	11.52^{ab}	145.0^{a}
Control	13.72^{b}	33.3^{c}	15.57^{b}	25.8^{b}	73.6^{a}	43.8^{b}	10.64^{b}	131.0^{b}
p-value	0.0082 (**)	<0.0001 (***)	0.0188(*)	<0.0001 (***)	0.0625 (ns)	0.0152 (*)	0.0287 (*)	0.0103 (*)

*Values within a column without a common letter are significantly different by LSD's test at 95% confidence level. Each value is the mean of nine biological replicates per treatment. * = P < 0.05, ** = P < 0.01, *** = P < 0.0001, and ns = not significant.*

Table 2: Soil nutrient contents amended by the inoculation of *P. fluorescens* 121, *S. rubidaea* 119, *S. rubidaea* 268 and *S. plymuthica* S412 in tomato and potato. TN: total nitrogen, P: phosphorus, K: potassium, EC: electrical conductivity, SOM: soil organic matter.

	Tomato						Potato					
Treatment	TN (mg/kg)	Available P (mg/L)	Available K (mg/L)	pH	EC (mS/cm)	S.O.M. (%)	TN (mg/kg)	Available P (mg/L)	Available K (mg/L)	pH	EC (mS/cm)	S.O.M. (%)
P. fluorescens 121	7080	36	140	5.9	1.7	42.7	5780	29	15	6.5	1.4	43
S. rubidaea 119	6190	34	140	5.9	2.1	43.4	6000	27	11	6.4	1.3	48
S. rubidaea 268	6360	34	130	5.9	2.4	39.6	6030	27	14	6.4	1.4	44
S. plymuthica S412	5510	34	150	5.8	2.3	41.0	6190	28	11	6.5	1.3	44
Control	6850	32	130	5.9	2.3	42.9	6160	28	9	6.5	1.2	46

The results suggested that growth promotion activity in tomato plants var. *Moneymaker* is strain dependent. *S. rubidaea* 119 and *S. plymuthica* S412 applications did not improve any growth variable, whereas plant height and total dry weight were increased after *P. fluorescens* 121 and *S. rubidaea* 268 applications. Yield parameters, total leaf number and chlorophyll content increased after *P. fluorescens* 121 application. Furthermore, *P. fluorescens* 121-treated plants had a higher leaf number (Table 1) with broad leaf surface area (Figure 2 and visual observation). These traits are associated with light interception and photosynthesis rate (Heuvelink et al., 2005). Along with high chlorophyll content, leaves of *P. fluorescens* 121-treated plants might have higher photosynthesis capacity than the other treatments, which explains high yield, leaf number and overall dry weight.

P. fluorescens 121 *S. rubidaea* 119 *S. rubidaea* 268 *S. plymuthica* S412 Control

P. fluorescens 121 *S. rubidaea* 119 *S. rubidaea* 268 *S. plymuthica* S412 Control

Figure 2: Tomato (above) and potato (below) plants subjected to treatment with *P. fluorescens* 121, *S. rubidaea* 119, *S. rubidaea* 268, *S. plymuthica* S412, and no inoculation (control) at 42 and 53 days after transplanting (DAT), respectively.

Additionally, TN, available P and K were slightly increased after *P. fluorescens* 121 treatments in soils planted with tomato (Table 2). This strain may have facilitated nutrient availability for plant uptake by producing organic acids. Apart from that, *P. fluorescens* 121-treated plants may have higher water use efficiency than other treatments, as illustrated by higher plant biomass and chlorophyll content, even though a similar amount of water was supplied in all treatments. This positive effect may have been attributed to EPS production that improved soil water retention capacity. However, this hypothesis

needs to be confirmed by investigating EPS production by *P. fluorescens* 121 and its effects on soil aggregation. Nevertheless, these results suggested that *P. fluorescens* 121 effectively formed an association with tomato plants compared to other strains and exerted beneficial effects on growth and productivity.

Table 1 shows that all strains increased at least one growth variable of potato plants var *Bintje* grown in pots. Moreover, the application of *P. fluorescens* 121 and *S. rubidaea* 119 increased all growth parameters measured (Figure 1 and Table 1). Interestingly, this result was consistent with their growth promoting activity on potato var *Desirée* tested under *in vitro* condition (Hanifah, 2022), showing their capability to promote the growth of different potato varieties. Nevertheless, all inoculated plants presented greater yield than those non-inoculated. This result showed that all rhizobacteria can greatly influence potato yield production. Their effect on dry matter accumulation was pronounced in the tuber, accounting for more than 67% of the total dry weight (data not shown). This result suggested translocation of photosynthate into tubers was enhanced with rhizobacteria application. In addition, the application of these bacteria might have facilitated continuous potassium (K) supply which is essential for starch production in potato tubers, by solubilising the mineral for plant uptake (Table 2) (Lindhauer and De Fekete, 1990). All bacteria can fix atmospheric nitrogen, however, TN was decreased at harvest, especially after *P. fluorescens* 121 and *S. rubidaea* 119 treatments. It was probably due to high nitrogen uptake to promote tuber growth and the above-ground plant part, as those processes require a high amount of N (Naumann et al., 2020).

Conclusions

All the rhizobacteria tested in this study increased potato yield, showing their potential as PGPR. Among all strains, *P. fluorescens* 121 consistently exerted beneficial effects on both test crops, which can be considered an interesting candidate for commercialisation. However, more research is needed prior to commercialisation which includes studies on its efficiencies as a PGPR in a wide range of production systems such as field, greenhouse, soilless culture and climatic conditions, its compatibility and interaction with other PGPR, its pathogenicity towards humans, and its impacts on the phyllosphere and rhizosphere microbial community and soil fertility.

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Calcium Biofortification Effects on Curly Kale Production Grown in a Plant Factory

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Introduction

Calcium (Ca) is an essential structural component in bones and teeth and takes part in a variety of biochemical and physiological processes. The concentration of Ca in foods of plant origin shows a wide range of variation. The lower Ca content is generally found in fruits (i.e.: apples, green pepper, tomato) and tubers (potato) with, on average, 10 mg of Ca/100g fresh matter (FM); higher values are present in leafy vegetables such as endive (93 mg/100g FM), kale (85 mg/100g FM) and spinach (78 mg/100g FM), (Martinez Ballesta et al., 2010). Therefore, leafy vegetables can be an excellent dietary source of Ca and are a good target for biofortification vegetables (Neeser et al., 2007). Data on the mineral content of foods are important and should be considered when recommending the daily intake of minerals, in particular, the RDA of Ca is considered in the wide range of 1000–1300 mg/day for adults, and 1300 mg/day for children above nine years old (Yates et al., 1998). Many studies have been performed with the aim of increasing the content of specific nutrients in edible portions (biofortification) of vegetables (Malorgio et al., 2009; Smolen et al., 2014 and Tomasi et al., 2015; D'Imperio et al., 2016). The biofortification of plants is an important method to increase nutrients in foods and improve the nutritional status of the target population, by using different approaches such as conventional plant breeding, genetic modification and agronomic methods (Carvalho and Vasconcelos, 2013). For Ca, the biofortification strategies provide increasing content in plants without adversely affecting plant growth or increasing production costs (White and Broadley, 2009). Calcium is the third-most important nutrient element available in the soil and is an essential element for plants. In soil, it comes from various sources such as weathering of a number of common minerals and rocks including feldspar, apatite, limestone and gypsum. Furthermore, Ca is a constituent of many compounds such as Ca carbonate, Ca phosphate, Ca sulphate, Ca gluconate, Ca chloride and others (Pilbeam and Marley, 2006). Some compounds are soluble, while most Ca compounds are generally insoluble and thus making the Ca unavailable to plants. In addition, soil application of Ca fertiliser has limited success, because of the problems associated with the transport of this element to phloem-fed tissue (Karley and White, 2009). In this context, the soilless cultivation, and in particular the floating system at the plant factory, utilised to obtain high yield and quality of leafy vegetables, allows appropriate monitoring of the plant nutrient uptake, and to produce leafy vegetables biofortified (Malorgio et al., 2009; Tomasi et al., 2015; D'Imperio et al., 2016). Regarding the Ca biofortification process, particular attention must be given to two different aspects: (1) choice of vegetable target and (2) level and chemical forms of Ca used to increase the Ca concentration in nutrient solution (NS) because many Ca salts used to prepare the nutrient solution have low solubility and could have a negative impact for vegetables and for human health. In normal practices, the standard Ca concentrations, for mostly leafy vegetables are 50 mg/L of Ca in NS. In addition, is more advisable not to use a high Ca level in order to avoid possible increase of its counteranion (Cl) and electric conductibility of NS, with possible reduction of yield, as reported by Borghesi et al. (2013). Curly kale is one of the superfoods. This green is packed with protein, vitamin C, iron, vitamin A, fibre, and anti-cancer properties and it is one of the vegetables with high calcium content. The addition of calcium elements in NS will further increase the calcium content in the plant. Since Curly kale vegetables are arousing more and more interest in consumers, the choice of species for growing was made

considering this aspect. Starting from these remarks, the aims of this study are to increase the Ca content of Curly kale vegetables without affecting vegetable growth and marketable quality.

Materials and Methods

Plant material and treatment application

The experiment was carried out with six nutrient solutions in a Plant Factory, MARDI Serdang. The seeds were sown in a sponge cell. After germination, the seedlings (with two true leaves) were transferred in a floating hydroponic system using six different NS with different levels of Ca: T1 (50 mg/L: control), T2 (100 mg/L), T3 (150 mg/L), T4 (200 mg/L), T5 (250 mg/L) and T6 (300 mg/L) calcium nitrate ($CaNO_3$) was added in order to reach different Ca level. Other micronutrients were added as defined in the nutrient solution proposed by Mohammad Abid et al. (2022). The treatments were arranged in a completely randomized design with three replications. Among important parameters that were considered are yield and physical measurements; fresh and dry yield (g) of plant, root-to-shoot ratio, leaf weight ratio, number of leaves, plant height (cm) and plant canopy diameter (cm), phytochemical content; TSS (°Brix) TTA (% Citric acid), ascorbic acid (mg/100g FW), total antioxidant (% DPPH), phenolic GAE (mg/100g FW), leaf texture (N) and inorganic cation and anion content (S, K, Mg, P and NO_3). The data was subjected to an analysis of variance (ANOVA) and differences among treatment groups were assessed using LSD using SAS software version 9.4. P values <0.05 were considered to indicate statistically significant results.

Results and Discussion

Effect of treatments on plant growth

A parameter of the fresh and dry yield of the plant (leaf, root, stem), plant canopy diameter and the number of leaves were significantly affected by the different Ca concentrations (Table 1 and 2). While plant height, root-to-shoot ratio and leaf weight ratio showed no significant difference as affected by the Ca treatments (Tables 1 and 3). Normally the agronomic approaches used for obtained biofortified vegetables did not reduce the yield, as reported in studies by D'Imperio et al. (2016), Malorgio et al. (2009) and Smolen et al. (2014), respectively, for biofortification in silicon, selenium and iodine. T1 which is control showed the least fresh yield and fresh leaves weight compared to other treatments.

Table 1: Plant height (cm), Pant canopy diameter (cm) and Number of leaves.

Treatments	Plant height (cm)	Plant canopy diameter (cm)	Number of leaves
T1	4.53^{b}	10.94^{b}	6.07^{c}
T2	5.133^{a}	13.10^{a}	6.83^{ab}
T3	4.79^{ab}	12.91^{a}	6.35^{cb}
T4	4.86^{ab}	12.71^{ab}	6.46^{bc}
T5	5.12^{a}	14.13^{a}	7.13^{a}
T6	4.96^{ab}	13.92^{a}	7.33^{a}
F-Test significant	ns	*	**

*Means with the same letters within a column are not significantly different from each other; (*p < 0.05, **p < 0.01,***p < 0.001, ns: not significant).*

Table 2: Effect of treatments on fresh yield (g), fresh root weight (g), fresh leaves weight (g), fresh stem weight (g), dry yield (g), dry root weight (g), dry leaves weight (g) and dry stem weight (g).

Treatments	Fresh yield/plant (g)	Fresh root weight (g)	Fresh leaves weight (g)	Fresh stem weight (g)	Dry yield (g)	Dry root weight (g)	Dry leaves weight (g)	Dry stem weight (g)
T1	17^{c}	2.59^{b}	13.15^{c}	1.26^{c}	1.35^{c}	0.15^{b}	1.10^{c}	0.09^{b}
T2	24.17^{ab}	3.94^{ab}	20.05^{ab}	1.83^{ab}	2.12^{a}	0.27^{ab}	1.70^{ab}	0.15^{a}
T3	29.23^{a}	4.60^{a}	22.29^{a}	2.12^{a}	2.41^{a}	0.29^{a}	1.97^{a}	0.16^{a}
T4	25.36^{ab}	4.61^{a}	19.19^{ab}	1.56^{bc}	1.91^{bc}	0.29^{a}	1.51^{bc}	0.12^{b}
T5	27.05^{ab}	4.57^{a}	21.33^{a}	2.10^{a}	2.42^{a}	0.30^{a}	1.96^{a}	0.17^{a}
T6	29.13^{a}	4.83^{a}	21.9^{a}	1.92^{a}	2.50^{a}	0.34^{a}	2^{a}	0.16^{a}
F-Test significant	***	*	***	***	*	*	***	***

*Means with the same letters between treatments per parameter are not significantly different from each other; (*p < 0.05, **p < 0.01,***p < 0.001, ns: not significant).*

Table 3: Root-to-shoot ratio and leaf weight ratio as affected by different Ca concentrations.

Treatments	Root-to-shoot ratio	Leaf weight ratio
T1	0.123^{a}	0.824^{a}
T2	0.147^{a}	0.802^{a}
T3	0.138^{a}	0.814^{a}
T4	0.169^{a}	0.796^{a}
T5	0.14^{a}	0.807^{a}
T6	0.155^{a}	0.803^{a}
F-Test significant	ns	ns

*Means with the same letters within a column are not significantly different from each other; (*p < 0.05, **p < 0.01,***p < 0.001, ns: not significant).*

Effect of treatments on calcium content and dry matter yield

The highest Ca content was found in T4 (440.57 mg/100 g), whereas the lowest Ca content was found in T1 (280.8 mg/100 g). Application of different levels of Ca did not show any significant difference in yield among the treatments except with 50 mg/L Ca (control) which showed the lowest yield (250 g/plant). The T4 treatment caused a significant increase in Ca content, with a Ca enrichment (Figure 1). Borghesi et al. (2013) reported a high increase in Ca content in lettuce cultivated in a floating system, after treatment with 800 mg/L of Ca (from $CaCl_2$). However, the use of such concentrations and $CaCl_2$ in nutrient solution caused an increase in chloride and electrical conductivity, with a reduction of yield of about 32% and a reduction of marketable quality (Borghesi et al., 2013). The increase of Ca content found in our research was in agreement with other authors that used agronomic (Neeser et al., 2007) and transgenic approaches (Park et al., 2009), without effects on production and marketable quality of vegetables. A higher increase of Ca content in the nutrient solution can be obtained using different chemical forms of Ca [$CaCl_2$ and $Ca(NO_3)_2$], however, this method could change some chemical parameters, such as an increase of Cl with negative impact for vegetables (Borghesi et al., 2013) and increase of NO_3 content with negative impact for human health (Santamaria, 2006).

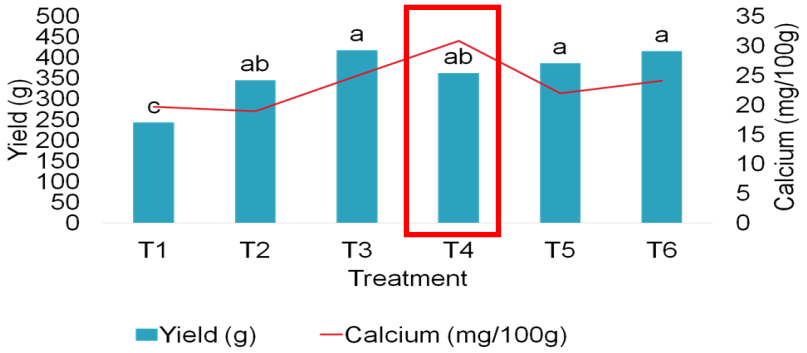

Figure 1: Effect of treatments on Ca content and dry matter yield.
*Means with the same letters between treatments are not significantly different from each other; (*p < 0.05, **p < 0.01,***p < 0.001, ns: not significant).*

Effect of treatments on cation content (S, Mg, K and P)

S, Mg and K content was found higher in T1, which is opposite to Ca content (Table 4). Borghesi et al. (2013) also stated that the content of Ca with Mg and S is antagonistic. The content of P was found to be higher in T4 and it is similar to Ca content (Table 4). This shows that the addition of Ca elements does not affect other elements in Curly Kale. The results obtained in this study are the same as the study by Borghesi et al. (2013), in which increasing Ca content in nutrients does not have a significant effect on other nutrients.

Table 4: Effect of treatments on inorganic cations: Sulphur (mg/100g), Magnesium (mg/100g), Potassium (mg/100g) and Phosphorus (mg/100g).

Treatments	Sulphur (S)	Magnesium (Mg)	Potassium (K)	Phosphorus (P)
T1	225.22^{a}	97.19^{a}	691.72a	79.72^{b}
T2	182.15^{c}	75.66b	649.30^{b}	68.69^{d}
T3	176.25^{d}	61.97^{e}	640.12^{c}	71.34^{c}
T4	189.56^{b}	62.00^{d}	637.43^{d}	79.81^{a}
T5	160.16^{f}	62.97^{c}	569.7^{f}	64.79^{e}
T6	170.77^{e}	61.59^{f}	593.73^{e}	59.83^{f}
F-Test significant	***	***	***	***

*Means with the same letters between treatments are not significantly different from each other (*p < 0.05, *p < 0.01,***p < 0.001, ns: not significant).*

Effect of treatments on anion content (NO_3)

The highest NO_3 was found in T5 (838 mg/kg) but not significantly higher as compared with T3, T4 and T6 (on average, 716.7, 761.1 and 721.9 mg/kg, respectively) (Table 5). The NO_3 contents in leaf vegetables are an essential parameter for marketable quality of ready-to-eat products, since, the presence of NO_3 in vegetables is a serious threat to man's health (Santamaria, 2006). However, in this study, the biofortification process did not influence the nitrate content in curly kale. Thus, the nitrate contents evaluated in all treatments in this research were lower with respect to the standard limit imposed by Commission Regulation (EU) No 1258/2011 for similar products, kale 2000 kg FM.

Table 5: Effect of treatments on NO_3 content.

Treatments	Nitrate (mg/kg)
T1	590.7[b]
T2	587.4[b]
T3	716.7[ab]
T4	761.1[ab]
T5	838.0a
T6	721.9[ab]
F-Test significant	*

*Means with the same letters within a column are not significantly different from each other; (*p < 0.05, **p < 0.01,***p < 0.001, ns: not significant).*

Effect of treatments on phytochemical content

Table 6 shows that T6 significantly gave the highest value of ascorbic acid (54.89 mg/100g FW), while T1 shows the highest value of phenolic content (26.15 mg/100g FW). Other parameters were not significantly influenced by Ca concentration treatments. Thus, the results of the present study suggest that the use of a nutritive solution with a Ca concentration with respect to the concentration usually used for growing vegetables allows for obtaining a yield without significant changes in phytochemical content.

Table 6: TSS (°Brix), TTA (% Citric acid), ascorbic acid (mg/100g FW), total antioxidant (% DPPH), phenolic GAE (mg/100g FW) and leaf texture (N) as affected by different Ca concentrations.

Treatments	TSS (°Brix)	TTA (% citric acid)	Ascorbic acid (mg/100g FW)	Total Antioxidant (% DPPH)	Phenolic GAE (mg/100g FW)	Leaves texture (N)
T1	5.08	0.147	45.51[b]	53.70	26.15[a]	41.89
T2	4.78	0.152	45.07[b]	55.16	23.74[b]	35.63
T3	5.31	0.125	52.18[ab]	56.40	22.58[bc]	32.16
T4	5.08	0.145	48.31[ab]	55.84	21.23[c]	39.28
T5	5.12	0.119	46.98[ab]	57.46	21.70[bc]	36.37
T6	5.59	0.128	54.89[a]	62.42	22.16[bc]	35.77
F-Test significant	ns	ns	*	ns	*	ns

*Means with the same letters within a column are not significantly different from each other; (*p < 0.05, **p < 0.01,***p < 0.001, ns: not significant).*

Conclusions

Treatment T4 (200 mg/L) showed the highest Ca value compared to other treatments without affecting yield. The application of 200 mg/100g of Ca to the nutrient solution allows for obtaining Ca biofortified. Consumption of biofortified curly kale in the diet is a reasonable recommendation to Ca-deficient populations for improving their Ca nutritional status. These products could be considered as an alternative strategy to provide Ca in vegan diets or diets destined to be intolerant to milk products.

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Yield Performance of Bell Pepper Varieties in the Malaysia Lowland Cultivation

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Introduction

Bell pepper [*Capsicum annuum* var. *grossum* (L.) Sendt.] is one of popular crop in Malaysia from species *C. annuum*, in Group Grossum. This cultivar is not pungent due to the absence of capsaicin content. The taste of this chilli is rather slightly bitter and grassy when it is green to sweet and mild fruity when it is matured. Therefore, it is usually consumed fresh, used in salad, or in western dishes. This crop has high nutritional value. One cup serving of red bell pepper (149g) provides 93% of the daily requirement for vitamin A, 317% Daily Value of vitamin C, 1% Calcium, and 4% iron. Fiber content of around 3.1g provides 13% of the daily fiber requirement. This dish also provides 39 calories of which 78% are carbs, 13% protein and 9% fat (Nutrition Data).

In Malaysia bell pepper is widely cultivated in highland agricultural areas such as in Cameron Highland, Pahang, Lojing Kelantan, and Ranau and Tambunan in Sabah as well as in Kinta Perak covering a total area of 122.3 ha with a production of 5623 Mt. However, this value is still insufficient for local consumers. In 2021, our country imports 44.8 Mt of commodity chillies under HS Code 090421 Peppers (Capsicum or Pimenta) - dried, whole which includes bell pepper with a value of around $ 107.6 million (Tridge 2022). One of the focuses of food security in the National Food Security Policy Action Plan (2021-2025) is to increase internal resources and diversify import sources (MAFI, 2022). Therefore, one of the strategies is to expand the agriculture sector including growing the temperate crop in lowland areas. These steps were taken to avoid dependency in the highland agriculture area which is already limited and preventing more deforestation that will damage the ecosystem. The idea to plant bell pepper in the lowland areas however will be challenging and require more information before it could be implemented.

Bell pepper needs sufficient light and well-drained soil and ideal temperature (day temperature 25-28 and night temperature 16-18) (Starke Ayres, 2014). Temperature has a very significant impact on bell pepper growth. Low night temperatures below 20 to 12 °C after flower bud initiation have a significant impact in increasing the size of bell pepper fruit (Cruz Huerta et al., 2011). However, exposure to temperatures beyond the ideal range, whether too cold or too hot usually caused adverse effects on the plant in term of germination ability, flower development, pollen viability and germination, plant growth, fruit set, fruit size, yield, seed number per fruit and fruit quality (Polowick and Sawhney, 1985; Mercado et al., 1997; Aloni et al., 2001; Saha et al., 2010; Diaz-Perez, 2014).

This paper presented the findings of an evaluation of bell pepper in the lowlands that has been carried out at the Vegetable Research Plot, MARDI Headquarters, Serdang. The purpose of the experiment was to evaluate the performance of commercial bell pepper varieties at lowland cultivation under Malaysian environment. This study is one of the initial steps to develop bell pepper production technology in the lowlands before manipulation of environments will be suggested.

Materials and Methods

Experiment was conducted at the Vegetable Experimental Plot of Horticulture Research Centre, MARDI Serdang from September 2021 to January 2022. All the seeds were obtained from the commercial market and recorded for research purposes. Most of the varieties used were not identified specially for lowland planting except SP06 and SP12. Seeds were sowed in a germination tray

containing peat moss. At the beginning, 19 varieties were sowed, but only 12 were germinated and evaluated (Table 1). Once the seedlings produced 3-5 true leaves, they were hardened and then transferred to polybags and placed in an open field. Polybag media consist of a soil mixture of mineral soil, sand and organic matter (at ratio: 3:2:1). The experiment was arranged in completely randomized design. The polybags were arranged at 0.5 m distant within the row and 1.5 m apart between rows. Each variety was represented by 10 plants. Granule fertilizer NPK were given at the rate 1.5 t/ha in 4 split applications. Watering was given twice a day using a drip irrigation method. Table 2 showed the temperature reading during the lifespan of the experiment.

Data were taken on yield and yield related traits. Fresh fruit weight was obtained from the average of 10 harvested fruits. Fruits were harvested at the recommended stage for each variety. Therefore, some varieties were harvested green or purple while some were harvested red. Fruit length and diameter were measured using a digital calliper. Number of fruits and yield per plant were recorded from each plant. The data were subjected to analysis of variance and the differences between means were compared using Duncan New Multiple Range Test. Analysis of variance was conducted using the SAS package.

Table 1: List of bell pepper varieties evaluated.

No.	Varieties	Original name and source	Recommended harvesting stage
1.	SP01	K77, JC Garden	Red
2.	SP02	F_1 Pepper California, Home Garden	Green
3.	SP04	SP299 Red Masta, GWG	Red
4.	SP05	113 Milano, Leckat	Green
5.	SP06	VE-018, Baba Smart Grow	Red
6.	SP07	CL 44 Overland; Crop Power	Red
7.	SP09	Capsicum; Hengchang Seed	Green
8.	SP10	Keystone Giant; Wellgrow Seed	Red
9.	SP11	Capsicum; JK Lifeng Seed	Green
10.	SP12	119 Jingle Bell; Crop Power	Red
11.	SP13	C062 Flower Goddess; no brand	Purple
12.	SP19	Capsicum; Tanegoodies	Green

Table 2: Mean monthly temperature (°C) during the experimental period.

Month in 2021	Temperature		
	Maximum	Minimum	Mean
September	35	22	28.4
October	36	23	29.6
November	35	23	28.5
December	34	23	28.2
January	35	23	28.7

Results and Discussion

Fruit weight, length and diameter

Fresh weight, fruit length, fruit diameter, number of fruits per plant and yield per plant significantly affected ($\rho < 0.01$) by different varieties evaluated. Table 3 showed the average fresh weight, fruit length and fruit diameter of twelve bell pepper varieties. The average fresh weight was 32.4 g. The highest average fruit weight obtained was 42 g from SP06. The maximum weight obtained so far is 85 g obtained from SP06 and SP11. While the lightest fruit weight was 4 g which was obtained from SP13. Bhattarai et al. (2014) recorded bell pepper fruit weight ranged from 29 g to 126 g while Buczkowska et al. (2014) recorded bell pepper fruit weight ranged from 88–124 g.

SP13 has a longer fruit length average which is 5 cm. Report by Bhattarai et al. (2014) showed 8.6 cm fruit length on California Wonders variety meanwhile Awuku and Egyir (2018) has obtained a mean 5

cm for the same variety. Average fruit diameter among all varieties is 4.9 cm. Fruit diameter could reach 7 cm as shown in some samples of SP12, SP05 and SP06.

Table 3: Fresh weight, fruit length and fruit diameter.

Gen.	Fresh weight (g)				Fruit length (cm)				Fruit diameter (cm)			
	Mean	Std Dev	Max.	Min.	Mean	Std Dev	Max.	Min.	Mean	Std Dev	Max.	Min.
SP01	38.9^{b}	14.1	79.54	13.89	4.3^{b}	0.86	6.4	2.8	5.2^{b}	0.81	6.9	3
SP02	27.2^{f}	8.5	44.55	6.13	4.2^{bc}	1	7.2	1.9	4.4^{d}	0.67	6.1	2.7
SP04	34.8^{bcd}	16.6	74.73	10.25	4.0^{bcd}	0.99	6.3	2	4.9^{bc}	0.99	6.5	3.3
SP05	31.8^{c-f}	13.1	79.78	7.11	4.0^{bcd}	0.82	6	2.5	4.9^{bc}	0.83	7.2	1.8
SP06	42.4^{a}	16.2	85.67	18.61	4.2^{bc}	1.06	7.8	2.6	5.5^{a}	0.79	7.3	3.8
SP07	37^{ab}	14	83.33	11.2	4.8^{a}	1.72	8.9	2.4	4.9^{bc}	0.86	6.6	3.1
SP09	29^{d-g}	11.8	63.65	11.17	3.9^{bcd}	0.74	6.4	2.2	4.9^{bc}	0.83	6.5	3
SP10	29.9^{def}	13.2	69.1	12.43	4.1^{bc}	0.95	5.8	2.6	4.7^{cd}	0.7	5.6	3.4
SP11	28.4^{efg}	12.5	85.43	9.88	3.9^{bcd}	0.9	7	3.1	4.7^{cd}	0.69	6.4	3.3
SP12	34.1^{b-e}	13.1	75.52	13.01	3.8^{cd}	0.91	6.5	2.2	5.1^{bc}	0.78	7	3.8
SP13	23.3^{g}	10	49.08	4.4	5.1^{a}	1.27	7.9	1.8	4.0^{e}	0.77	6.4	2.3
SP19	34.5^{bcd}	15	71.21	14.95	3.6^{d}	0.87	5.8	1.9	5.0^{bc}	0.77	6.4	3.2
Mean	32.4**				4.1**				4.9**			

Means in each column with the different letters within the column indicate significant differences at p ≤ 0.05 level according to Duncan New Multiple Range Test.
*** significant different at ρ ≤ 0.01.*

Number of fruits per plant and yield per plant

Table 4 showed the result on fruit number and yield per plant. The average number of fruits per plant is 7 fruits. Variety SP05 gives the highest average fruit number per plant which is 12 fruits. The maximum number of fruits obtained is 27 which is from SP19 while some varieties such as SP01, SP02, SP04, SP06, SP07, SP09 and SP10 have plants with no fruits at all. High fruit numbers however do not contribute high yield per plant. This is related to the small fruit size which has had an impact on the yield. The average yield is 204.6 g per plant. The highest average was obtained from SP05 which was 358.8g per plant. The maximum yield of this variety is 611.6g. This result however is still far from the true potential of bell pepper when compared to those grown at low temperatures. Depending on the variety, the potential yield per plant for common bell pepper is between 2.0 kg–2.5 kg per plant (Vikaspedia). Some varieties could also reach up to 4 kg / plant (Mohd Anim, 2010).

Table 4: Number of fruits per plant and yield per plant.

	Number of fruits per plant				Yield per plant (g)			
Gen.	Mean	Std Dev	Max.	Min.	Mean	Std Dev	Max.	Min.
SP01	6.6^{bcd}	4.5	15	0	230.7^{bc}	136	412.1	0
SP02	5.3^{cd}	3.9	13	0	133.5^{cd}	113.8	346.6	0
SP04	5.4^{cd}	4.1	12	0	159.9^{bcd}	126.2	320.9	0
SP05	12.3^{a}	5.4	23	3	358.8^{a}	133.9	611.6	122.8
SP06	6.8^{bcd}	4.1	16	0	269.6^{ab}	155.2	490.9	0
SP07	4.7^{cd}	3.5	10	0	161.7^{bcd}	142.3	349.1	0
SP09	4.8^{cd}	3.3	9	0	128.4^{cd}	90.2	264.8	0
SP10	2^{d}	2.3	8	0	58.2^{d}	49	149.1	0
SP11	10.5^{ab}	7.3	26	2	258.2^{abc}	146.6	511.5	69
SP12	7.3^{bc}	3.7	12	1	235^{abc}	126.4	417.2	45.1
SP13	8.9^{abc}	5.1	18	2	208.6^{bc}	127	420.7	43.2
SP19	8.7^{abc}	7.1	27	2	253.1^{abc}	132.7	454.8	71.2
Mean	6.9**				204.6**			

Means in each column for each trait with the different letters within the column indicate significant differences at p ≤ 0.05 level according to the Duncan New Multiple Range Test.
*** significant different at ρ ≤ 0.01.*

Conclusions

From this experiment, it is concluded that bell pepper could be planted in lowland areas using conventional planting methods. However, the size of the fruit and yield will be reduced from the commonly marketed bell pepper which is usually planted in recommended temperature. Each variety will give different performance and the environment's factors contributed crucial impact. The findings from this experiment provide an overview on the performance of these plants in the lowlands when there is no intervention of environmental manipulation to suit the plants' needs. Technology such as the root zone cooling system, controlled environment system coupled with fertigation techniques may be suitable to improve the yield of bell pepper planted in Malaysia lowland. Other options are to plant a heat tolerance variety and at the same time we need to develop a heat tolerance bell pepper variety that is suitable with Malaysia lowland temperature.

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Effects of Citric Acid Treatment on Green Liberica Coffee Beans

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Introduction

Coffee bean products are popular as roasted coffee rather than green coffee products. The roasted coffee is one of the most popular coffee ingredients worldwide as whole roasted beans, processed beans (powdered) and instant coffee because of its unique taste with desirable odour properties of refreshing and stimulating characters. The green coffee beans (GCBs) are often used as cosmetic ingredients rather than a beverage ingredient, and there are a lot of GCB supplements such as in capsules or tablets, pre-mixed functional drinks and high antioxidant materials. GCBs contain chlorogenic acids (CGA) and other derivatives between 3.5-7.5% and 7.0-4.0% (dry basis) for Arabica and Robusta coffee beans, respectively (Narita and Inouye, 2011). The three compounds known as 3-CQA, 4- CQA and 5-CQA are dominated as CGA derivatives in the GCBs and their extracts (Perrone et al, 2008; Dziki et al., 2015). Caffeine contents vary considerably relying on the coffee species, method of bean-roasting and beverage preparation. Caffeine has numerous pharmacological effects, including its effects on central nervous system and vascular tissue (Echeverri et al., 2010).

The chemical compositions of coffee beans are very complex consisting of volatile and non-volatile compounds. The GCB components are mostly consisting of carbohydrates, lipids, proteins, peptides, amino acids, alkaloids, organic acids and phenolic compounds. Most of these components are important precursors for the coffee aroma especially during roasting. In GCBs, CGA is a specialty compound which is responsible for the coffee bitterness, while sucrose is responsible for the coffee sweetness. Chlorogenic acids (CGA) are esters of quinic acid and various class of hydroxycinnamic acids such as caffeic acid (3, 4-dihydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), *p*-coumaric (4-hydroxycinnamic acid), and sinapic acid (3, 5-dimethoxy-4-hydroxycinnamic acid) (Manach et al., 2004). The CGA is the main phenolic compound in the coffee beans. This study evaluated and determined the physical, chemical and assay characteristics of the MARDI's Liberica coffee beans by investigating its GCB morphology, yields, scavenging activities against DDPH radicals, ferric reduction, total phenolic (TPC), flavonoid contents and the coffee beans' active phyto-chemicals (caffeine and CGA).

Materials and Methods

A sampling was carried out at MARDI Kluang, Johor, Malaysia. Three clones of Liberica coffee were harvested and filled into special containers and freshly transported to MARDI HQ Serdang on the same day after harvesting. The cleaning process was done under a tap water, followed by rinsing with filtered RO water. Each sample was air-dried followed by citric acid (C.A.) treatments and analysis. The three Liberica coffee clones namely clone 213, clone 222 and clone 224 are illustrated in Figure 1.

Figure 1: Liberica coffee clones: MKL 213, MKL 222 and MKL 224.

An amount of 100 g sun-dried coffee beans were weighed and soaked in 0% (control), 2.5% and 5.0% C.A. solution (2 L). The coffee beans were soaked for 48 hours. The treated coffee beans were toasted and its berry layers and parchments were removed to obtain green coffee beans. Then, the coffee beans were washed with tap water and rinsed with distilled water to remove all the residuals of acids and other gummy materials. The green beans were dried in an oven at 55-60 ^{o}C for 24 hours in order to achieve 10-12% moisture content. The dried green beans were packed in glass bottles and frozen prior to analysis.

In this study, the green Liberica coffee beans were investigated for GCB yields, morphology (Stereo Microscope, Olympus) and phytochemicals (Scavenging activities IC_{50}, ferric reduction, phenolic and flavonoid contents (Mirfat et al., 2020), while caffeine and CGA contents were determined using a HPLC methods (MARDILab in-house laboratory procedure).

Results and Discussion

Yield and colour of GCBs after C.A. treatment

In Figure 2, an increased in the C.A. concentration changed the treated coffee bean parchment and mucilage into darker red in colour as compare to control coffee beans. In the control samples (water treatment), coffee bean parchment and mucilage turned into brownish and darker colour than those treated with C.A.. The acidic condition has changed the coffee bean pulps and parchments into darker red due to the acidic condition which retarded the enzymatic browning processes during C.A. treatments.

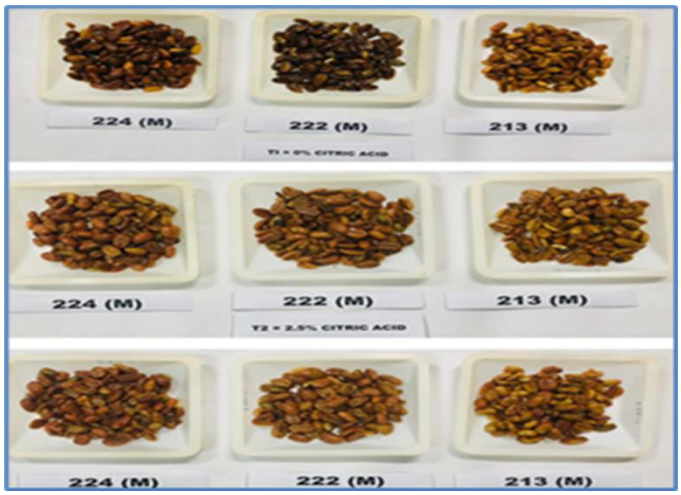

Figure 2: Appearance of 3 Liberica GCBs parchments after treatment with C.A. solution.

Liberica coffee clone 224 treated with 5.0% C.A. gave the highest GCBs yield of 37.9% with a significant difference ($P < 0.05$) as compared to coffee clone 213 (without treatment). There was no significant difference ($P > 0.05$) for all other coffee clones with other treatments (Figure 3).

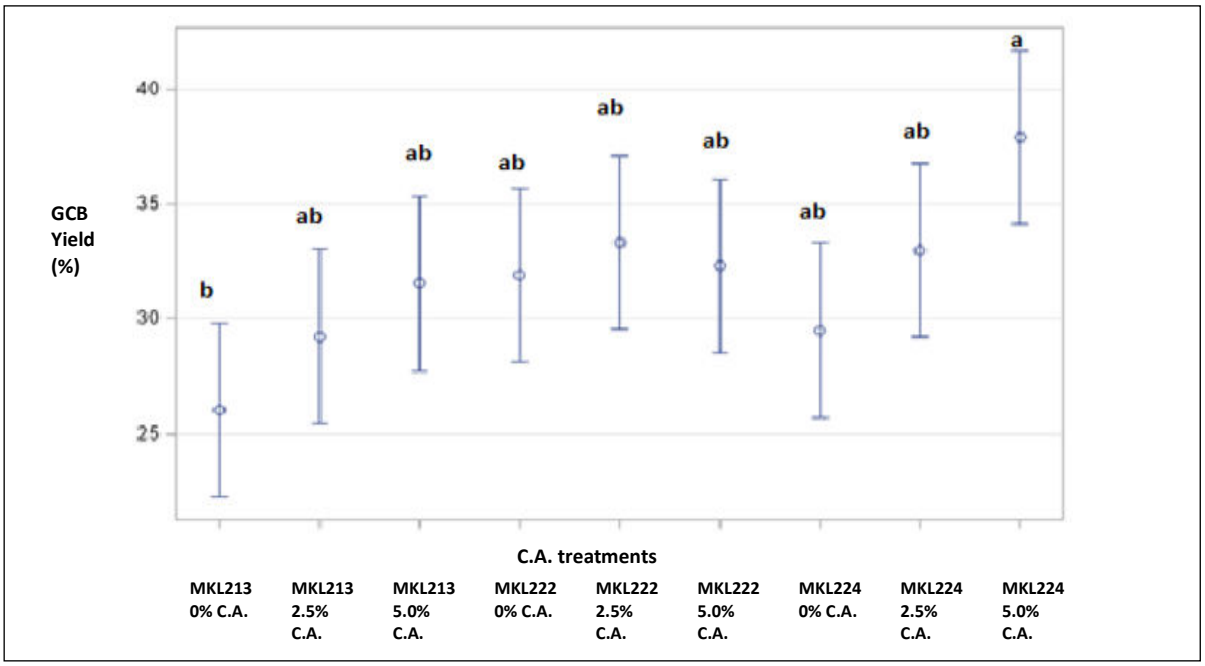

Figure 3: Changes in yield of GCBs after C.A. treatment; means with the same letter are not significantly different at $P < 0.05$.

Changes in morphology of GCBs

The morphology of GCBs treated with C.A. changed to crystalized appearance (Figure 4). The image was captured with the aid of a stereo microscope. The acidic condition made the silver skins of GCBs softer and easily released from GCBs.

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Figure 4: Changes in morphology of GCBs after C.A. treatment.

Phytochemical properties of GCBs

In Table 1, all the Liberica coffee berries soaked in water (control) were found having higher antioxidant scavenging activities (IC_{50} mg/mL) as compared to the C.A. treatments, and the findings were relevant to the highest ferric reduction activity and phenolic content in green Liberica coffee beans, especially with coffee clone 222. The CGA in coffee clone 222 was the highest in control (water soaking), and also in both 2.5 and 5.0% C.A. treatments. The phenolic contents in GCBs increased with 5% C.A. treatment because these compounds were preserved by acidic condition. The outer part of GCBs changed to red colour after treatment, and the beans' aroma became that similar to matured tamarind fruits. According to Farah et al. (2005), CGA is the main phenolic compound in coffee beans and its concentration is the highest in coffee beans than other coffee parts. Antioxidant activity of coffee beans depends on the characteristics of phenolic compounds, especially CGAs which possess *in vitro* and *in vivo* antioxidant capacity (Shahidi and Chandrasekara, 2010).

Table 1: Comparison of phytochemical analyses on GCBs treated with C.A.

Liberica coffee clone	C.A. treatment (%)	DPPH radical scavenging assay (IC_{50} mg/mL)*	Ferric reducing antioxidant power - FRAP (mM)	Total phenolic content (mg CGAE/g)	Total flavonoid content (mg RE/g)
MKL213	0.0	0.803^{a}	1.535^{c}	30.53^{gh}	14.894^{b}
MKL222	0.0	0.708^{a}	2.701^{a}	112.908^{a}	17.617^{a}
MKL224	0.0	0.782^{a}	1.482^{c}	73.701^{f}	12.117^{c}
MKL213	2.5	0.828^{a}	0.612^{d}	32.566^{g}	16.469^{ab}
MKL222	2.5	1.278^{bc}	0.698^{d}	93.733^{e}	15.709^{ab}
MKL224	2.5	0.921^{ab}	2.418^{b}	102.732^{c}	15.771^{ab}
MKL213	5.0	1.137^{bc}	0.196^{e}	28.709^{h}	15.367^{b}
MKL222	5.0	1.686^{c}	1.416^{c}	109.909^{b}	16.275^{ab}
MKL224	5.0	1.222^{bc}	1.467^{c}	97.375^{d}	15.491^{b}

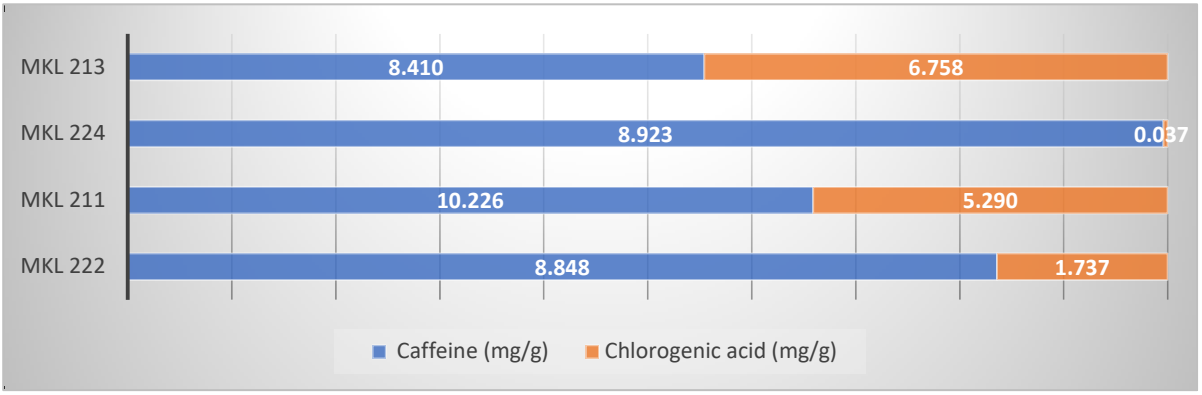
Means with the same letter are not significantly different at $P < 0.05$.
**A lower value of IC_{50} mg/mL for DDPH radical scavenging assay indicates the highest scavenging activities against the free radicals in the sample extract.*

Changes in caffeine and CGA contents after soaking treatments

Other phytochemicals obtained as caffeine and CGA contents were also discussed in this study. The CGA of these coffee beans is still in the investigation and the further mechanism in phytochemical changes will be observed in the next roasting process especially in these treated and untreated GCBs. Figure 5 presents a low amount and varied CA contents in the fresh-dried GCBs. The GCBs of MKL 222, MKL 211, MKL 224 and MKL 213 contained 1.737, 5.290, 0.037 and 6.758 mg/g CGA, respectively. As mentioned in literature, the unroasted Malaysian Liberica and Arabica coffee beans contained CGA of 1.6 and 2.2 mg/g, respectively (Mubarak et al., 2019).

Liberica coffee is one of the identified coffee species, despite being less popular in market compared to *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* P. (Robusta coffee) (Adepoju et al., 2017). The caffein contents in the Liberica coffee were reported low among the other GCB varieties in the world. In this study, GCB Liberica samples contained between 8.410-10.226 mg/g caffeine (Figure 5), which was close to 12-16 mg/g as reported by Mubarak et al. (2019) and was lower compared to those in the Robusta and Arabica coffee beans (Weinberg and Bealer, 2001; Magdalena et al., 2016). Past research mentioned that the caffeine contents in the Robusta and Arabica coffee extracts were 8.16% (81 mg/g) and 3.41% (34 mg/g) per dry weight basis, respectively (Magdalena et al., 2016). Similarly, Weinberg and Bealer (2001) also reported a double amount of caffein in the Robusta coffee amounting to 2.4% (24 mg/g) as compared with the Arabica coffee containing 1.3% (13 mg/g) in dry weight basis. In fact, the Arabica and Robusta coffee beans have higher caffeine content as compared to the Malaysian Liberica coffee beans.

As reported by Stellar Market Research (2022), Liberica coffee is mainly produced in Indonesia, Malaysia, and the Philippines; these coffee beans make up about 2% of the world's coffee. Surprisingly, more than 95% of the Malaysian coffee crop is Liberica. However, it is not commonly found in the North American and European markets. Thus, even though the caffeine of Liberica coffee beans is sightly lower but the taste and aroma of the roasted coffee drinks are acceptable for Malaysian people.



Liberica coffee MARDI Kluang Johor with an additional new coffee clone sample - MKL 211.

Figure 5: Initial caffeine and CGA contents (mg/g) in the Liberica GCB.

A comparison between fresh GCBs and those after treatment with C.A is illustrated in Table 2 and Figure 5. Caffeine contents of GCBs changed slightly, being between 8.30-10.4 mg/g caffeine, after treatment with C.A.. Treated GCBs (Table 2) also had increased GCA contents as compared to the initial GCBs without treatments (Figure 5). The contents of CGA were lower than caffeine contents for all Liberica coffee clones. GCBs (clone 224) increased their caffeine content from 8.9 mg/g (Figure 5) to 9.7 mg/g caffeine (Table 2) after treatment with 5% C.A.. The MKL 222 increased its caffeine content from 8.8 mg/g to 10.16 mg/g caffeine after treatment with 2.5% C.A., and MKL 213's caffeine content only increased slightly from 8.4 mg/g to 9.4 mg/g caffeine after the acid treatment. The acidic condition in these treatments had increased the CGA content and changed slightly the caffeine content in the GCBs.

Table 2: Changes in caffeine and CGA contents in GCBs after soaking treatments.

Liberica coffee clones*	C.A. treatment (%)	Caffeine (mg/g)	CGA (mg/g)
MKL213	0.0	8.740±0.170	47.935±5.169
MKL213	2.5	7.000±0.071	46.885±12.735
MKL213	5.0	9.365±0.035	51.015±9.313
MKL222	0.0	8.700±0.085	47.910±1.909
MKL222	2.5	10.165±0.007	43.595±3.062
MKL222	5.0	8.305±0.049	39.030±10.267
MKL224	0.0	10.470±0.071	32.125±3.359
MKL224	2.5	8.760±0.042	21.515±1.252
MKL224	5.0	9.720±0.057	52.490±5.869

**Batch 1- Liberica coffee berries; means with the same letter are not significantly different at P < 0.05.*

Conclusion

Soaking GCBs with C.A. at 2.5-5.0% had a significant effect in changing the caffeine and CGA contents. The beans also changed in colour, appearance and produced a broken silver skin, which may reduce the waste during roasting process. The high CGA obtained in the treated GCBs can be utilized in the GCBs supplement and cosmetic industries in order to obtain a high CGA in their raw materials as high phyto-nutrients of CGAs.

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Growth and Yield Responses of Grain Corn as Affected by Application of Biofertilizer

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Introduction

Grain corn has been given higher priority in Malaysia as one of the new sources of national wealth. It is due to its important component in the formulation of animal feed, where Malaysia has relied heavily on corn imports for more than 50 years (Nor Amna et al., 2020). The grain corn industry in Malaysia is relatively small. However, the development of the livestock industry, especially the ruminants, broilers and swine, needs millions of tons of grain corn as the main component for feeds. One of the factors that determine the viability of the livestock industry is the source of livestock feed involves the cost of 60-70% of the total cost of livestock production. Budget need for livestock feed for the livestock industry in this country is as much as 7 million tons per year. The main ingredient of animal feed consists of 50% to 55% grain corn and 25-35% soy bean. In 2020, as much as 3.8 million tons equivalent to RM 3.3 billion of grain corn has been imported to meet the needs of the domestic market (UNComtrade, 2022). The National Grain Corn Development Blue Print Target, is to reduce grain corn imports by 30% by 2032. Therefore, grain corn production should reach 1.44 million tons which requires a total planting area of 80,000 hectares (ha).

The productivity of grain corn is dependent on its nutrient requirement and management particularly on the availability of macro nutrients: nitrogen, phosphorus and potassium (Arunkumar, 2007). In Malaysia, local farmers rely heavily on the usage of chemical fertilizer (90%) throughout the farming systems. Increased cost of inorganic fertilizers including their pollution effects on the environment has directed attention to other sources of fertilizer to enhance grain corn production. Therefore, intensive farming practices that aim at producing higher yield require extensive use of nitrogen fertilization which are costly and create environmental pollutions (Baser et al., 2012).

Biofertilizers which are eco-friendly play an important role for supplementing the essential plant nutrients for sustainable agriculture and economy (Mugilan et al., 2011). Moreover, microbial fertilizers can clean the environment, enhance the productive capacity of land, reducing the amount of chemical fertilizer consumption (Hossein and Farshad, 2013) and improve plant growth and health (Raaijmakers et al., 2002). Biofertilizers are preparations of containing live or latent cells of efficient nitrogen fixing, phosphate solubilizing algae, bacteria or fungi. Enhancement of cereals yields by inoculation with nonsymbiotic nitrogen fixing bacteria was recorded by many researchers (Naureen et al., 2005; Khaliq et al., 2006; Salantur et al., 2006). The application of biofertilizer, can be either to the seed or soil, could speed up the microbial processes in the soil thereby augmenting the availability of nutrients which can be assimilated in grain corn production (Wu et al., 2005).

Proper fertilization is required to achieve optimum grain corn production. The profit potential for farmers depends on producing enough grain corn per hectare to keep the production costs below the selling price. One of the important keys to profitable corn yield is meeting the nutrient requirement by applying the suitable types and amounts of fertilizers. Therefore, the objective of this study was to evaluate the effects of Biofertilizer (N-bio booster) application towards growth and yield responses of grain corn.

Materials and Methods

Study area and planting materials

The experiment was carried out at research field at MARDI Headquarters, Serdang using a total area of 80 m × 20 m. The soil type of experimental site was the alluvial soil. The variety of grain corn used was hybrid Dupont P4546 planted at the rate of 17 kg ha^{-1}.

Land preparation and agronomic practices

The experimental plots were disc ploughed, harrowed, leveled and the ridges were built and the experimental plots were marked using rope, pegs and tape. Each experimental plot had a dimension of 5 m x 5 m and two seeds were sown per hole at a depth of 3-5 cm with planting distance of 75 x 20 cm. Organic fertilizer was applied at 7 days before planting at the rate of 3 t ha^{-1} distributed on the ridged-plot and incorporated into the soil. Plants were irrigated immediately after sowing and thinned to one plant/hole a week after sowing. Weeding was carried out manually when needed.

Treatments and experimental design

There were seven treatments which comprised of combination of chemical and N-biobooster fertilizers in the present study as stated in the Table 1, and arranged in a randomized complete block design (RCBD) with three replications. Each plot had an area of 25 square meters consisting of six (6) between rows, 25 plants in one row and total sample 150 plants. A one (1) meter distance between blocks and plots was provided.

Table 1: Treatment combination of chemical and N-biobooster fertilizer application to increase the growth and yield of grain corn production.

Treatment		0 DAS	N-Biobooster (10 DAS)	15 DAS	30 DAS	N-Biobooster (35 DAS)
100% rate NPK + Urea (Control)	T1			NPK 100%	Urea	
NPK 75% rate + Urea	T2			NPK 75%	Urea	
NPK 50% rate + Urea	T3			NPK 50%	Urea	
NPK 100% rate + Urea + 2 (N bio booster)	T4		1 L/ha	NPK 100%	Urea	1 L/ha
NPK 75% rate + Urea + N biobooster	T5		1 L/ha	NPK 75%	Urea	1 L/ha
NPK 50% rate + Urea + N biobooster	T6		1 L/ha	NPK 50%	Urea	1 L/ha
100% rate NPK + Urea	T7	NPK 100%			Urea	

Data collection

Five plants were randomly selected from each treatment. Parameters were taken during experimental period on growth performance at 90 DAS which were plant height (cm), stem diameter (mm), number of leaves, and leaf area (cm^2). After harvesting at 115 DAS, measured variables of moisture content (%), total cob/plot, cob length (cm), cob diameter (mm), cob weight cob (g), number of grain/cob, 1000-grain weight (g) and grain yield (before and after drying) were obtained.

Statistical analysis

Data obtained were analyzed using ANOVA in the SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) and differences among treatments means were compared using Duncan Multiple Range Test (DMRT) at $P \leq 0.05\%$.

Results and Discussion

Effect of chemical fertilizer and N-Biobooster on growth performance and dry matter of grain corn at 90 DAS

The vegetative responses in stem diameter (cm), leaf length (cm), leaf area (cm2), leaf dry weight (g) and root dry weight (g) showed significant differences ($p < 0.05$) among all treatments applied at 90 days after sowing (DAS). Meanwhile, plant height (cm), leaf number and stem dry weight (g) showed no significant difference ($p > 0.05$) among all treatments. The effects of chemical fertilizer and N -Biobooster on stem diameter showed the T4 also did not show any significant difference with T1, T2, T5, and T7. As well as T3 showed no significant difference with T1, T2, T5, T6 and T7. The highest radial growth recorded at T4 (24.82 mm) while the least growth was recorded for T3 (20.23 mm). Other than that, leaf length also showed no significant difference with other treatments (T1, T2, T5, T6 and T7. The longest of leaf length recorded with T4 (96.30 cm) while the shortest length was for T3 (89.40 cm). Mary et al. (2013) had reported that the application of biofertlizers (*Azotobacter, Azospirillum* and PSM) alone or in combination increased the growth parameters of maize seedlings in terms of plant height, stem base diameter as well as fresh and dry weight of plants. This agrees with Zahir et al. (2004) that biofertilizer (*Azotobacter* and *Azospirillum*) are the most important plant growth promoting rhizobacteria which affects the growth and development of crops. Dry matter of grain corn at 90 DAS also showed there was no significant difference with T2, T3, T5, T6 and T7 on leaf dry weight as well as T4 in root dry weight also no significant difference with T1, T2, T3and T7. The highest dry weight for leaves and roots in the T4 treatment combination of biofertilizer with the resulting leaf dry weight (499.95 g) and the root dry weight (65.76 g). The application of biofertilizer N-Biobooster between treatment T4, T5 and T6 showed no significant different in all parameters.

Effect chemical fertilizer and N-Biobooster on yield and yield of grain corn

The effects of chemical fertilizer and N -Biobooster on grain corn yield showed the T4 did not show significant difference with T5, T6 and T7 as well as T1 no significant difference with T2, T3, T5, T6 and T7. The highest grain yield was obtained from 100% NPK + Urea + 2 (N -Biobooster) (T4) with 8530.0 kg ha^{-1} which was significantly different from the lowest yield value which was obtained from 100 % NPK + Urea (Control) (T1) with 6356.0 kg ha-1 (Table 3). The same results were indicated by Mahato and Neupane (2017), which showed that the combination of chemical fertilizers and biofertilizer provides higher yields of corn than the use of chemical fertilizers, thus reducing the amount of chemical fertilizers used during cultivation. According to Zarabi et al. (2011), adding organic fertilizer will increase water use efficiency, optimize photosynthesis and stimulate plant growth and development.

Table 2: Effect of chemical fertilizer and N-Biobooster on growth performance and dry matter of grain corn at 90 DAS.

Treatment	Plant height (cm)	Stem diameter (mm)	Leaf number	Leaf length (cm)	Leaf area (cm^2)	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)
T1	197.80^{a}	22.65^{ab}	20.35^{a}	95.93^{a}	5109.10^{ab}	463.05^{b}	60.28^{a}	48.92^{ab}
T2	197.59^{a}	23.52^{ab}	13.05^{a}	93.75^{ab}	6545.90^{a}	493.55^{a}	76.46^{a}	51.45^{ab}
T3	184.80^{a}	20.23^{b}	12.50^{a}	89.40^{b}	4075.60^{b}	495.1^{a}	78.73^{a}	45.73^{ab}
T4	193.40^{a}	24.82^{a}	13.25^{a}	96.30^{a}	5842.80^{ab}	499.95^{a}	66.20^{a}	65.76^{a}
T5	196.61^{a}	22.28^{ab}	13.00^{a}	92.35^{ab}	4817.50^{ab}	483.95^{a}	59.01^{a}	38.63^{b}
T6	203.50^{a}	$21.38a^{b}$	12.60^{a}	93.01^{ab}	4427.30^{b}	473.18^{ab}	109.44^{a}	38.78^{b}
T7	198.39^{a}	22.58^{ab}	12.50^{a}	95.10^{a}	5188.60^{ab}	499.65^{a}	63.95^{a}	55.92^{ab}
p-value	ns	*	ns	*	ns	*	ns	*

**Means in each column with the same letters are not significantly different at $p \leq 0.05\%$ level according to Duncan's Multiple New Range Test (DMNRT) (Mean ± SE, n=3). ns= not significant.*

Table 3: Effect chemical fertilizer and N-Biobooster on growth and yield of grain corn.

Treatment	Moisture content (%)	Total cob/plot	Cob length (cm)	Cob diameter (mm)	Weight cob (g)	No. of grain/cob	1000-grain weight (g)	Grain yield before drying (kg ha^{-1})	Grain yield after drying (kg/ha^{-1})
T1	26.93^{a}	122.00^{a}	16.36^{a}	161.55^{a}	34.19^{c}	463.05^{a}	302.64^{a}	7539^{b}	6256^{b}
T2	28.17^{a}	115.75^{a}	16.13^{a}	167.18^{a}	37.11^{abc}	493.55^{a}	338.85^{a}	8336^{b}	6964^{b}
T3	28.17^{a}	120.25^{a}	16.21^{a}	189.17^{a}	35.66^{abc}	495.1^{a}	360.26^{a}	7487^{b}	6261^{b}
T4	27.56^{a}	132.25^{a}	16.21^{a}	182.02^{a}	40.73^{ab}	499.95^{a}	368.38^{a}	10129^{a}	8530^{a}
T5	23.15^{a}	130.75^{a}	21.87^{a}	163.54^{a}	42.61^{a}	483.95^{a}	367.86^{a}	8934^{ab}	7485^{ab}
T6	28.13^{a}	124.5^{a}	17.06^{a}	141.83^{b}	36.85^{a}	473.18^{a}	384.89^{a}	8909^{ab}	7423^{ab}
T7	28.65^{a}	136.25^{a}	16.35^{a}	184.13^{a}	40.19^{abc}	499.65^{a}	363.28^{a}	8853^{ab}	7342^{ab}
p-value	ns	ns	ns	*	*	ns	ns	*	*

**Means in each column with the same letters are not significantly different at P ≤ 0.05% level according to Duncan's Multiple Range Test (DMRT) (Mean ± SE, n=3). ns= not significant.*

Conclusions

The use of renewable resources is one of the basic principles of sustainable agriculture allowing for maximum crop productivity and minimal environmental risk. Biofertilizers play an important role plays a role in increasing soil fertility by increasing the nutrients required for plant growth. Other than that the use of bioafertilizers can be considered as an economic way to minimize the use of inorganic fertilizers, in addition, the cost price, the technology can be engineered into organic farming for better sustainable agriculture solutions for environmentally friendly progress.

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Effects of Different Growth Media in Hydroponic and Aquaponic Cultivation Methods on Biomass and Phytochemical Contents of Radish Microgreens (*Raphanus sativus* L.)

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Introduction

High-quality and nutritional foods are necessary to sustain human growth, development, and survival. However, not everyone has access to high-quality and healthy food products. This crucial situation is called food insecurity. Food insecurity was considered a major global issue embedded in the Sustainable Development Goals (SDGs) of the United Nations. Consequently, there is an increasing demand for high-quality and healthy food products, especially fruits and vegetables. Microgreens are an emerging class of produce consisting of vegetables, herbs or grains seedlings that have gained increasing popularity for consumers concerned about their health. These fast-growing fresh produce have been recognized as health-promoting foods for their high content of phytonutrients compared to mature plants. However, several studies have revealed that microgreens' nutritional quality, growth performance and phytochemical compositions are affected by various environmental factors, including growth medium, temperature, and light quality (Kyriacou et al., 2020; Turner et al., 2020). Gioia et al. (2017) demonstrated that the physicochemical properties of growth medium could influence the microgreens' yields and nitrate content. The addition of nutrient solution has also affected the microgreen yield, especially in *Brassica* sp. (Palmitessa et al., 2020).

Radish (*Raphanus sativus* L.) is a major root crop belonging to the Brassicaceae family and is among the most common plants grown as microgreens. Radish microgreens have a spicy and peppery flavour that can add a unique flavour to salads, sandwiches, and other dishes. They are easy to grow at home and can be ready to harvest in just a few weeks, making them a popular choice for home gardeners and health enthusiasts. Radish microgreens have been regarded as a novel functional food exhibiting significant antioxidant, anti-diabetic and anti-obesity properties (Wojdylo et al., 2020). In addition, the consumption of radish microgreens has a potential to lower the risk of cancer as it has significant amounts of cancer-fingting glucosinolates (Xiao et al., 2016).

The development of aquaponic system through the integration of aquaculture elements and hydroponic system has gained popularity in sustainable agriculture. Several studies have reported the potential of aquaponic system to sustain water quality and increase the productivity of fresh produce like vegetables and herbs (Yamane et al., 2021). Various vegetables have been grown using aquaponic system, but limited studies have been conducted to evaluate microgreens production (Nicoletto et al., 2018). Therefore, this project was undertaken to establish a small-scale aquaponic system and investigate different cultivation systems' influence on biomass and phytochemicals accumulation in microgreens. The radish microgreens were used in this project due to their high germination rate, high nutrient contents, and bioactive compounds with anti-inflammatory and anticancer properties (Palmitessa et al., 2020).

Materials and Methods

Plant materials and growth conditions

The radish microgreens (*Raphanus sativus* L.) were used in this study. The seeds were purchased from a local seeds distributer, CityFarm Malaysia. 3.0 g of radish seeds were used for each replication of the experiments. The seeds were soaked in filtered water for one hour and sowed evenly on a wet growing

medium in the growth container. Two different growth mediums were used in this study; peat moss and layers of rock wool. The seeds were sprayed with filtered water before incubation at room temperature under dark conditions for three days. The seeds were sprayed twice a day to maintain the moisture of the medium during the incubation period. After three days, the germinated seeds were maintained under white LED illumination for ten days. The microgreens seedlings were sprayed with filtered water twice a day to maintain the humidity in the indoor environment.

Cultivation methods and establishment of aquaponic system

The microgreens were cultivated in two different growing medium, peat moss or layers of rock wool, using three different methods: conventional, hydroponic, and aquaponic. In the conventional method, the radish seeds were sowed on a peat moss medium inside a growth container, as shown in Figure 1(a). Peat moss was considered the main substrates used for cultivation of microgreens as reported by Gioia et al. (2017) and this growing method was used as control. The rock wool and peat moss were used as the growing medium in both hydroponic and aquaponic methods. The hydroponic system used filtered water, as shown in Figure 1(b). The hydroponic growth container for microgreens manufactured by Baba, a local gardening company, was used in the hydroponic method (Figure 1(c)).

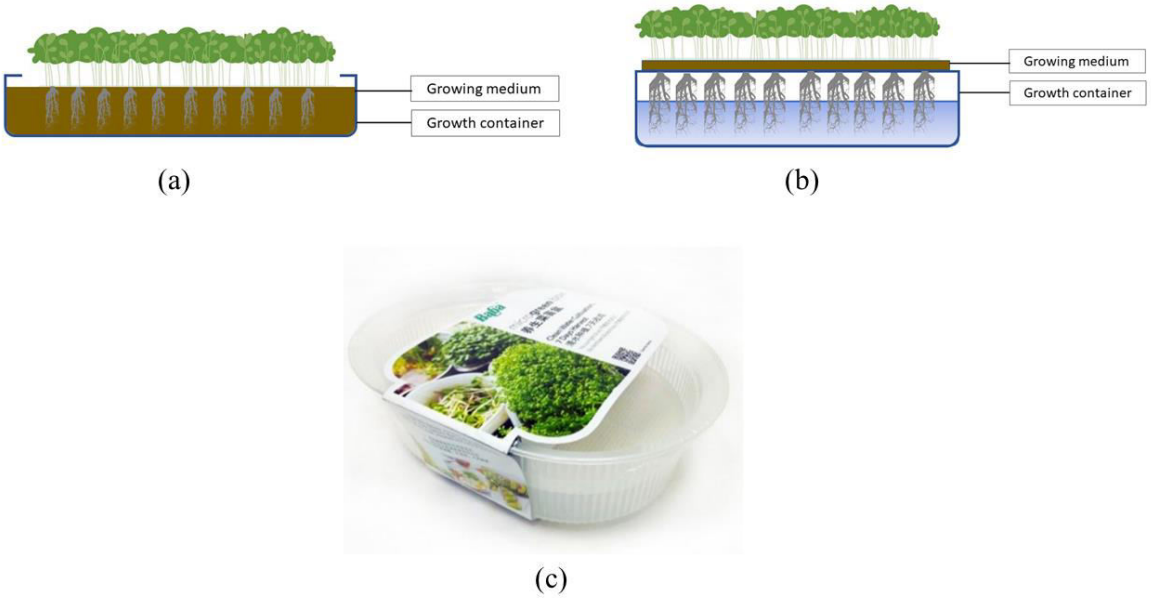

Figure 1: Different cultivation methods and growth container. (a) Schematic diagram of conventional cultivation method. (b) Schematic diagram of hydroponic cultivation method. (c) Hydroponic growth container for microgreens.

In the aquaponic method, the hydroponic container was modified by making some holes at the bottom to establish the aquaponic system and allow water from the aquarium to enter the container (Figure 2(a)). The modified container containing the medium and seeds was placed on top of the aquarium tank (15 litres). The three-month-old goldfish (*Carassius auratus*) were used in the aquaponic system. Six fish were maintained in the aquarium, which was also provided with a water pump and water filter machine, as shown in Figure 2(a). The goldfish were fed twice a day. A few drops of API STRESS ZYME bacterial cleaner were added to the aquarium water to provide the beneficial microbes to establish an effective aquaponic system.

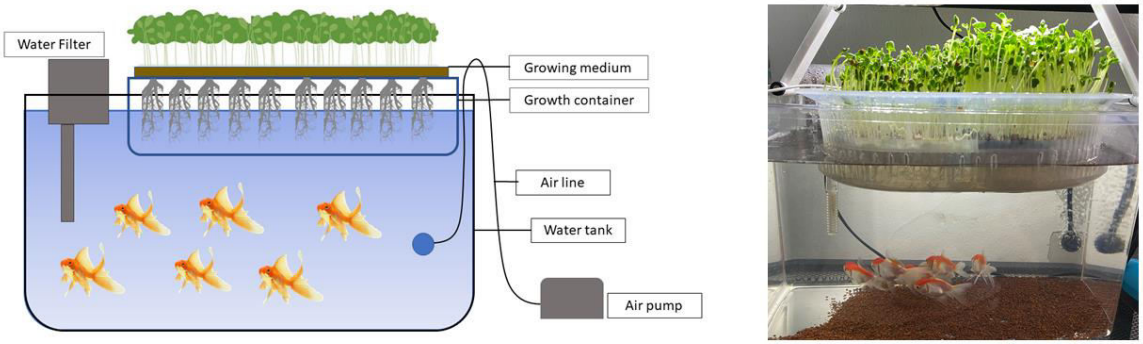

Figure 2: Establishment of small scale aquaponic system. (a) Schematic diagram of an aquaponic system, (b) Established aquaponic system with modified growth container.

Determination of biomass

The microgreens were harvested after 10 days of sowing by cutting the hypocotyl part, as shown in Figure 3. The fresh weight (FW) and length of the dicotyledon leaf were determined using an electronic analytical balance and a ruler, respectively. The harvested radish microgreens were dried at 50°C for 48 hours to obtain the dry weight (DW). The FW and DW were expressed as the weight for ten microgreens seedlings.

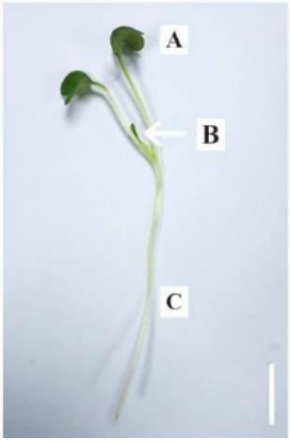

Figure 3: The harvested radish microgreens after 10 days of sowing. (a) Cotyledon leaf. (b) True leaf. (c) Hypocotyl. (bar = 2.0 cm)

Extraction of plant materials

The methanol extraction and maceration method were used in this study. The dried microgreens were ground into powder form with a mortar and pestle, followed by soaking in 80% methanol (w/w) inside an Erlenmeyer flask. The flasks containing the extracts were incubated on an orbital shaker at 110 rpm at 25 °C for two days. The extracts were filtered using filter paper Grade 1 to remove the residues. The filtered extracts were stored at –20 °C for further analysis.

Determination of total phenolic and flavonoid contents

The total phenolic content (TPC) was determined using Folin–Ciocalteu assay as described by Brazaityte et al. (2019) and expressed as mg of gallic acid equivalents/g dry weight (mg GAE/100 g

DW) of extract. The aluminium chloride colorimetric assay was used to quantify the total flavonoid content (TFC). Quercetin (Sigma-Aldrich) was used as a reference substance, and the results were expressed as quercetin equivalents in mg per gram of dry weight (mg/100 g DW) of extract.

Statistical analysis

All data were subjected to analyses of variance (ANOVA) using Duncan's multiple range test (DMRT) with SPSS Version 21 and presented as means ± standard error (SE) of three replications. The significance of the differences among the treatments was compared using DMRT at $P \leq 0.05$.

Results and Discussion

Growth and biomass of microgreens

In this project, the aquaponic system was developed using a modified growth container to provide better water aeration to support microgreens' growth and protect the root system. The growth container is crucial to ensure the aquaponic system can work effectively and efficiently in this project. The results show a higher yield of microgreens biomass produced using the aquaponic method with peat moss medium (Table 1). The microgreens grown using layers of rock wool in the hydroponic method had the lowest percentage of germination and biomass. The presence of humic acids in peat moss potentially affects the germinated seedlings' primary and secondary metabolism. Bulgari et al. (2021) suggested that humic substances in peat moss enhance root growth and nutrient uptake in microgreens. The low porosity of layers of rock wool and low water-holding capacity has affected the development of roots and the growth performance of the microgreens (Gioia et al., 2016). In particular, the present results indicated that the growing substrate choice significantly influenced the microgreens' yield. The present results also demonstrated that high nitrate content in the aquarium tank is suitable for cultivating the studied species. Adding beneficial microbes and dissolved organic matter from fish faeces could play essential roles in promoting the growth of microgreens (Nicoletto et al., 2018).

Table 1: Effects of different cultivation methods on the germination percentage and yield of radish microgreens.

Cultivation method	Percentage of germination (%)	Fresh Weight (g)	Dry weight (g)
Conventional method (control)	95	2.08 ± 0.10^{b}	0.11 ± 0.00^{b}
Hydroponic method + rock wool	75	1.42 ± 0.12^{c}	0.09 ± 0.00^{c}
Hydroponic method + peat moss	95	2.16 ± 0.15^{b}	0.11 ± 0.01^{b}
Aquaponic method + rock wool	80	2.20 ± 0.27^{b}	0.10 ± 0.01^{bc}
Aquaponic method + peat moss	95	2.95 ± 0.10^{a}	0.13 ± 0.01^{a}

Data are the mean of three replications. Means followed by different lowercase letters within each column are significantly different ($P < 0.05$) by Duncan's multiple range test.

Total phenolic and flavonoid contents

Different cultivation methods significantly affect the accumulation of phytochemicals in radish microgreens. High total phenolic and flavonoid content were recorded in microgreens cultivated using the aquaponic method and peat moss. However, less significant differences of TPC were observed in microgreens cultivated using rock wool and peat moss in the aquaponic system. The radish microgreens grown using the hydroponic method with rock wool had the lowest total phenolic and flavonoid contents compared to the conventional growing method. Overall, the present results demonstrated that using a peat moss medium increased the accumulation of phenolic and flavonoid contents in microgreens. According to Kyriacou et al. (2020), peat moss medium provided optimal physicochemical conditions to support the growth and increased various microgreens' total minerals and nitrate content. The higher bulk density, total pore space, and air-holding capacities contribute to the growth performance of microgreens (Kyriacou et al., 2020; El-Nakhel et al., 2021).

Table 2: Effects of different cultivation methods with peat moss and rock wool on total phenolic and flavonoid content of radish microgreens.

Cultivation method	Total Phenolic Content (mg GAE/ 100g DW)	Total Flavonoid Content (mg Quer/ 100g DW)
Conventional method (control)	211 ± 1.53^{c}	4.37 ± 0.05^{bc}
Hydroponic method + rock wool	211 ± 4.84^{c}	3.72 ± 0.13^{d}
Hydroponic method + peat moss	231 ± 7.00^{b}	4.11 ± 0.07^{c}
Aquaponic method + rock wool	269 ± 3.79^{a}	4.41 ± 0.11^{b}
Aquaponic method + peat moss	279 ± 6.49^{a}	5.48 ± 0.07^{a}

Data are the mean of three replications. Means followed by different lowercase letters within each column are significantly different ($P < 0.05$) by Duncan's multiple range test.

Conclusion

The preliminary results of this study suggested aquaponic method improves microgreens' biomass and enhances their phytochemical contents. Further studies to determine the nutrient contents in the aquaponic water, as well as the growth performance of the fish, are necessary to evaluate the effectiveness of the aquaponic system. Moreover, it is essential to conduct a study on several types of microgreens. This project is the first to report the alternative growing method for sustainable production of high-quality microgreens in terms of growth rate, biomass as well as total phenolic and flavonoid contents using a small aquaponic system. The small-scale aquaponic system could be upscaled to be used in the fish farm or any freshwater area for sustainable microgreens production. It can reduce land consumption and promote food self-sufficiency in many countries.

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Chapter 6

Seed Technology and Quality Planting Materials

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Yield Performance of Various Oil Palm Planting Materials from Seed-producers in Malaysia

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Introduction

Oil palm (*Elais guineensis*) is an important commercial agricultural crop, supplying over 40% of world traded vegetable oil. This perennial crop also had a long life span of at least 25 years, played a key dietary component as a palm oil and consumed by over three billion people worldwide. Mainly plantation grown in Asia, has various non-food uses in industrial applications and downstream products (Denis et al., 2021). In 2019, world oil palm production reached about 74.58 million tonnes (mt), with harvested amount of 415.90 mt of oil palm fruits and almost 84% were concentrated oil in Asia (FAOSTAT, 2022). The major oil palm producers in the world are Indonesia and Malaysia at 42.87 and 19.86 mt respectively (FAOSTAT, 2022). The world production trend of oil palm fruits supply is expected to increase in 2021 due to high demands, with production up to 418.44 mt (FAOSTAT, 2022).

As the demand for palm oil increases, the need for high-quality planting materials also increases, either by expanding a cultivated area or through replanting programs. In general, there are three types of raised oil palm seedlings used in the market: (1) advanced DxP seeds, (2) semi-clonal seeds and (3) *E. oleifera* x *E. guineensis* (OxG) hybrids seeds. At present there are nine major oil palm seedling producers in the world: (1) ASD Costa Rica, (2) Palmeite-CIRAD®, (3) Sime Darby Plantation, (4) AAR, Malaysia, (5) FGV-Felda, (6) Sawit Kinabalu Seeds, (7) IOI, Malaysia, (8) Asian Agri, Indonesia, and (9) PT Dami Mas Sejahtera, Indonesia (Rethinam and Murugesan, 2018). Other than the advanced DxP seedlings, the oil palm industry also exploited the high-performance oil palm clones. According to DKAN (2021), oil palm plantations in Malaysia require 10% of clonal planting materials for its yearly replanting program and there are broad varieties of commercially available planting materials. This study was conducted to observe the yield performance of various DxP and other clone planting materials from five different seed producers in Malaysia.

Materials and Methods

A trial, T265 was set up at Stage 1 Replanting area and situated at FGV Agri Services Sdn Bhd (FGVAS) Plantation, Sahabat 17, Lahad Datu, Sabah. The location of the latitude is N 5° 8’ 32.381”E 119° 0’ 0”. The total trial area consists of 2.82 ha and measurements were from 2016-2021. A total of 384 were plants were planted and the soil used was Sahabat l series. The experiment comprises of nine progenies with four replications each. The planting density was 136 palms/ha. The planting materials planted are as listed in Table 1. The experimental design used was Completely Randomized Design. The data was analysed using ANOVA and mean comparison was conducted on the data collected using the Statistical Analysis System (SAS). The mean was conducted using Duncan Multiple Range test at $p \leq 0.05$ significant level.

Table 1: Various commercial planting materials planted in Trial T265.

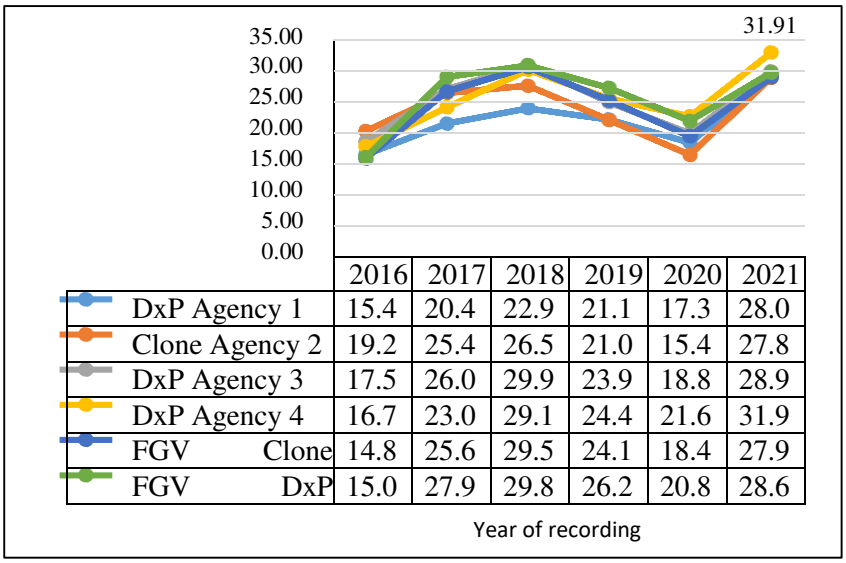
No	Progeny	Parent material	Replicates	Number of palms
1	DxP Agency 1	Unknown	4	64
2	Clone Agency 2	Unknown	4	64
3	DxP Agency 3	Unknown	4	64
4	DxP Agency 4	Unknown	4	64
5	FGV Clone FC4628	Deli-NPM x Yangambi	4	64
6	FGV DxP TK3873	Deli-NPM x Yangambi	4	64
		Total planted palms		384

Results and Discussion

Bunch yield component

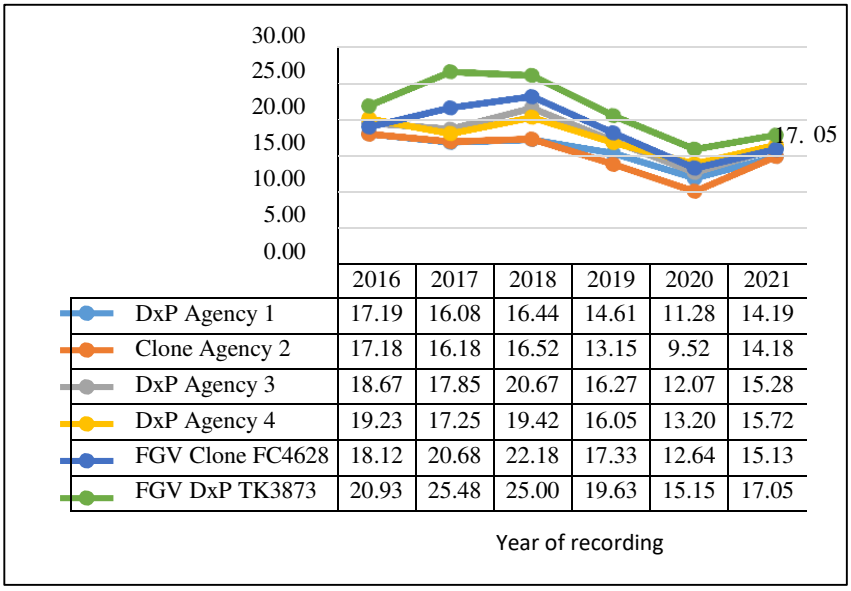
Results obtained from fresh fruit bunch (FFB) showed that the production at year one and two were highest using Clone Agency 2 and material FGV DxP TK3873) with 19.25 t/ha and 27.97 t/ha respectively (Figure 1). There was also an increment of 86% in the FGV material used as compared to the Agency clone by 31% within a year. At year three (2018), the highest FFB produced by DxP Agency 3 was 29.93 t/ha, followed by FGV DxP TK3873 with a subtle difference of 0.08 t/ha while at later phase (2019), the FFB for FGV DxP TK3873 clone seems to be consistent and remained highest with an increased to 26.23 t/ha as compared to others. Meanwhile, the DxP Agency 1 give the lowest FFB with less than 23 t/ha among others over a period of five years. In 2020, FFB yield for all planting materials showed slightly lower with production between 2.86-5.76 t/ha while the highest FFB was produced by DxP Agency 4 clone. This is possible due to implementation of Covid-19, Movement Control Order 1.0 (MCO). However, from 2016-2019, DxP Agency 1 was the least performer with production less than 23 t/ha.

The average number of bunches (BNO) and bunch weight (ABW) showed that FGV DxP TK3873 produced the highest BNO at 15.15-25.48 number/palm/year (Figure 2). The highest ABW was found to be from year one to four and this was achieved by Clone Agency 2 with results ranging from 8.29 to 11.83 kg/p/yr, while within a single year in 2020, this DxP Agency 4 showed production of 12.04-14.63 kg/p/yr. However, lowest ABW was obtained in FGV DxP TK387 at nine years after planting (Figure 3).



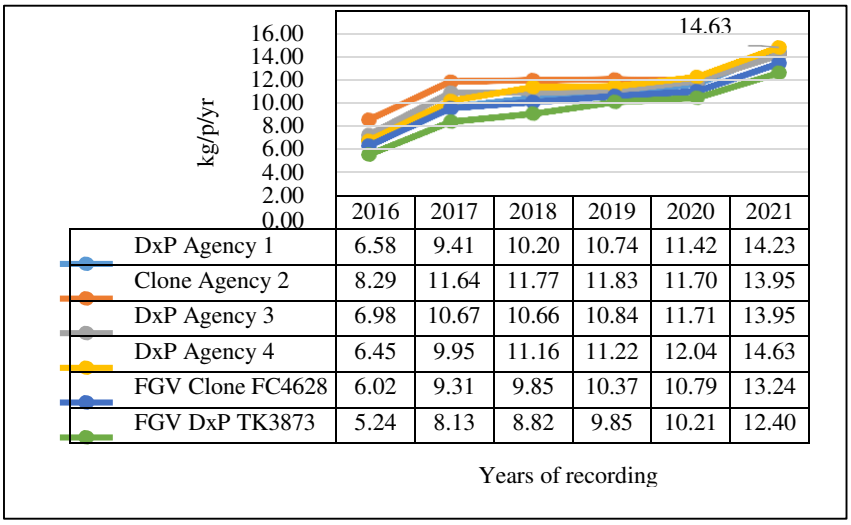
	2016	2017	2018	2019	2020	2021
DxP Agency 1	15.4	20.4	22.9	21.1	17.3	28.0
Clone Agency 2	19.2	25.4	26.5	21.0	15.4	27.8
DxP Agency 3	17.5	26.0	29.9	23.9	18.8	28.9
DxP Agency 4	16.7	23.0	29.1	24.4	21.6	31.9
FGV Clone	14.8	25.6	29.5	24.1	18.4	27.9
FGV DxP	15.0	27.9	29.8	26.2	20.8	28.6

Figure 1: Six years of FFB yield record at Trial T265.



	2016	2017	2018	2019	2020	2021
DxP Agency 1	17.19	16.08	16.44	14.61	11.28	14.19
Clone Agency 2	17.18	16.18	16.52	13.15	9.52	14.18
DxP Agency 3	18.67	17.85	20.67	16.27	12.07	15.28
DxP Agency 4	19.23	17.25	19.42	16.05	13.20	15.72
FGV Clone FC4628	18.12	20.68	22.18	17.33	12.64	15.13
FGV DxP TK3873	20.93	25.48	25.00	19.63	15.15	17.05

Figure 2: Six years of BNO record at Trial T265.



	2016	2017	2018	2019	2020	2021
DxP Agency 1	6.58	9.41	10.20	10.74	11.42	14.23
Clone Agency 2	8.29	11.64	11.77	11.83	11.70	13.95
DxP Agency 3	6.98	10.67	10.66	10.84	11.71	13.95
DxP Agency 4	6.45	9.95	11.16	11.22	12.04	14.63
FGV Clone FC4628	6.02	9.31	9.85	10.37	10.79	13.24
FGV DxP TK3873	5.24	8.13	8.82	9.85	10.21	12.40

Figure 3: Six years of ABW record at Trial T265.

According to Kushairi et al. (2013), the FFB yield profile can be estimated based on palm age and the yield profile, which is typically increased from aged 4 to aged 11 and categorized as high for the yield of 15.5 t/ha/yr, 22.0 t/ha/yr and 26.0 t/ha/yr and above for young palm aged 4, 5 and 6 years, respectively. As such, all planting materials (clone or DxP) at four years old are considered high yield except from FGV. However, thereafter the FGV Clone FC4628 and FGV DxP TK3873 exhibited high growth performance by producing high FFB yield while three other materials except for DxP Agency 1 fell under medium category (18.0 to 22.0 t/ha/yr) after the next two consecutive years. Here, only FGV DxP TK3873 reached the medium category for FFB yield at seven years old palm. There are three dry period phases that occurred in the Sabah south regions: early February-April 2019, August-September 2019 and February-April 2020. During these drought periods, the yields are affected in the following manners: inflorescences abortion incident over 9-11 months, bunch failure for 4-6 months, and other immediate effects such as bunch weight reduction for 1-2 months (Balamurugam and Roslan, 2020). As such, the results obtained during the course of the trials can significantly affects the FFB yields in the regions.

Relatively, FGV DxP TK3873 overall has the highest in bunch producer for the six consecutive years of recording as higher BNO usually has a direct effect on FFB. There are several factors that can determine the optimum number of ripe bunches for harvest: 1) the number of inflorescences initiated, depending on leaf production, 2) sex ratio, a ratio of the female inflorescence to total inflorescence number, 3) bunch failure, the abortion of a bunch before fully ripe that occurs 2-4 months after anthesis. This bunch failure is reported to be due to poor pollination or acute and severe shortage of water supply or radiation (Lotte et al., 2017).

Meanwhile, the highest ABW producer is Clone Agency 2 for four consecutive years except during 2020 and 2021 periods for DxP Agency 4. Possible factors that can determine the bunch weight include: the number of spikelets, fruit set, fruitlet weight and non-fruit components (Lotte et al., 2017). As such, it was also reported that fresh bunch weight normally increases with palm ages, starting from 3-5 kg at 24 months after planting to over 30 kg by 25 YAP (Rajanaidu et al., 1996) and this fruit set is mainly determined by pollination efficiency. However, the bunch weight and oil content are less responsive to environmental stress but has significant impact on yield (Lotte et al., 2017).

Bunch quality component

Results obtained showed that FGV Clone FC4628 (Figure 4a and 4c) produced the highest percentage of Oil to Bunch (%O/B) (27.64%) and percentage of Oil Extraction Rate (%OER) (23.63%) and followed by FGV DxP TK3873 and Clone Agency 2 (Table 2).

Table 2: Bunch quality component from Trial T265.

No	Progeny	Parent material	Mean 2017-2021				
			O/B (%)	OER (%)	OY (t/ha)	KY (t/ha)	TEP (t/ha)
1	DxP Agency 1	Unknown	25.35^{b}	21.67^{b}	5.27^{d}	0.86^{b}	5.78^{c}
2	Clone Agency 2	Unknown	26.36^{ab}	22.54^{ab}	5.94^{c}	0.98^{a}	6.53^{b}
3	DxP Agency 3	Unknown	24.90^{b}	21.29^{b}	5.98^{bc}	1.03^{a}	6.60^{ab}
4	DxP Agency 4	Unknown	25.48^{b}	21.79^{b}	6.20^{abc}	0.75^{c}	6.65^{ab}
5	FGV Clone FC4628	Deli-NPM x Yangambi	27.64^{a}	23.63^{a}	6.45^{ab}	0.84^{b}	6.95^{ab}
6	FGV DxP TK3873	Deli-NPM x Yangambi	26.46^{ab}	22.62^{ab}	6.50^{a}	0.93^{ab}	7.06^{a}

**Mean with the same letter in the same column are not statistically significant by Duncan Multiple Range Test.*
$P \leq 0.05$: a significant difference. O/B: Oil to bunch, OER: oil extraction rate, OY: oil yield, KY: kernel yield, TEP: total economic product.

As FGV DxP TK3873 (Figure 4b and 4d) exhibited the highest Oil Yield (OY) at 6.50 t/ha, the FGV Clone FC4628 (6.45 t/ha) production showed no significant difference between them. Material from DxP Agency 1 is the lowest OY producer as compared to all other planting materials. In contrast, the DxP Agency 3 and Clone Agency 2 outperformed others in Kernel Yield (KY) at 1.06 t/ha and 0.98 t/ha, respectively. The DxP Agency 4 however, produces less KY of 0.75 t/ha and was significantly lower than other clones and DxP materials planted as shown in this study. FGV DxP TK3873 give the highest value in terms of Total Economic Product (TEP) as at 7.06 t/ha while the lowest was by DxP Agency 1 at 5.78 t/ha.

Figure 4: Phenotype character of FGV planting materials (a) FGV Clone FC4628 (9 YAP) (b) FGV DxP TK3873 (9 YAP) (c) Fruit cross-section of FGV Clone FC4628 (d) Fruit cross-section of FGV DxP TK3873.

It has been reported that any differences in oil content are generally due to planting material backgrounds. For example, using *Tenera* hybrids, a cross between *Dura* (thick shell) and the *Pisifera* (shell-less) has produced an intermediate shell thickness with a significant oil extraction rate (OER) of 22-30% (Rajanaidu and Kushairi, 2006). Further studies by Hoong and Donough (1998) on OER and Kernel Extraction Rate (KER) with data collected from 21 mills in Sabah showed that both biological and management factors should be taken into considerations. The biological factors are climatic changes that can affect the palms physiology, age of palms, pollination processes, including amount of rainfall and solar radiation while the management factors are mainly involved manpower constraints that affects harvesting and processing efficiency.

Similarly, clone's oil yield has successfully contributed to a higher oil yield than the DxP seeds. Sharma et al. (2006) reported that at United Plantation Berhad (UPB), semi and bi-clonal DxP seeds oil yields ranged between 7.95 to 9.52 t/ha/yr. This was also found in MPOB where seven high-yielding clones such as P164, P203 and P162 (not included in this study) can produce more than 7 t/ha/yr of oil (Kushairi et al., 2013).

Conclusion

FGV DxP TK3873, was ranked highest producer for various components (FFB, BNO, OY and TEP values). The Clone Agency 2 was also found to yield highest in ABW, FGV Clone FC4628, outperformed others in terms of %OB and %OER while DxP Agency 3 has the highest KY. These findings demonstrated that FGV planting material performed better and is recommended as one of the best planting materials for the oil palm industry.

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Handling Effects on Seedling Quality of Several Dipterocarp Species

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Introduction

Dipterocarpaceae (Dipterocarp) forests are one of the most important natural resources in Southeast Asia. It is a foremost ecologically component of the lowland rain forests. As cited in Ashton (1982), members of this family dominate the forests, establish up to 10% of all tree species and 80% of all emergent individuals. Normally, Dipterocarps included both of small and large, and mostly evergreen trees (Ghazoul, 2016). It is the most highly desirable timber species group in the tropical forests for commercial utilization such as manufacturing wood, fruit, essential oil, camphor, fat, balsam, bark, resin and charcoal (Widiyono, 2021). Its products have very significant roles for domestic practice and export needs. It comprises of 10 genera which are *Anisoptera*, *Cotylelobium*, *Dipterocarpus*, *Dryobalanorps*, *Hopea*, *Neobalanocarpus*, *Parashorea*, *Shorea*, *Upuna* and *Vatica* (Ashton, 1982; Nor Asmah et al., 2017).

In 2021, mass flowering followed by mass fruiting occurred at the Forest Research Institute Malaysia (FRIM), Kepong, Selangor. Dipterocarps produce abundance of seeds during this period. When trees start fruiting, procurement planning has to be started immediately, so that, good quality planting material can be achieved. Recalcitrant seeds (short-lived) are difficult to store due to survival of seeds in this family is limited even under optimal conditions. Temperatures under 16 ℃ or above 32 ℃ must to be avoided because seeds will die in dried condition and also damage in low temperatures (Krishnapillay and Tompsett, 1998). The period between collection and storage or sowing should be as short as possible to decrease coincidental seed deterioration. Any delay in the handling process will result in further loss of viability and decrease the amount of available planting material (Marzalina et al., 2004). Therefore, this paper focused on the seeds quality of eight dipterocarp species being delivered by local commercial courier services.

Materials and Methods

Site of study

The study site was located at the Greenhouse Nursery of FRIM Mata Ayer Research Station, Perlis.

Diptercocarp seeds collection

Freshly ripened Dipterocarp seeds of eight dipterocarp species (*Shorea mecistopteryx, Shorea macrophylla, Shorea singkawang, Shorea macroptera, Shorea leprosula, Shorea multiflora, Dryobalanops aromatica* and *Dipterocarpus crinitus*) were collected in FRIM Kepong, Selangor. The seeds were cleaned from impurities, weighed and put into a paper box, attached with the seed information sheet containing information for plants asset management. The upper part of the paper box was labelled with the information of recipient before delivery. Then, it was transported by local commercial courier services to FRIM Mata Ayer Research Station, Perlis which was about 515 kilometres by road. Unboxing was done the day after receiving the seeds. Batch of seeds were weighed and 10 seeds were taken and the weight recorded to determine the average seed weight.

Germination of seeds

Seeds collected were manually dewinged with care for ease of handling before sowing activity. Dewinged process was done to ensure good contact with the germination medium. Then, seeds were arranged in lines accordingly and pressed into the sand bed and covered with thin layer of fine sand.

Seeds were sown using semi course riverine sand and watered immediately. Automatic watering was done twice daily by using a water timer.

Figure 1: Dewinged process was done before sowing activity.

Figure 2: During sowing, seeds were arranged in rows to provide a good spacing.

Data collection

The number of seeds that germinated during the experiment was recorded when the radicle emerged through the seed coat, heading into the sowing media and hypocotyl elongated. The data were recorded after 2 weeks, at weekly interval until 8 weeks. The calculation for germination potential was as follows:

$$\text{Germination (\%)} = \frac{\text{No. of germinated healthy seeds}}{\text{No. of seeds being tested}} \times 100$$

Results and Discussion

The seeds started to germinate on the second week after sowing and ended by eighth weeks. The germination rates of collected Dipterocarp seeds were as recorded in Table 1.

Table 1: Germination rate of collected Dipterocarp seeds.

No.	Species	No. of seeds tested	No. of seeds germinated	Germination percentage (%)	Duration (week)
1	*Dryobalanops aromatica*	288	166	57.64	8
2	*Shorea leprosula*	70	55	78.57	8
3	*Shorea macroptera*	88	80	90.91	8
4	*Dipterocarpus crinitus*	0	0	0.00	0
5	*Shorea mecistopteryx*	33	33	100.00	8
6	*Shorea macrophylla*	49	49	100.00	8
7	*Shorea multiflora*	60	36	60.00	8
8	*Shorea singkawang*	142	142	100.00	8

Table 1 shows that the highest and best performance of germination percentage (100%) was recorded in three dipterocarp species, *S. mecistopteryx, S. macrophylla*, and *S. singkawang* followed by *S. macroptera* (90.91%), *S. leprosula* (78.57%), *S. multiflora* (60 %) and *D. aromatica* (57.60%). No germination (0%) was recorded for *D. crinitus*, as the seeds were not viable. The germination of seeds is not successful if the seeds are not viable (Nor Asmah, et al., 2017).

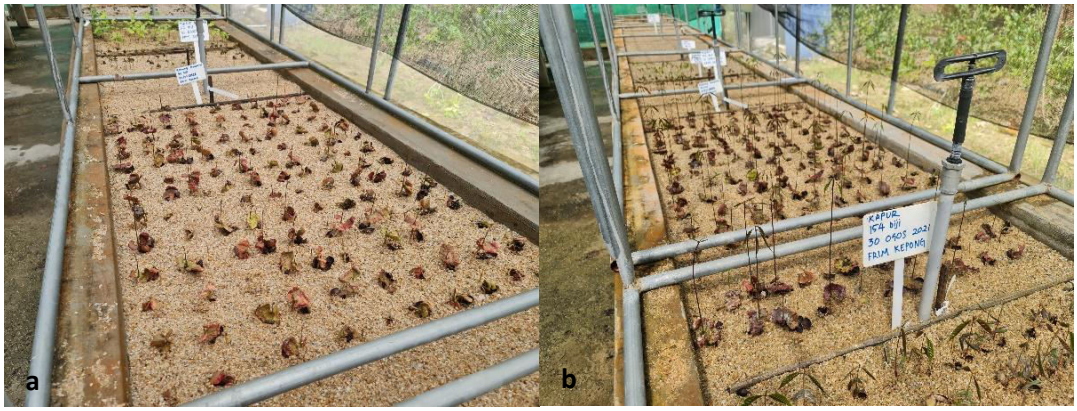

Figure 3: a and b shows the germinated of dipterocarps seeds.

Conclusions

Delivery by using local commercial courier services can be considered as one of the reliable methods for transporting seeds for long distance because duration of delivery of only three to five days does not affect the seed germination rate.

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Early Screening of Selected MARDI *Coffea liberica* Clones for Potential Quality and Uniform Rootstock

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Introduction

Coffee is the second most consumed drink in the world, after water. More than 100 species of coffee were documented in the family Rubiaceae currently (Davis, 2010; Davis et al., 2011). However, only three species are widely used as drinks namely Arabica, Robusta and Liberica. *Coffea liberica* plant is native in west tropical parts of Africa (Gomez et al., 2009). Liberica coffee is less produced and constitutes only 1% of total world production. Currently there is a rise in the demand for Liberica coffee in the specialty coffee market. This species of coffee is planted mainly in Malaysia, Indonesia, The Philippines and several areas in Surinam and in Guyana regions (Lim, 2013).

The production of Liberica coffee starts with the propagation of rootstocks using seeds. Clonal planting material is then obtained by grafting scion of Liberica clones on top of rootstocks. Seed is a vital component for cultivation of coffee plants. However, germination pattern of coffee seeds is very slow and erratic. Observations done in MARDI Pontian showed that *C. liberica* seedling emergence from the soil occur at 60 days after sowing with about 60% germination over a period of two months. This slow and uneven growth makes it difficult to plan seedling production for clonal coffee planting material. Rootstock is a very important part in plant propagation and plays a vital role in the overall growth, development and fruiting of the plant. Seed germination and seedling emergence are the most important and vulnerable phases in plant growth. Slow and asynchronous germination of seeds will make seedling production of coffee difficult to plan. Rootstocks with good growth and resistance to disease are usually selected for clonal propagation. However, early screening can be done to identify Liberica clones that are of high, faster and uniform germination to get better seedling establishment.

Therefore, this study was conducted to screen seeds from clonal Liberica coffee produced by MARDI as suitable rootstock for Liberica seedling production.

Materials and Methods

Assessment of MKL 1, MKL 5, MKL 6 and MKL 7 Liberica coffee germination ability

Four types of clones namely MKL 1, MKL 5, MKL 6 and MKL 7 were used in this experiment. Fruits were harvested fresh from Liberica coffee plot in MARDI Kluang and sent to Technology Commercialisation and Business Centre planting material nursery in MARDI Headquarters, Serdang, Selangor. Collected fruits were of orange red to full red colour. The fruits had their exocarps removed manually, and the seeds with remaining fruit parts, mesocarp and endocarp were soaked in water at 30 °C for a period of 24 h. After this period, the mucilaginous mesocarp was easily removed by washing with water. After drying, seed coats were removed by hand and the seeds were sown in germination plastic boxes using sand as the media. The number of seeds germinated was recorded after 45 days. Experiment was conducted in a completely randomised design arrangement with 4 replications.

Effect of seed harvested at different maturity stages on germination Liberica coffee

Three Liberica coffee clones namely MKL 5, MKL 6 and MKL 7 seeds harvested at four different maturity stages which were stage 1 for immature unripe at 210 days after anthesis (DAA) with green

fruit, stage 2 for mature unripe at 260 DAA with greenish red orange fruit, stage 3 for mature ripe at 310 DAA with fully red fruit, and stage 4 for overripe at 360 DAA with brown to black fruit, were used in this experiment. Processed seeds were germinated on sand as media and the number of seeds germinated was recorded after 45 days. Experiment was conducted in a two factor randomized complete block design (RCBD) with 4 replications.

For both experiments, seed germination percentage (GP) and mean germination time (MGT) were calculated using the following formulas:

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

$$\text{Mean germination time (MGT)}, \quad t = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i},$$

where ti, time from the start of the experiment to the i^{th} observation (day for the example);
ni, number of seeds germinated in the i^{th} time (not the accumulated number, but the number correspondent to the i^{th} observation), and k, last time of germination.

Data obtained was analysed using ANOVA in the SAS software (Version 9, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatments means were compared using Tukey's Honest Significant Difference (HSD) at $P \leq 0.05$.

Results and Discussion

Assessment of MKL 1, MKL 5, MKL 6 and MKL 7 Liberica coffee germination ability

Seeds from MKL 5 had the highest germination percentage (GP) of 93.25% and mean germination time (MGT) of 29.06 days compared to MKL 1 (76.66% GP, 34.94 MGT), MKL 6 (78.33% GP, 34.93 MGT) and MKL 7 (84.16% GP, 33.48 MGT) (Table 1). Seed germination is the continuation of growth of the seed embryo from quiescence state to the development of seedling (Bradford, 2013). Germination starts with the process of water imbibition and ends with the emergence of the embryonic axis, which is usually the radicle. Seeds within the same species have different germination ability due to genetic factors. Arabica coffee seed was known to have different germination percentage when compared among different clones (Wibowo et al., 2020).

Table 1: Comparison of germination percentage (GP) and mean germination time (MGT) for MKL 1, MKL 5, MKL 6 and MKL 7 Liberica coffee seeds.

Clone	Germination percentage (GP)	Mean Germination Time (MGT)
MKL 1	76.66^{c}	34.94^{a}
MKL 5	93.25^{a}	29.06^{b}
MKL 6	78.33^{c}	34.93^{a}
MKL 7	84.16^{b}	33.48^{a}

Means with same letter are not significantly different.

Effect of seed harvested at different maturity stages on Liberica coffee germination

Seeds taken from stage 1 (immature unripe) regardless of clones fail to germinate altogether. At stage 1, embryo is still not developed, making it impossible to germinate. Stage 3 (mature ripe) is the best stage to harvest since all clones scored high germination percentage. MKL 5 gave the best germination percentage at 95% compared to MKL 6 (77.5%) and MKL 7 (84.16). When harvested at stage 4 (overripe), gemination percentage dropped to 76.66% for MKL 5, 77.5% for MKL 6 and 69.16% for

MKL 7 (Figure 1). Coffee seeds have been known to have higher value in germination when harvested at cherry (ripe) stage compared to seeds harvested at greenish-yellow stage (Rosa et al., 2011).

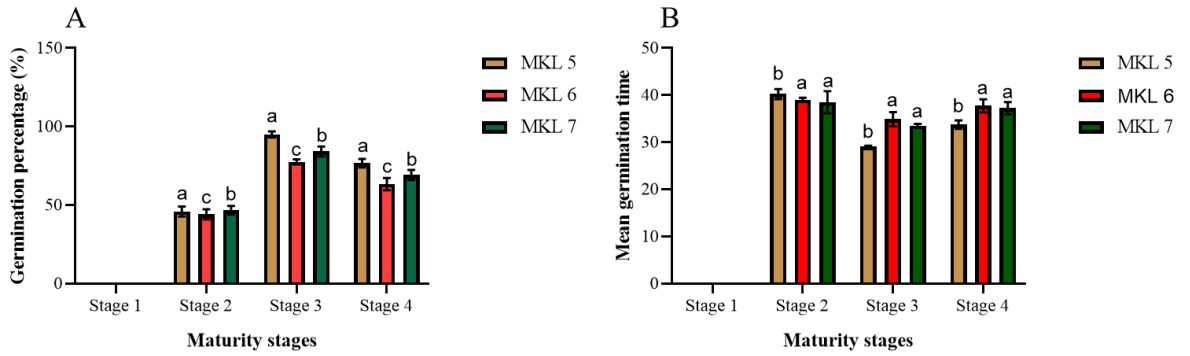

Figure 1: Germination percentage (A) and mean germination time (B) at different maturity stages for MKL 5, MKL 6 and MKL 7 Liberica coffee seeds. Means with the same letter are not significantly different.

Conclusions

From both experiments conducted, it can be concluded that the seeds from MKL 5 Liberica coffee clones have the potential to be used as rootstock for clonal propagation. Having the highest germination rate and low mean germination time will help produce rootstocks that are uniform in growth. However, further study, especially of the growth of seedlings after transplanting into polybags, is needed to confirm this finding.

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The Effect of Different Drying Techniques of Grain Corn for Seed Production

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Introduction

Grain corn (*Zea mays* L.*)* is a major cereal crop grown commercially worldwide. It is a major component of livestock feed and one of the primary cereal crops used to provide nutrients to humans. Grain corn can also be used as a raw material in the production of fuel and processed into a variety of industrial products (Anjum et al., 2016). This plant can be grown all year in Malaysia and other Asian countries with tropical climates. In Malaysia, the livestock and food industries play an important role in ensuring that the country's food supply is always sustainable, safe, and available.

According to the latest statistical report, world grain corn production exceeded 1.03 billion metric tons in 2017/2018, followed by rice and wheat (Anon, 2018). Malaysia imports 93% of grain corn from Argentina and Brazil, and the remaining 7% is imported from other countries (USDA, 2017). The country's grain corn import trend increased significantly from 1.0 million tons in 1987 to 2.0 million tons in 1996, and then to 4 million tons in 2018 (Index Mundi, 2019). In 2018, the trade balance value for livestock feed imports was RM 7,309 million while the value of livestock feed exports was RM 2,192 million (DVS, 2019). As a result, Malaysian government has taken the initiative to develop the country's grain corn industry over the long-term plan by involving all industry players throughout the supply chain, considering the domestic needs and the country's significant reliance on grain corn imports. The demand and interest in grain corn continue to increase in line with the progressive growth of the livestock and food industry in Malaysia. In relation to that, post-harvest handling research, particularly drying studies, has been conducted to determine the drying characteristics of grain corn, as most of the livestock feed is sold and marketed in dry form.

Post-harvest operations of grain corn such as drying, and storage are among the critical activities along the production chain for farmers and smallholders in Malaysia. Drying of the kernels for seed and fodder production is required to extend the shelf life of the product, especially in hot and humid countries like Malaysia. Freshly harvested grain corn has relatively high moisture content (MC) between 20-25%, making it perishable if not dried immediately (Nor Amna A'liah et al., 2020). The drying process is required to reduce the MC to a safe level in order to keep the product stable during storage. As a result, proper post-harvest handling techniques are necessary because the commodity is susceptible to fungal infection and contamination (Appell et al., 2009). The MC of grain corn must be reduced to 13%-14% to prevent the growth of fungi which is capable to produce aflatoxin.

Accordingly, this study was conducted to evaluate the performance of grain corn drying using various drying strategies. Many studies on the drying of grain corn kernels have been carried out but information on drying in the form of corn cobs is very lacking and limited. Drying the corn cobs is necessary to maximize seed production due to minimal injury to corn kernels. The kernel will be removed from the corn cob manually after the drying process without damaging the embryo. Corn cob drying is also a common practice for smallholder farmers in Malaysia due to the lack of mechanical separator machines in rural areas. Traditionally, the cobs are sun-dried openly. However, due to the unpredictable drying periods, this traditional method may have a negative impact on grain corn quality.

The drying of corn kernels separated by the separator machine for seed preparation revealed a low germination rate due to mechanical damage to the kernel embryo. So far, no Malaysian research document has documented drying studies for grain corn cobs for seed production. Therefore, this drying study has been proposed to evaluate the influence of different drying strategies on seed germination performance of grain corn. The primary goal of this research is to maximise seed germination rate in order to promote future commercial grain corn production in Malaysia.

Materials and Methods

Drying protocols

Grain corns were harvested at an optimum maturity of 110 days after planting. Whole grain corns with cobs were dried by adopting different drying strategies as shown in the flow chart of Figure 1. Two different drying strategies namely slow drying at low temperature between 35-40 ℃ and fast drying at moderately high temperature of 50 ℃ were applied to assess the effectiveness of these two drying methods on seed germination rate of grain corn. Drying treatments were carried out using both solar dryer and a mechanical oven. The reduction of MC during the drying process was recorded to determine the drying kinetics for each strategy implemented. Then, the dried kernels with a MC of less than 12% were germinated in the laboratory. The percentage of germination, germination index as well as mean germination time were evaluated to determine the most appropriate drying strategy with the highest rate of germination for seed production. The drying strategies used and overall methodology are depicted in Table 1 and Figure 1 below.

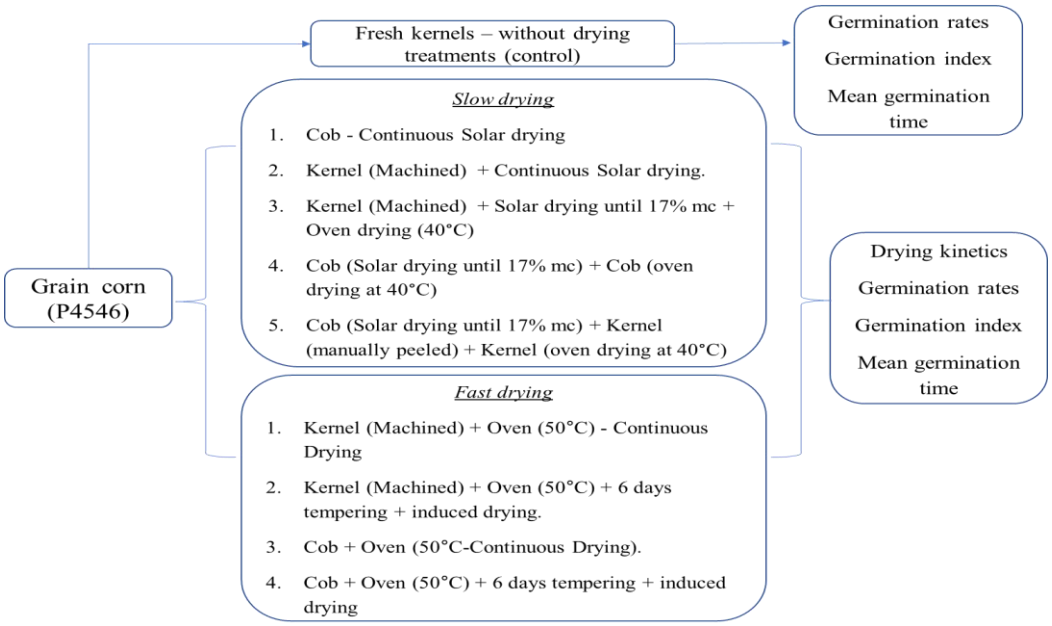

Figure 1: Flow chart of methodology.

Table 1: Drying strategies of grain corn prior to seed production.

Treatment	Drying strategies
T1	Fresh kernels without drying treatments (control)
T2	Continuous drying of cobs in the solar dryer until 10% MC
T3	Continuous drying of machine separated kernels in the solar dryer until 10% MC
T4	Drying of machine separated kernels in the solar dryer until 17% MC, followed by oven drying at 40 ℃ until 10% MC.
T5	Drying of cobs in the solar dryer until 17% MC, followed by oven drying at 40 ℃ until 10% MC.
T6	Drying of cobs in the solar dryer until 17% MC and then the kernels from the cobs were manually separated, followed by oven drying at 40 ℃ until 10% MC.
T7	Continuous drying of machine separated kernels in the oven at 50 ℃ until 10% MC.
T8	Drying of machine separated kernels in the oven at 50 ℃ until 10% MC, followed by 6 days tempering period and oven drying at 40 ℃ until 5% MC.
T9	Continuous drying of cobs in the oven at 50 ℃ until 10% MC
T10	Drying of cobs in the oven at 50 ℃ until 10% MC, followed by 6 days tempering period and oven drying at 50 ℃ until 5% MC.

Statistical analysis

The effects of different drying strategies on seed germination rate, germination index and mean germination time were determined by ANOVA followed by mean separation by Duncan's Multiple Range Test (DMRT) using Minitab 12 software (Minitab Inc., State College, PA, USA).

Results and Discussion

Figures 2-10 illustrate how each drying strategy affected the drying kinetics of grain corn. Drying in cob form took a longer time because the MC of the cob and the kernel differed significantly. This resulted in a longer time to reach moisture balance between kernel and cob due to the continuous migration of moisture from cob to kernel. Drying of the cobs in the solar dryer took the longest drying period of 525 hours when compared to the other drying strategies. The corresponding drying behaviours of grain corn also depended on the drying strategy as the migration of water vapour from the interior of the cell to the environment through the diffusion process relied on the ambient temperature, relative humidity, wind movement and speed, the type of crop and micro-structure of the cell.

Table 2 shows the effect of different drying strategies on seed germination rate of grain corn. Drying treatments which combined both solar dryer and oven drying at a low temperature of 40 ℃ (slow drying) was recommended. The treatments of T5 and T6 (Table 2) resulted in the highest seed germination rate at 98% (T5) and 98.5% (T6), respectively. Continuous drying of the cobs in a solar dryer was the best with a relatively high seed germination rate of 97.5%. However, this technique was entirely dependent on the unpredictable weather condition which could make the drying period longer for almost 22 days. The extended drying period due to the inconsistent environmental conditions especially the fluctuation in relative humidity will increase the risk of fungal infestation and subsequently will damage the quality of the seeds that will negatively impact the plant's growth performance. For this reason, continuous drying of grain corn in the solar dryer prior to seed production is not advisable due to poor quality of seed kernels.

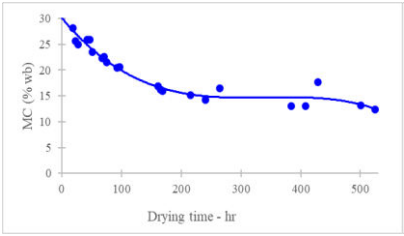

Figure 2: Seed MC with T2.

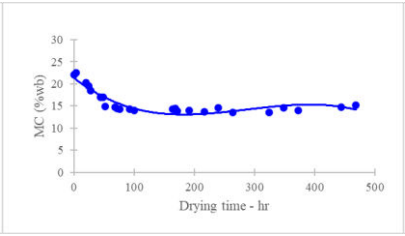

Figure 3: Seed MC with T3.

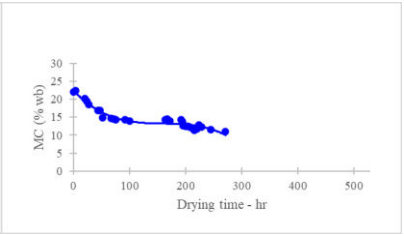

Figure 4: Seed MC with T4.

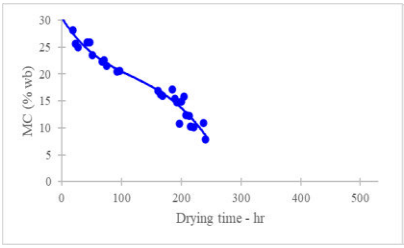

Figure 5: Seed MC with T5.

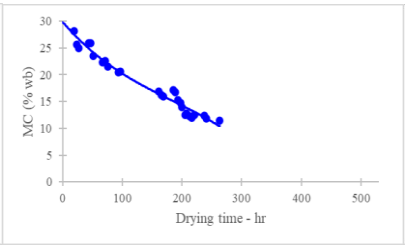

Figure 6: Seed MC with T6.

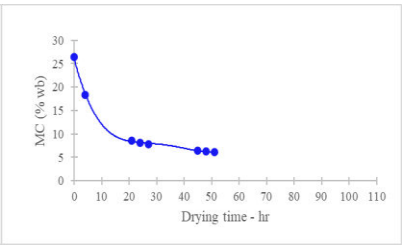

Figure 7: Seed MC with T7.

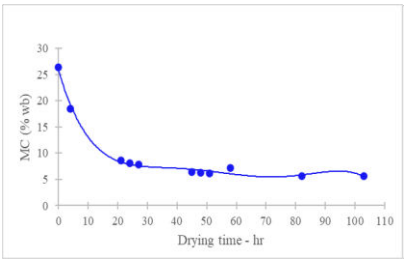

Figure 8: Seed MC with T8.

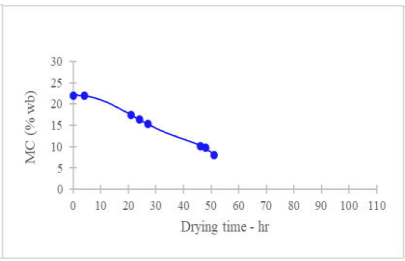

Figure 9: Seed MC with T9.

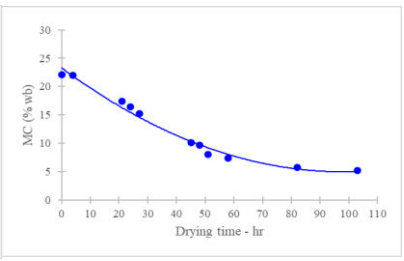

Figure 10: Seed MC with T10.

Table 2: The effect of different drying strategies on seed germination rate, germination index and mean germination time of grain corn.

Treatment	MC (%)	Drying period (Days)	Germination rate (%)	Germination index	Mean germination time (day)
T1	-	-	98.0^{a}	16.54^{a}	3.05^{e}
T2	12.31	21.88	97.5^{ab}	13.11^{cd}	3.83^{bc}
T3	13.60	13.5	96.0^{abc}	11. 86^{e}	4.14^{ab}
T4	11.23	8.92	96.0^{abc}	12.33^{de}	3.40^{ab}
T5	11.14	10.04	98.0^{a}	12.43^{de}	3.40^{ab}
T6	11.40	10.02	98.5^{a}	13.8^{bc}	3.94^{ab}
T7	6.20	1	92.0^{c}	13.88^{bc}	3.55^{cd}
T8	5.26	4.29	93.0^{bc}	14.52^{b}	3.33^{de}
T9	8.08	2.13	95.5^{abc}	12.14^{de}	4.24^{a}
T10	5.65	4.29	96.0^{abc}	12.70^{cde}	4.07^{ab}

Means with similar letter within column are not significantly different.

Conclusion

The germination rate of grain corn seed depended on the drying strategy because different techniques contributed to different drying kinetics and simultaneously affected the germination rate of the seed. Therefore, drying treatments with a combination of solar dryer and oven drying at a low temperature of 40 °C were recommended due to high germination rates and shorter drying period of 10 days with

the increase in seed germination rate to 98% (T5) and 98.5% (T6). High seed germination rates will boost productivity and promote commercial grain corn cultivation in Malaysia.

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Different Stage Maturity and Seed Germination of Merawan Siput Jantan (*Hopea odorata)*

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Introduction

Hopea odorata, a member of the Dipterocarpaceae, is popularly known locally as Cengal pasir or Merawan siput jantan. The genus name, *Hopea*, honours Dr John Hope (1725-1786) who was a Scottish botanist at the Edinburgh Botanic Garden, United Kingdom, while the species name, *odorata*, refers to the fragrant flowers. *Hopea odorata* is distributed from Bangladesh, the Andaman and Nicobar Islands, Indo-China, Thailand, and Malaysia to Indonesia. In Peninsular Malaysia, it is a well-known tree in Pulau Langkawi, Perlis, Kedah, northern Perak, Kelantan and Terengganu (Symington, 2004). It is found in lowland dipterocarp forest and seasonally dry tropical rain forests, mainly growing in riparian areas, and is rarely found far from streams.

This species is very common in Peninsular Malaysia because it has been widely planted, especially as a roadside tree. However, in the wild it is rare, and the species is considered as Vulnerable (VU) for Malaysia (Chua et al., 2010) due to forest clearance. Members of the Dipterocarpaceae are known to have winged fruits and also produce resin. The fruit of this species is an ovate-conical nut with two-wings. The flowers are pale yellow and leaves unequal-sided. The main characteristic for identification of this species is the presence of the pore-like domatia in the leaf axils. The tree has scaly bark and the inner bark is dull brown or greenish yellow and sometimes tinged very faintly pink. *Hopea odorata* is widely grown as a shade tree in the villages of Kelantan and Terengganu. It is a popular roadside tree species, especially in Kuala Lumpur, because of its fast growth, and tall, straight and clear bole.

These tall and big trees of Dipterocarpaceae are largely restricted to moist and wet tropical seasonal habitats where most of them produce seeds that belong to the recalcitrant category family (Schmidt, 2000; Tweddle et al. 2003; Berjak and Pemmenter, 2008). This type of seeds cannot be dried and storage is only possible for a short period of time. Maturity of seed at proper stage is very essential to attain better germination. Excellent performance of seed germination could be characterized by the seed maturity stage. The objective of this study was to determine the suitable stage of physiological maturity for collecting *H. odorata* seeds in order to produce high quality seed production of this species. Results obtained can be further used in planning seed harvesting.

Materials and Methods

Collection of seeds

Hopea odorata seeds were collected from the tree planted on the roadside at Rawang, Selangor on 20th of May with an average of 5 m in height and 20 cm in diameter. It takes three months from flower (in March) to fruiting. The colour of the wings that turns brown is determined as maturity. Seeds were classified into four groups based on different colours of the wings which are fully brown, half brown half green, quarter green and fully green when the seeds arrived at the laboratory.

Germination test

The seeds were categorised into four groups based on different colors of the wings and wings were removed manually. Seeds were tested for germination in petri dish by using moist tissue which is 25 seeds for each petri dish and were observed daily for two weeks. A seed with a radicle extension of 2 mm or more was considered germinated.

Results and Discussion

Figure 1 shows the number of days required for *H. odorata* seeds at various maturity stages to germinate. Fully brown-winged seeds germinated faster than other maturity stages where complete germination was observed on the 5th day; where more than 64 seeds germinated after one day. This followed by the quarter-winged seeds where 63 seeds germinated on the third day, before it reduced and complete the germination process on the 9th day. As for the half-brown half-green seeds the first germination occurred on the third day and reached its peak on the fourth day with only 23 seeds germinated. Germination was completed on the 11th day. A much longer time was required (15 days) for the germination of fully green-winged seeds. These seeds started to germinate on the fourth day, and the highest number of seeds germinated was observed on day 6 but this reduced gradually until the final day.

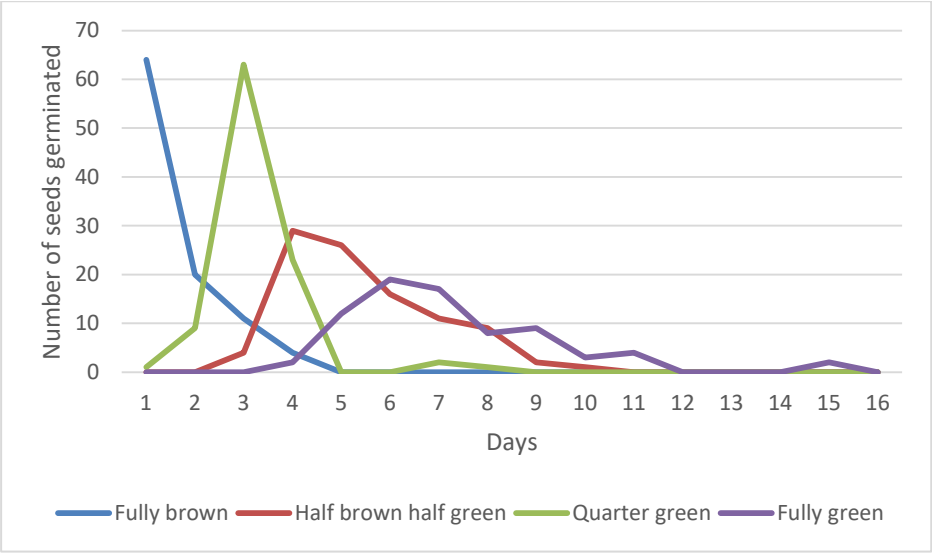

Figure 1: The number of seeds germinated at different stages of maturity.

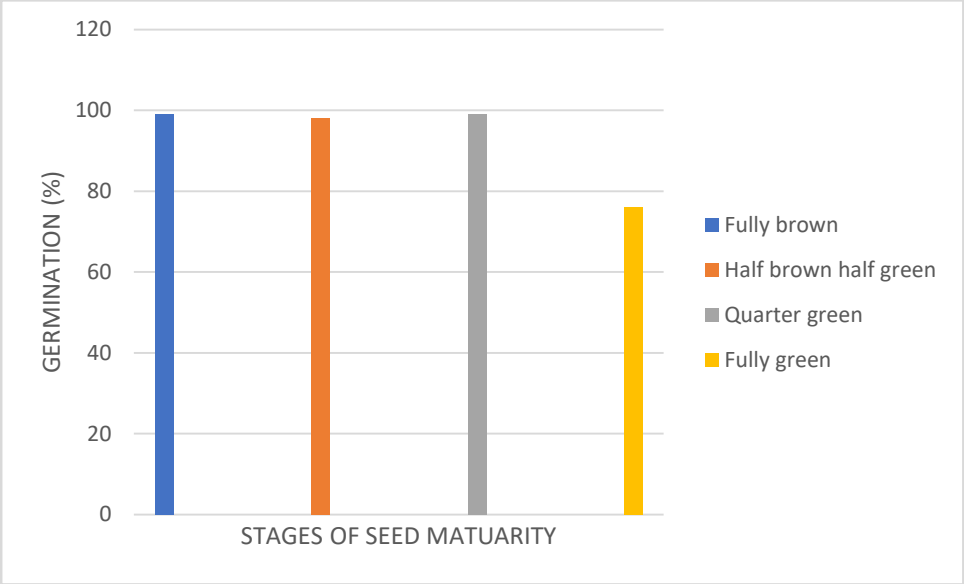

Figure 2: Germination percentage of *H. odorata* seeds at various maturation stages.

Seed germination increased from fully green-winged (76%) to the fully brown-winged stage reaching a maximum of 99% (Figure 2). Similar germination percentages were observed for quarter green-winged and half brown-winged seeds; with 99% and 98% of seeds germinated; respectively. Germination time as an indicator of seed quality implied that the seed from the fully developed fruits with more consistency morphological and physiological characteristics would be more favourable for planting. However, the seed from a fully green wing could germinate and a long time was needed to achieve this. Harvesting of the seed at proper stage of maturation is very essential to attain better seed quality. Excellent seed performance could be characterized by the seed viability and vigor, as well as their actual performance in the field. The timing of harvest is very critical because the reduction of seed quality could be due to seed immaturity and weathering. As mentioned by Seshu and Dadlani (1989), the major aspects of seed quality are genetic and physical maturity, high germination and vigor, and the absence of seed-borne pests'.

Conclusion

Based on the results obtained in this work, we identified the best stage for collecting *H. odorata* seeds was when the wings had turn to full brown where the germination rate was the highest (99%). In addition, at this maturity stage, germination was the fastest compared to other stages.

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Preliminary Assessment of Seed Protein Content of Soybean [*Glycine max* (L.) Merrill] Accessions

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Introduction

Soybean [*Glycine max* (L.) Merrill] is an annual crop that belongs to the legume or pea family of the Leguminosae. Leguminosae, also called Fabaceae, consists of 12,000-18,000 species and produces pod with seeds. These species are grown across the globe, although only some are of economic importance as human feed and as forage for livestock. Unlike most legumes, soybean is unique because it provides exceptional source of protein, making soybean and its food products are an excellent plant-based protein. To date, soybean is the most preferred source of high quality plant-based protein for livestock and human nutrition (Ali et al., 2020).

Soybean is native to East Asia and was first domesticated by Chinese farmers around 1100 BC. It has then gradually spread all over the world and widely consumed for its edible bean (Lee et al., 2021). Soybean protein is one of the cheap sources of dietary protein and contains most of the essential amino acids required for animal and human nutrition. It is also considered to be a good dietary replacement of animal protein sources, due to the similar nutritional profile except for sulfur amino acids (methionine and cysteine), to that of animal protein (Hassan, 2013).

The economical and high nutritive values of soybean have led to an increasing demand for consumable soybean. This has made soybean as one of the most important and valuable legume crops cultivated worldwide. Unfortunately, Malaysia does not produce soybean and has to fully rely on imports to meet the supply-demand gap for its supply. It is estimated that world production increased from approximately 160 million tonnes on 70 million ha in 1998 to 350 million tons on 131 million ha in 2019 (Ksi¸ezak and Bojarszczuk, 2022).

Many studies on soybean protein have been reported, but research to discover useful genetic resources for the development of high protein varieties is still lacking. Therefore, this study was conducted to evaluate the variation of seed protein in ten (10) soybean accessions using Bradford assay method, aiming to identify the best accession with high protein. Morphological analysis was also conducted to provide supporting scientific evidence on the soybean accessions to select useful genetic resources.

Materials and Methods

Plant materials

Seeds of ten (10) soybean accessions (Figure 1) were obtained from several agencies and universities such as World Vegetable Centre (WorldVeg), Universiti Putra Malaysia (UPM) and International Islamic University Malaysia (IIUM). The seeds were represented as accession 5 (ACC5), accession 7 (ACC7), accession 19 (ACC19), accession 21 (ACC21), accession 22 (ACC22), accession 27 (ACC27), accession 28 (ACC28), accession 29 (ACC29), accession 30 (ACC30) and accession 31 (ACC31). They were grown under both rain shelter and netted structure in a randomized complete block design (RCBD) with three replications. The commercially available soybeans (SB), adzuki beans (AB) and black eyed pea beans (BEPB) were also purchased from a local market and used as a

comparison. The soybean samples were finely ground to get a uniform size and stored in a -20 ºC freezer and prior to protein extraction.

Figure 1: Soybean seeds from ten different accessions.

Morphological characterization

After reaching full maturity, the morphological traits and yield components; the number of pods per plant and the number of seeds per pod were measured. The observations of the pods and seeds were conducted to the colour, length and width on day 90 after sowing. The seed yield and weight were also determined upon harvest.

Protein extraction

Salt/alkaline extraction method was performed as described by Maehre et al. (2018), with minor modifications. Briefly, 0.5 g of the samples was homogenized with 30 mL of 0.1 M sodium hydroxide (NaOH) in 3.5% sodium chloride (NaCl) using a homogenizer. The homogenates were incubated at 60 °C for 90 min in a water bath before centrifugation at 10,000 rpm for 10 min at 4 °C. The resulting supernatants (extracts) were kept at -20 °C until analyses.

Protein estimation by Bradford method

Protein content of the extracts was measured using Coomassie Plus (Bradford) protein assay kit (Thermo Scientific, USA), according to the manufacturer's instruction. The procedure is based on the formation of a complex between the Coomassie brilliant blue G-250 dye in the Bradford reagent and the protein present in the sample. An aliquot of 10 µL of the seed extracts was mixed with 300 µL of Bradford reagent. They were fully mixed for a few seconds using a shaker. After incubation for 10 min at room temperature, the absorbance of the mixture was read at 595 nm with a microplate reader (Eon Biotek, VT, USA). Protein concentration was determined based on a calibration curve that was generated using Bovine Serum Albumin (BSA) (Sigma-Aldrich) as the standard reference.

Statistical analysis

All experiments were carried out in triplicates and presented as means ± standard deviations (SD). The data were statistically analysed by one-way analysis of variance (ANOVA) and Tukey's posthoc test using SPSS software, version 20 (SPSS Inc; Chicago, IL, USA). A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

In order to assemble superior varieties with high quality seeds, a collection of genetic resources, also known as a germplasm collection, plays an important role. The characterization of the resources can be done by studying the morphological characteristics. The evaluation is also important to identify soybean lines and the diversity level of the plant in a certain area (Ningsih et al., 2019).

In the present study, morphological characterization was conducted on ten different accessions of soybean seeds. Results (Table 1) showed that ACC29 had the highest developed pod (61.22/plant) resulting in a better seed weight (10.37g/pod) and total number of seeds (111.11/plant). Considerable results were also observed in ACC27. The accession had high weight of plant with pod (16.66 g) and also total weight of seed (6.25 g/plant). However, the differences between the two accessions were subtle. According to Ksi˛ezak and Jolanta Bojarszczuk (2022), morphological characteristics such as number of pods, number of seeds in a pod and seed weights are essential yield components in plants. Therefore, from the results, it can be suggested that ACC29 and ACC27 had better yields as compared to other accessions.

Table 1: Comparative morphological characteristics of ten soybean accessions.

Accession	Seed Colour	Developed Pod	Number of Seed/ Pod	Pod Width (mm)	Pod Length (mm)	Total Weight of Plant with Pod (g)	Total Weight of Seed with Pod (g)	Total Number of Seed/Plant	Total Weight of Seed (g)
ACC5	Yellow	5.89^{e}	1.33^{c}	33.31^{ab}	7.64^{b}	6.99^{d}	4.59^{cd}	2.67^{e}	0.75^{d}
ACC7	Yellow	4.11^{e}	1.77^{bc}	41.94^{a}	10.39^{a}	5.88^{d}	2.77^{d}	2.44^{e}	0.59^{d}
ACC19	Yellow	15.53^{de}	2.66^{a}	38.70^{ab}	8.42ab	8.74^{cd}	6.23^{abcd}	22.55^{de}	3.57^{abc}
ACC21	Yellow	19.00^{cde}	3.00^{a}	41.54^{a}	9.18^{ab}	11.48^{abcd}	6.95^{abc}	28.56^{cde}	2.74^{cd}
ACC22	Yellow	12.11^{e}	1.33^{c}	33.91^{ab}	6.79^{b}	9.08^{cd}	6.19^{abcd}	18.56^{de}	3.06^{bcd}
ACC27	Yellow	35.00^{bc}	2.89^{a}	37.61^{ab}	7.94^{ab}	16.66^{a}	9.45^{ab}	93.33^{ab}	6.25^{a}
ACC28	Yellow	35.00^{bc}	2.78^{a}	41.32^{a}	8.39^{ab}	14.08^{abc}	8.93^{ab}	61.78^{bc}	5.08^{abc}
ACC29	Yellow	61.22^{a}	2.66^{a}	33.35^{ab}	7.60^{b}	16.36^{ab}	10.37^{a}	111.11^{a}	5.73^{ab}
ACC30	Yellow	15.33^{de}	2.11^{b}	34.74^{ab}	7.67^{b}	7.13^{d}	4.06^{cd}	33.56^{cde}	2.76^{cd}
ACC31	Black	30.66^{bcd}	1.77^{bc}	27.72^{b}	7.32^{b}	10.94^{bcd}	5.66^{bcd}	48.67^{cd}	3.18^{bcd}

**Values are means of triplicate determination (n = 3) ± standard deviations which, with different letters within the same column are significantly different at P < 0.05 according to Tukey's test.*

Soybean is called as the 'Golden Bean' for being the high quality protein source for human and animal nutrition (Ali et al., 2020). It has been reported that 85% of soybean cultivation destined for animal feed and the remaining destined for direct human consumption (Ksi˛ezak and Jolanta Bojarszczuk, 2022). There are a number of different analytical methods available for estimating total protein concentrations, which include titration-based methods, chromatographic methods and spectrophotometric methods. Among them, Coomassie dye binding assay is the fastest and easiest to perform (Nouroozi et al., 2015; Okeniyi et al., 2015).

The Bradford dye assay is one of the preferred methods for quantifying protein in many laboratories. The assay is based on the binding of Coomassie brilliant blue G-250 dye at acidic pH to protein to produce a complex absorbing at 595 nm. The amount of absorption is proportional to the protein present. The dye has specific binding requirements for to basic amino acid residues such as arginine, histidine, phenylalanine, tryptophan, tyrosine residues, and hydrophobic interactions in proteins which results in a colour change to blue. The advantages of the Bradford assay include rapid, convenient, inexpensive, relative sensitivity and low interference by other substances (Nouroozi et al., 2015).

Table 2 showed a comparison of protein content of commercial beans as measured by Bradford and Kjedahl methods. Kjeldahl method is one of the most common analyses performed in the nutrition

laboratory. From the results, it can be observed that soybeans (SB) remained as the best source of protein. There were no significant differences observed in adzuki (AB) and black eyed pea beans (BEPB). Interestingly, both Bradford and Kjedahl methods showed a similar trend of the protein concentration. Therefore, it can be suggested that Bradford method can be used as an alternative measurement of protein to the widely used Kjedahl.

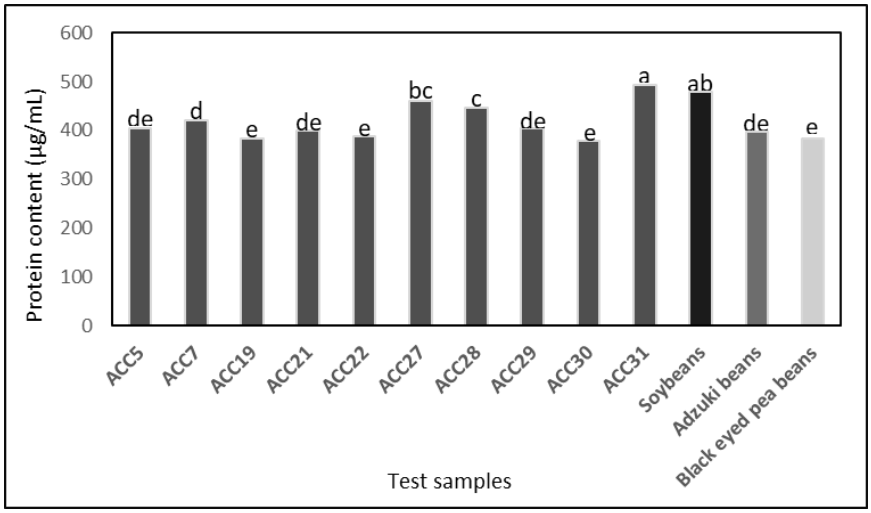
Table 2: Protein content of commercial beans as measured by Bradford and Kjedahl methods.

Sample	Protein content	
	Bradford (µg/mL)	Kjedahl (%)
Soybeans	478.82[a]	35.40[a]
Adzuki beans	397.85[b]	22.50[b]
Black eyed pea beans	383.31[b]	21.93[b]

Values are means of triplicate determination (n = 3) ± standard deviations which, with different letters within the same column are significantly different at P < 0.05 according to Tukey's test.

The Bradford method was further used to determine protein content of the ten soybean accessions. Comparison was also made to the commercially available beans SB, AB and BEPB. As displayed in Figure 2, among the soybean accessions, ACC31 showed the highest amount of seed protein (492. /mL) followed by ACC27 (459.8 µg/mL) and ACC28 (446.8 µg/mL). The protein contents of ACC31 and ACC27 were also significantly comparable to SB (479.32 µg/mL). Subtle differences were evident in other soybean accessions and commercial beans.

The variation in our results could be due to the different accessions and types of the soybean seeds. This is in line with previous studies that protein content of soybeans may differ according to genetic variability, cultivars (Lee et al., 2021; Ksi˛ezak and Bojarszczuk, 2022), source (location) and colours (Ciabotti et al., 2016; Lee et al., 2021). Ksi˛ezak and Jolanta Bojarszczuk (2022) reported that chemical composition (protein), yields and structural yield components of soybeans are largely attributed to the cultivars in addition to the environmental factors. The high protein content of the black colour seed ACC31 was also in agreement with Ciabotti et al. (2016). The researchers described that soybean grains with black seed coat are recognized for being an excellent source of high quality protein and have been associated with a wide range of health benefits.



Values are means of triplicate determination (n = 3) ± standard deviations which, with different letters are significantly different at P < 0.05 according to Tukey's test.

Figure 2: Comparative protein content of ten soybean accessions and commercial beans as measured by Bradford method.

Conclusions

ACC31 and ACC27 can be suggested as the best soybean accessions with promising seed protein content, which provide new possibilities to exploring them for other nutritional qualities in the future. Interestingly, ACC27 possessed both high yield and high protein characteristics, which would be attractive for plant-growers and end-users. These findings provide a useful reference for those selecting genetic resources for the development of high quality soybean varieties. Further studies on the potential soybean accessions are also critical to sustain the productivity and empower the livestock feed industry to achieve self-sufficiency level.

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Chapter 7

Biotechnology

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Development of Surface Sterilization Protocol for the *Tinospora crispa* (Patawali) Aseptic Culture Establishment

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Introduction

Tinospora crispa (L.) Hook. f. & Thomson (Menispermaceae), generally known as Patawali, Akar Patawali, Seruntum, or Akar Seruntum in Malaysia (Noor et al., 1989). In Malaysia, *T. crispa* is used traditionally for numerous therapeutic purposes like diabetes, hypertension, stimulation of appetite, and protection from mosquito bites (Ahmad et al., 2016). More than 65 compounds of diverse chemical structures, such as alkaloids, flavonoids, lignans, furanoditerpenes, steroids and lactones, have been identified in the extract of *T. crispa* (Haque et al., 2020). Ahmad et al. (2016) stated that *T. crispa* possesses a broad spectrum of activities, including antidiabetic, antioxidant, antitumor, antiinflammation, antimicrobial, antiosteoporosis, and immunostimulation effects.

Plant tissue culture is the most popular technique of plant biotechnology, which has diverse applications in various fields and is generally used for disease-free plant propagation. There are several types of plant tissue culture, such as protoplast culture, cell suspension culture, tissue and organ culture, and anther or pollen culture, which are later inoculated on an appropriate nutrient medium for growth and differentiation into complete plantlets (Kumar and Loh, 2012). Plantlets obtained were then acclimatized in the nursery before being planted in the field.

Explant surface sterilization is a procedure that involves immersing the explants in an appropriate concentration of chemical sterilizers or disinfectants for a certain period, resulting in a contamination-free culture or an aseptic culture (Oluwakemi, 2008). The main objective of this study is to surface sterilize *T. crispa* clones from identified mother plants.

Material and Methods

Plant samples

Plant samples of *T. crispa* (Figure 1) were collected from FRIM's tissue culture nursery.

Figure 1: *T. crispa* plant samples.

Culture media and condition

Murashige and Skoog (1962) (MS) basal medium added with 3% (w/v) sucrose and 0.3% (w/v) Gelrite supplemented with 0.1 mg/L BAP was used in this study. The medium pH was adjusted to 5.8

using 1 N NaOH. The culture medium in the test tube was steam sterilized in an autoclave at a pressure of 1 kg/cm^2 at a temperature of 121 °C for 15 minutes. All cultures were incubated in the culture room at 22±2 ℃ under a 16/8 h photoperiod.

Surface sterilization and culture initiation

Nodal segments (10-12 cm) of *T. crispa* (Figure 2) were collected and cut into small pieces. Afterwards, the nodal segments were washed with fungicide plus a drop of Tween 20 and then rinsed with sterile distilled water. Three different treatments were employed for surface sterilization. Method A comprises of 70% (v/v) ethanol for 3 min, 50% (v/v) Clorox® for 15 min, Method B comprises 70% (v/v) ethanol for 3 min, 60% (v/v) Clorox® for 15 min and Method C comprises of 70% (v/v) ethanol for 3 min, 50% (v/v) Clorox® for 20 min. Then the explants were washed three times with sterile distilled water and dried in the laminar flow before being inoculated onto MS basal medium supplemented with 0.1 mg/L BAP for culture initiation. All cultures were incubated in the culture room with a temperature of 22±2 ℃ under 16 hours of light and 8 hours of dark conditions.

Figure 2: Nodal segments of *T. Crispa.*

Results and Discussion

After 3 weeks of culture, it was observed that Method A produced 100% aseptic culture and 63% new shoot induced meanwhile, Method B produced 87% aseptic culture and 87% new shoot induced. For Method C, only 27% aseptic culture and 13% new shoot induced were obtained (Table 1). The establishment of aseptic culture is a crucial stage in the tissue culture process for culture initiation. Surface sterilization protocol varies among species depending on the type of explant used.

Table 1: The percentage of *T. crispa* aseptic culture and new shoot induced after 3 weeks of culture.

Method	Aseptic culture (%)	New shoot induced (%)
A	100	63
B	87	87
C	27	13

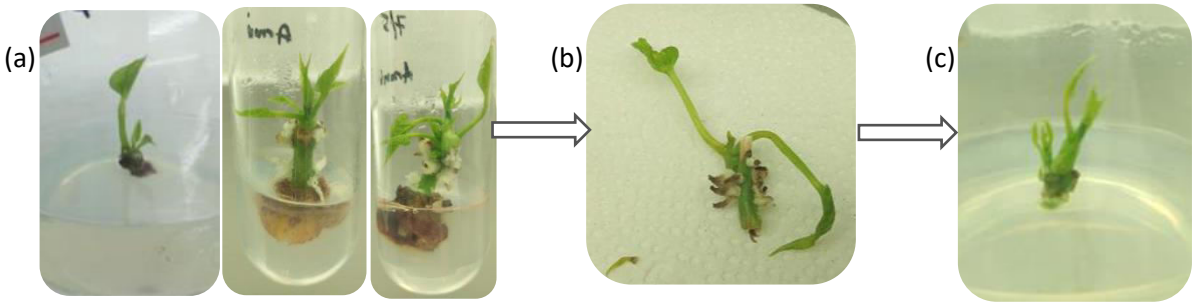

Figure 3: The proliferated shoots of *T. crispa* after 4 weeks of culture were subcultured onto MS basal medium supplemented with 0.5 mg/L BAP for the shoot multiplication.

Contamination in tissue culture probably can originate from two sources: carry-over of microorganisms on the surface or in the explants tissues or through faulty procedures in the laboratory.

From the above result, both fungal and bacterial contaminations were observed in the cultures during the surface sterilization stage. Method C recorded a lower aseptic culture percentage, probably due to a low sterilization agent concentration that could not remove the existing contaminant on the explants' surface. Meanwhile, Method A produced the highest percentage of aseptic culture. It could be due to the surface sterilization technique using suitable bleaching agent and disinfectant concentration. However, the percentage of new shoot induced in Method A is lower than in Method B. It was observed that some of the aseptic cultures from Method A did not produce new shoots. This could be explained by the lethal browning of the explant in the culture and resulted in the death of explants. Browning is the output from the oxidation of phenolic compounds released from the explants' cutting ends by polyphenol oxidases. The oxidized products, quinones, are known to be highly reactive and inhibit enzyme activity leading to the death of explants (Bhat and Chandel, 1991).

Ervin and Wetzel (2002) noticed that a high sterilant concentration can destroys plant tissues. Surface sterilization should not destroy or break the biological activity of the explants but rather the contaminants. Explants should only be sterilized with an appropriate concentration of sterilization agent for a specific period of time (Oyebanji et al., 2009). The profilerated shoots of *T. crispa* (Figure 3) were subcultured onto MS basal medium supplemented with 0.5 mg/L BAP for the shoot multiplication and subsequently *in vitro* rooting in medium containing auxin before acclimatizing in the nursery.

Conclusions

Surface sterilization method B gave the highest percentage of new shoots induced. The proliferated shoots were then multiplied in MS basal medium containing 0.5 mg/L BAP for further research on the development of tissue culture protocol for *T. crispa*.

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Establishment of *In vitro* Propagation Media for Leaves Explant of *Hevea brasiliensis* Clone RRIM 3001

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Introduction

RRIM 3001 belongs to Group 2 under LGM Clone Recommendation 2013. Latex timber clone (LTC) of *Hevea brasiliensis*, RRIM 3001, has shown impressive yield productivity, up to 2,276 kg/ha/yr, with high clear bole volume at 0.6 m^3/tree at the age of 21 years. The highest increase in girth measurement after the 6^{th} year of cultivation was at 56.3 cm, i.e., at an average of 10.6 cm/yr. The tree trunk is straight, with minimum branches at the crown part (Nurmi-Rohayu et al., 2015).

In the previous report, the presence of zeatin and kinetin has maintained somatic embryogenesis and plantlets regeneration of RRIM 2025 (Nor Mayati, 2015). Zeatin added at 0.5 mg/L in differentiation media enriched with IBA, $AgNO_3$ or NAA, and BA showed a synergistic effect on embryogenesis and plantlets regeneration of this clone. Meanwhile, kinetin at 0.3 mg/L was found to have synergistic effects in enhancing embryogenesis and induced plantlets recovery while applied in the media containing IBA and BA (Nor Mayati, 2015).

Tissue culture research on *H. brasiliensis* Clone RRIM 3001 has been established since 2010 as a viable methodology to propagate RRIM 3001 while complementing the planting material and rootstock. This technique also implies a platform for the genetic engineering of this clone. It will serve as a vehicle to produce proteins of high value, thereby enhancing their agronomic characteristics. This study aimed to redevelop specific media to enhance callogenesis, embryogenesis, and the survival of the RRIM 3001 plantlets. Due to current morbidity and unexpected situations that have global impacts on the demand for rubber, research on rubber has been accelerated accordingly. The limited flower sources for the anther explant have allowed the vegetative tissue culture to re-instigate, and several woody plant media are tested.

Materials and Methods

Vegetative tissue culture of available clones RRIM 3001 (43 trees) is carried out from the samples collected from source bush seedling trees planted in the lawn of the netted house at Genetic Resources and Improvement Unit (UPSG), in SPSB (3°10'02.1"N 101°33'26.3"E). The seedlings were designated for sampling by cut back, manured following common practice in MRB's recommendation.

The seedlings were pruned and treated with fungicide two weeks prior to sampling. Bronze-stage young shoots were harvested and brought to the laboratory for sample preparation. The shoots were cleaned under running tap water and surface sterilized with 70% (v/v) alcohol with two drops of Teepol for 1 min. The material was then rinsed with distilled water, followed by 100% commercial bleach (Clorox) with two drops of Teepol for 20 min. The explants were then rinsed three times with distilled water, followed by washing them in sterile water using an ozone sterilizer at ozone output M 0.065 for 15 min.

The leaves were aseptically cut into a small disk using a sterile dissecting knife inside the sterilized laminar flow (Figure 1). The explants were inoculated onto 17 types of woody plant media (Table 1). The media were selected based on successful media used in meristem culture research carried out earlier (Nor Mayati and Jamnah, 2014). They comprise a series of modified WPM (Llyod and McCown, 1980; Minh and Thu, 2001) and DKW (Driver and Kuniyuki, 1984). The culture plates

containing the shoot explants were kept in the darkroom at 25-27 °C and monitored for 45 days before being transferred to differentiation media to induce embryos.

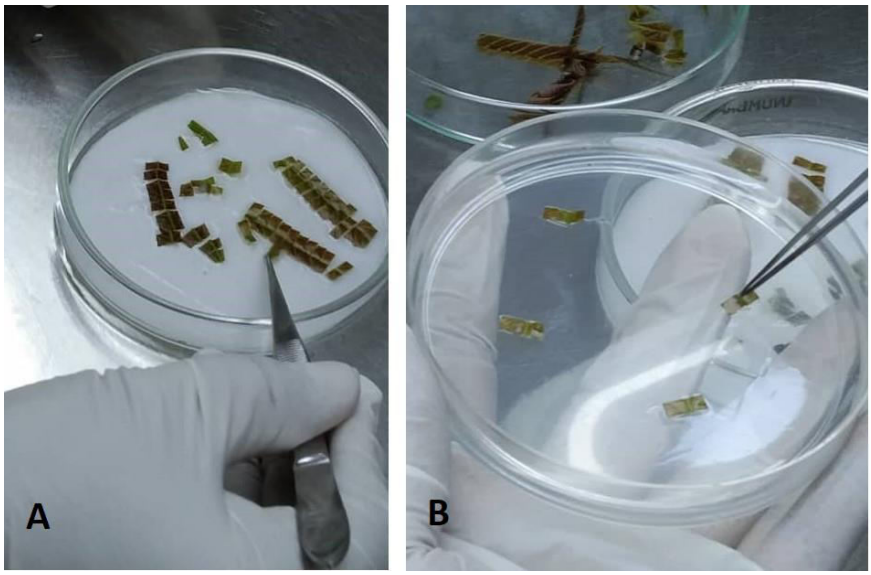

Figure 1: Bronze stage shoot explant: (A) cut in smaller disk, and (B) inoculated on woody plant media.

Table 1: Woody plant media tested for vegetative cell culture of *H. brasiliensis* clone RRIM 3001.

Reference	Media	Media Component
Modified Woody Plant Media (Minh and Thu, 2001)	MS(ID)Z -Control	MS + 0.5 mg/L zeatin
	WPM A	0.1 mg/L BA + 0.5 mg/L 2-4-D + 10 % coconut water (cw) + 7 % sucrose
	WPM B	0.1 mg/L BA + 0.1 mg/L NAA + 10 % cw + 7 % sucrose
	WPM C	0.5 mg/L BA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose,
Modified Woody Plant Media (Llyod and McCown, 1980)	WPM D	0.1 mg/L BA + 0.1 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10% CW + 7% sucrose
	WPM E	0.2 mg/L BA + 0.1 mg/L NAA + 0.5 mg/L 2-4-D + 10 % coconut water (cw) + 7 % sucrose
	WPM F	0.4 mg/L BA + 0.1 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose
	WPM G	0.1 mg/L BA + 0.2 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose
	WPM H	0.1 mg/L BA + 0.4 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose
Modified DKW (Driver and Kuniyuki, 1984)	DKW A	0.1 mg/L BA + 0.5 mg/L 2-4-D + 10 % coconut water (cw) + 7 % sucrose
	DKW B	0.1 mg/L BA + 0.1 mg/L NAA + 10 % cw + 7 % sucrose
	DKW C	0.5 mg/L BA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose
	DKW D	0.1 mg/L BA + 0.1 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10% CW + 7% sucrose
	DKW E	0.2 mg/L BA + 0.1 mg/L NAA + 0.5 mg/L 2-4-D + 10 % coconut water (cw) + 7 % sucrose
	DKW F	0.4 mg/L BA + 0.1 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose
	DKW G	0.1 mg/L BA + 0.2 mg/L NAA + 0.5 mg/L 2,4-D +

	0.5 mg/L zeatin + 10 % cw + 7 % sucrose
DKW H	0.1 mg/L BA + 0.4 mg/L NAA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose

MS(ID)Z = Murashige and Skoog (Indian modification) + zeatin, WPM = Woody Plant Media, DKW = Driver and Kuniyuki media, BA = Abscisic acid, 2,4-D = 2,4-Dichlorophenoxyacetic acid, NAA = Naphthalene acetic acid.

Results and Discussion

The tissue culture study of RRIM 3001 began in 2010, and success was later achieved in 2013 [callogenesis (47%); embryogenesis (9%); 3 plantlets], and 2016 [callogenesis (43%); embryogenesis (22%); 8 plantlets] (Nor Mayati and Izilawati, 2017; Nor Mayati, 2020). Calluses that were embryogenic and potentially complete regeneration were obtained mostly from differentiated cells using control RD1 (0.5 mg/L zeatin), RD1-E1 (0.3 mg/L kinetin), and RD1-E2 (0.8 mg/L kinetin) (Nor Mayati and Izilawati, 2017).

The vegetative leaf cultures were redeveloped using 17 woody plant media above. Three experiments were carried out in 2020 for 1377 explants producing self-contained calluses at an average of 35% with 53% contamination rate (Table 2). The callogenesis was recorded below 50% in most of the media tested except for WPM-D (70.7%) that significant (P = 0.0561). The contamination rate was also observed high (Table 2). The differentiation using RD1-C1 and RD1-E1 induced moderate embryogenesis rate at > 70% but did not significant (P = 0.4079), indicating that both C1 (containing 0.5 mg/L zeatin) and E1 (containing 0.8 mg/L kinetin) are potentially produce a promising regeneration of leave culture of RRIM3001 (Table 2). An attempt to culture leaves successfully induced rooted embryos for this clone (Figure 2).

Table 2: Callogenesis and contamination rate of RRIM3001 leave culture carried out in the year 2020.

Experiment		Callogenesis rate (%)	Contamination rate (%)
	TCIV 1/20	47.29^{a}	70.12^{a}
Leave culture	TCIV 2/20	33.12^{b}	49.71^{b}
	TCIV 3/20	23.77^{b}	37.71^{b}
CV		33.2	
F-test probability		<.0001	
$LSD_{0.05\ (n=17)}$		10.4	12.18
Media			
MS(ID)Z	control	37.00^{b}	59.33^{ab}
WPM	A	38.00^{bc}	48.33^{b}
	B	40.67^{bc}	48.33^{b}
	C	45.67^{b}	63.33^{ab}
	D	70.67^{a}	81.67^{a}
	E	31.00^{bc}	37.00^{b}
	F	26.00^{bc}	44.67^{b}
	G	27.00^{bc}	48.00^{b}
	H	25.00^{bc}	40.33^{b}
DKW	A	37.00^{bc}	59.33^{ab}
	B	20.00^{c}	44.33^{b}
	C	33.33^{bc}	51.67^{b}
	D	28.33^{bc}	55.67^{ab}
	E	36.00^{bc}	51.67^{b}
	F	32.00^{bc}	55.33^{ab}
	G	43.00^{bc}	59.33^{ab}
	H	19.67^{c}	44.33^{b}
F-test probability		0.0561	
$LSD_{0.05\ (n=3)}$		24.76	28.99

MS(ID)Z = Murashige and Skoog (Indian modification) + zeatin, WPM = Woody Plant Media, DKW = Driver and Kuniyuki media.

Table 3: Embryogenesis rate of RRIM3001 leave culture carried out in the year 2020.

Experiment		Callogenesis rate (%)
Leave culture	TCIV 1/20	86.79[a]
	TCIV 2/20	79.91[a]
	TCIV 3/20	52.94[b]
CV		53
F-test probability		0.0015
$LSD_{0.05\ (n=34)}$		18.95
Differentiation Media		Embryogenesis rate (%)
RD1	C1	74.52[a]
	E1	71.91[a]
F-test probability		0.4079
$LSD_{0.05\ (n=51)}$		45.11

CV = Coefficient of variation, LSD = Least Significant Difference.

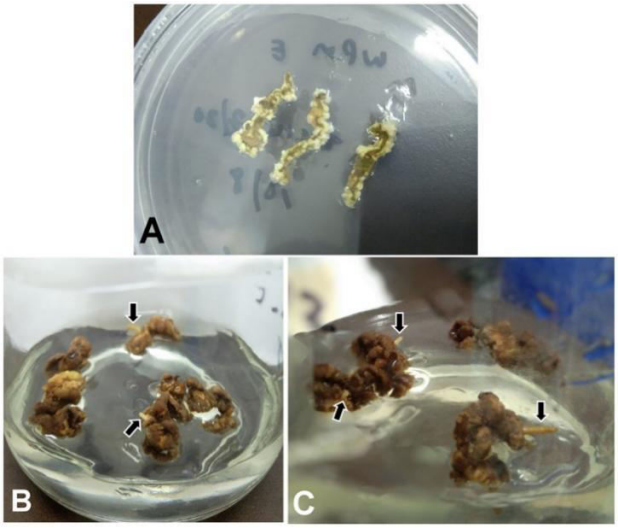

Figure 2: (A) Callogenesis of RRIM 3001 clone leaf culture on woody plant media. (B and C) Rooted embryos resulted for TCIV 1/20 initiated on WPM-C media and differentiated on RD1-C1 differential media (indicated by arrows).

Further, the study was repeated in April 2021 (1206 shoot explants) and October 2021 (234 shoot explants), producing self-contained calluses at an average of 84% and a contamination rate at an average of 21% (Table 4). Media tested included WPMA (0.1 mg/L BA + 0.5 mg/L 2-4-D + 10 % coconut water (cw) + 7 % sucrose), WPMB (0.1 mg/L BA + 0.1 mg/L NAA + 10 % cw + 7 % sucrose), and WPMC (0.5 mg/L BA + 0.5 mg/L 2,4-D + 0.5 mg/L zeatin + 10 % cw + 7 % sucrose). WPMA and WPMC gave the highest callus survival potential (P = 0.0208) and the lowest contamination rate (P = 0.0227). These findings indicate that the presence of 2-4-D produces the highest callus and performance is maintained although slightly decreased with the presence of zeatin. Zeatin is expected to be able to produce embryogenic calluses that have the potential to develop into trees at a later stage.

Table 4: Mean % survival survived and contamination rate of RRIM 3001 leaves culture in 2021.

Media	% Survival Callus	% Contamination
WPMA	91.67[a]	11.00[b]
WPMB	73.67[b]	35.83[a]
WPMC	87.50[a]	16.50[b]
F-test probability	0.0208	0.0227
$LSD0.05_{(n=6)}$	12.28	17.28
CV	11.32	63.61

WPM = Woody Plant Media, CV = Coefficient of Variation.

Acknowledgment

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Shoot Multiplication of Red Ginger, *Zingiber officinale* var. *rubrum* using Rhizome Buds as Explant

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Introduction

Zingiber officinale Roscoe, or ginger, is a member of the Zingiberaceae family. It is an important spice crop used in various culinary and medical preparations and is widely used in Asia. Based on its size, colour of the rhizome, and chemical constituents, *Z. officinale* is further classified into three varieties: big white or giant ginger (*Z. officinale* var. *officinale*), small white ginger (*Z. officinale* var. *amarum*) and small red ginger (*Z. officinale* var. *rubrum*) (Supu et al., 2018). Compared to other *Zingiber officinale* species, the base of the leaf shoot of red ginger is red, with a yellow to pink cross section on the outside of the rhizomes. It is an annual plant that reaches a height of 50 to 100 cm. The rhizomes are thick and reddish-brown in colour. It morphologically resembles common ginger morphologically. The leaves are small, lance-shaped, and 5-25 cm long by 8-20 mm wide. The plant has an ovoid-shaped composite that develops from the rhizomes, a stem length of 10-25 cm, and tiny leaves at the base of the bloom. The red ginger has 2.0-2.5 cm long funnel-shaped, dark purple corollas with creamy golden dots on them. The petals are tridentate, tubular, and small. Unlike normal ginger, it has a reddish petiole and a scarlet red lip (Sivasothy et al., 2011).

Aside from morphology, the chemical constituent of red ginger has a distinct value that aids in the classification of *Z. officinale*. The uses of the *Z. officinale* varieties vary depending on their chemical constituent content. Essential oils, which give rise to the gingery odour and taste (shogaols and gingerol) were found in higher concentrations in red ginger than in the other two varieties. These two compounds, compared to those in common ginger, caused red ginger to have a pungent or stronger smell. Previous studies reported that red ginger contains about 169 chemical constituents, and red ginger has cytotoxic, antibacterial, antihypertensive, antihyperlipidemic, and immunomodulatory properties; these biological activities are the underlying causes of the red ginger therapeutic benefits (Zhang et al., 2022).

Like other *Z. officinale* species, the red ginger is vegetatively propagated by utilising underground rhizome buds; however, this method is limited depending on the number of rhizome buds that emerge, which is considerably low in a year. Therefore, an alternative propagation process, tissue culture, is required to produce a large quantity of high-quality planting materials for commercial use. However, the success of tissue culture is influenced by several factors, such as plant species, explant selection, culture medium, growth regulator, and culture conditions (Sathyagowri and Seran, 2013). The processes for developing a successful tissue culture protocol include surface sterilization, shoot multiplication, rooting induction, and acclimatisation.

In this study, red ginger rhizome buds were used as explant sources for tissue culture establishment. Two types of basal culture media with different ranges of benzylaminopurine (BAP) were used to determine their effects on shoot production and multiplications. The selected culture media will be used for multiple plantlet production for later experiments on rooting induction and acclimatisation.

Materials and Methods

Surface sterilisation of red ginger

Young nodes from the red ginger rhizome were used as a sample source for tissue culture establishment. The red ginger rhizomes were washed under tap water and then incubated for a few days on the wetted Whatman papers, which were placed in petri dishes at room temperature to induce the new bud. The rhizomes were sprayed with distilled water to maintain moisture. The freshly emerged rhizomes (later referred to as explants) were removed after nearly two weeks and surface-sterilized using sterilising solutions to produce clean cultures. The explants were washed under running tap water to remove any macro-contaminants on the surface. Later, in a clean working environment in laminar flow, the explants were further cleansed with distilled water and a surfactant, Tween 20, for 10 minutes to thoroughly remove the macro-contaminant.

The explants were soaked in fungicide for 1 hour, followed by 50% (v/v) ethanol for 1 minute. The double-sterilisation method was conducted to remove the micro-contaminants with different concentrations of Clorox®, which are 50% v/v (2.6% Sodium hypochlorite, NaOCl) and 20% v/v (1% NaOCl). The explants were first soaked in 50% (v/v) Clorox for 25 minutes, excised into smaller pieces, leaving the bud area of around 1 cm^2, before being soaked again in Clorox® at a concentration of 20% (v/v) for 10 minutes. The samples were left to dry in laminar flow for approximately 3 hours prior to culture on Murashige and Skoog (MS) media supplemented with 0.5 mg/L BAP for shoot induction.

Shoot multiplication

Two basal media, MS and Gamborg B5, were prepared and supplemented individually with a range of BAP at a concentration of 0, 0.5, 1.0, 2.5, and 5.0 mg/L. In all media, 30% (w/v) of sucrose has been added as a carbon source and 0.3% (w/v) of gelrite has been added as a solidifying agent. All media were set at pH 5.8 prior to autoclaving. In the MS basal media containing 0.5 mg/L BAP, the initial rhizome buds' shoots were multiplied to create sufficient explants for the shoot multiplication experiment. Next, shoots approximately 2 cm long from the basal, with all leaves excised were cultured on the experimental medium. Each medium was cultured with four explants and was repeated six times. All cultures were incubated in a growth room with 16/8 h light and a temperature set at 22±2 ^{o}C for 8 weeks. The new shoot growth, height, and condition were observed on the 6^{th} and 8^{th} weeks. Contaminated cultures were recorded and considered as missing values.

Statistical analysis

Duncan's Multiple Range test (DMRT) was performed when significant differences among treatments were detected by analysis of variance (ANOVA). The analyses were performed with SAS version 9.1.2 (SAS Institute Inc., Cary, NC, 2000).

Results and Discussion

Surface sterilisation of red ginger

Surface sterilisation of the red ginger was successfully achieved with 50% of clean cultures, and 30% of the cleaned rhizome buds were considered viable when shoot growth was observed after 1 week. After 3 weeks, the red ginger culture showed no signs of bacterial contamination or fungus growth. Figure 1 shows the clean culture of red ginger and the new shoots emerging from the rhizome bud after 3 weeks of surface sterilization. According to past research, several surface sterilisation methods have been implemented to obtain clean cultures of rhizome-type explants. Given the natural habitat of the rhizome, which is in the soil, the soil microorganisms highly adhere to the surface of the rhizome. Hence, the difficulty of sterilisation is much greater compared to shoot tips or nodal segments. In a

study on other *Zingiber* spp. with a range of NaOCl concentrations, 2-5% (50% - 100% Clorox®) produced more than 70% clean culture after 4 weeks, and the survivability dropped from low to higher NaOCl concentrations (Khatun et al., 2016). Even though the percentage of clean culture was increased, higher NaOCl concentrations (an active ingredient in Clorox®) resulted in fewer viable rhizome buds.

The new shoots grew, and the basal thickened and turned reddish colour (Figure 2A). During the first few subcultures, the new reddish thickened basal were excised and separated into smaller pieces. These new pieces later grew into new shoots, as shown in Figures 2.

Figure 1: New shoot emerged from *Z. officinale* var. *rubrum* rhizome bud after surface sterilization.

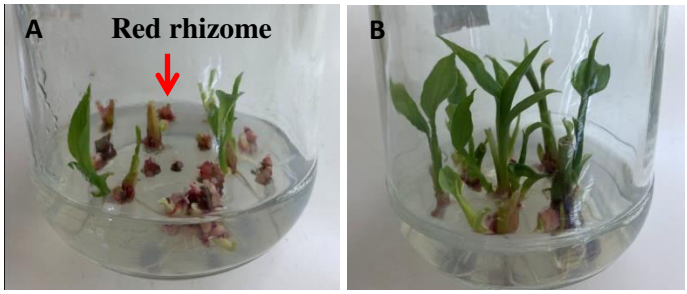

Figure 2: (A) New shoot emerged from *Z. officinale* var. *rubrum* rhizomes after 1-2 weeks of culture. (B) New shoots with leaves multiplied after 4 weeks of culture.

Shoot multiplication

Two basal media, MS and B5, have been used for the red ginger shoot's multiplication and growth. The number of new shoots produced after 8 weeks and their height were shown in Table 1. Statistical analysis indicated that there were significant differences between the means of the number of shoots and average shoot height in B5, but only in the average shoot height in MS. According to the results, both MS and B5 basal media supplemented with BAP positively induced the shoot multiplication of red ginger at 0.5 and 1.0 mg/L BAP. The number of new shoots increased with the increment of BAP until 1.0 mg/L and decreased thereafter. However, the number of new shoots was nearly similar in both MS and B5 at 1.0 mg/L BAP, which were 5.28 and 5.33 new shoots per explant, respectively (Table 1). The main difference between MS and B5 media is that the B5 medium has a lower NH_4^+/NO_3^- ratio (Russowski et al., 2006). However, this difference seems to have no significant effect on the shoot multiplication of red ginger. The shoot elongation, however, was better in B5 media, where the shoot height increased with the increasing concentration of BAP until 2.5 mg/L and then decreased at 5.0 mg/L.

In the MS media supplemented with BAP, the shoot growth was stunted and the shoot height was lower than the control (Table 1). Shorter shoots, fewer shoots, and abnormalities have been associated with increased BAP concentrations in *in vitro* plant culture (Jafari et al., 2011; Sathyagowri and Seran,

2011). However, BAP supplementation increased shoot growth in the Gamborg B5 medium to the greatest extent (2.67±0.21[a] in 2.5 mg/L BAP) and was significantly different from the BAP-free medium. This is probably due to the interaction effects between different levels of ammonium in different basal media types and cytokinin concentration. According to previous studies, there are interaction effects between basal media and cytokinin concentrations. The addition of high cytokinin concentrations to higher ammonium levels in the medium caused hyperdydricity in the shoot and reduced normal shoot production (Ivanova and Van Staden, 2008; Zahid et al., 2021). Because the ammonium concentration in MS medium is higher than in Gamborg B5, high concentrations of BAP reduced shoot height but not in Gamborg B5, where the shoots height increased with increased BAP.

Figure 3 shows the shoot multiplication of *Z. officinale* var. *rubrum* in MS and Gamborg, B5 basal media with different BAP, ranging from 0-5.0 mg/L concentration after 8 weeks. In this study, roots were grown from all shoot clumps in MS and B5 media at all concentrations with at least 2 roots per shoot clump, indicating the red ginger has no rooting problem in culture media.

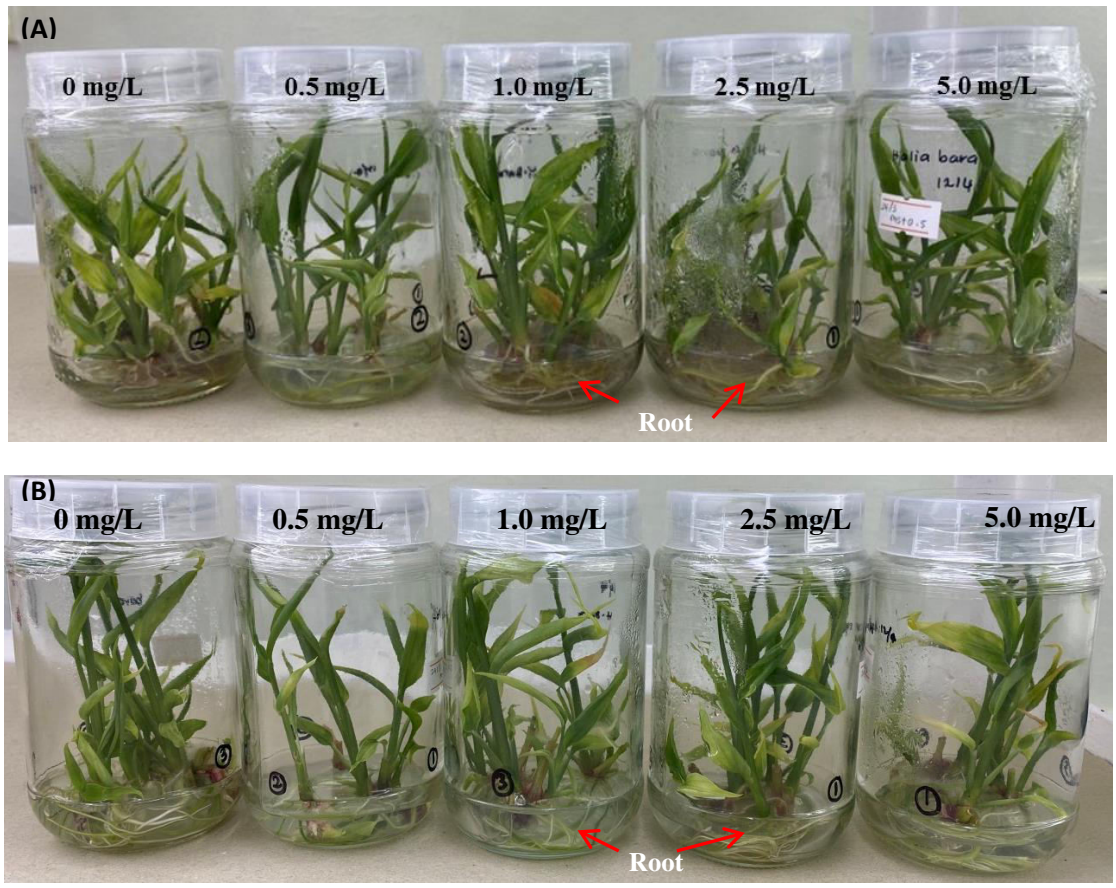

Figure 3: Shoot multiplication of red ginger, *Z. officinale* var. *rubrum* in (A) MS and (B) Gamborg B5 basal media with different BAP concentrations after 8 weeks in culture.

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Table 1: Effect of different basal media and BAP concentration on the shoot multiplication of red ginger, *Z. officinale* var. *rubrum* after 8 weeks in culture.

	MS basal media				Gamborg, B5 basal media			
BAP (mg/L)	Percentage of shoot multiplied (%)	No. of micro shoot per explant	Range of new shoot height (cm)	Average shoot height (cm)*	Percentage of shoot multiplied (%)	No. of micro shoot per explant*	Range of new shoot height (cm)	Average shoot height (cm)**
0	94.4	4.11± 0.37^{a}	1.0-6.0	2.18± 0.26^{a}	100	4.40± 0.29^{bc}	2.0-6.0	2.27± 0.14^{bc}
0.5	94.1	4.76± 0.48^{a}	0.4-6.0	1.86± 0.17^{ab}	91.67	4.58± 0.30^{ab}	1.0-3.5	1.84± 0.16^{c}
1.0	94.4	5.28± 0.50^{a}	1.0-5.0	1.56± 0.13^{b}	100	5.33± 0.28^{a}	1.0-4.5	2.45± 0.14^{ab}
2.5	100	4.40± 0.38^{a}	0.7-4.0	1.55± 0.16^{b}	100	3.92± 0.31^{c}	1.0-6.0	2.67± 0.21^{a}
5.0	88.9	3.94± 0.49^{a}	1.0-3.0	1.54± 0.13^{b}	100	4.53± 0.26^{bc}	1.0-4.5	2.09± 0.11^{bc}

Each value presented in this table is the mean of 6 replicates. Different letters within the column indicate significant differences at $p \leq 0.05$(*) and $p \leq 0.01$ (**) levels according to DMRT.

Conclusions

The establishment of red ginger tissue culture has been successfully obtained with 50% clean and 30% viable cultures. The shoot multiplication experiment has also indicated that both Murashige and Skoog (MS) and Gamborg B5 basal media supplemented with 1.0 mg/L BAP are suitable for red ginger shoot multiplication. These media can be used for shoot multiplication of red ginger and to produce a sufficient number of plantlets for *in vitro* rooting experiments, as well as for acclimatisation of red ginger. Later, the establishment of complete and hardened tissue culture plantlets of red ginger will be required to meet commercial needs.

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High Throughput Stock Plant *In vitro* in *Labisia pumila* var. *alata* from Nodal Explant using Thidiazuron

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Introduction

Labisia pumila (locally known as Kacip Fatimah) is an herbaceous plant that has been extensively used in south East countries as a health tonic for the maintenance of women's health as it is good for their reproductive organ (Nik Hussain and Kadir, 2013). It is a member of genus *Labisia* in the family Myrsinaceae (Muhammad et al., 2019). The phytoestrogenic properties of Kacip Fatimah have led to its wide medicinal and pharmacological users. In Malaysia, Kacip Fatimah is popularly known as a traditional herbal medicine to facilitate and ease childbirth and vital for post-partum care as it promotes recovery after childbirth by regaining body strength (Hairi et al., 2016). Moreover, it is generally used by women to improve fertility and maintaining their appearance as this plant can function as an anti-aging agent by promoting skin collagen synthesis (Chua et al., 2012; Nik Hussain and Kadir, 2013). Also, it is highly beneficial for relieving and reducing menopausal symptoms in women having hormonal imbalance. This medicinal plant also possesses antioxidant and anti-inflammatory activities that can reduce the risk of estrogen-deficient disorders such as cardiovascular diseases and osteoporosis in menopausal women. This is possible as this plant exhibits high contents of phenolic compounds including flavonoids and phenolic acid which can be used to treat oxidative stress-related disease (Nik Hussain and Kadir 2013; Zakaria et al., 2021). The advancement of biotechnology in Malaysia has revealed the benefit of *L. pumila* in a variety of applications and more demand for the raw material and may cause insufficient supply of local raw materials to feed the growing industry. Over the last few decades, micropropagation of *L. pumila* has been mostly achieved. Due to the diversity of the methods and application of available culture techniques, plant tissue culture of *L. pumila* is extensively covered in the existing literature. However, an improvement of the existing technique is needed to optimize the growth performances of *L. pumila* under *in vitro*. Other than improvements of the plant growth and development for production purposes, good management of plant stock *in vitro* is as crucial whereby a mass amount of stock plant is needed to be kept in the laboratory to ensure plant production consistency. The present study aimed to perform a technique for efficient plant stock on a large scale to ensure sustainable supply of *L. pumila* var. *alata.* However, it was not always convenient as plants have its maturity and subjected to subculture at specific timeline otherwise the performances of plants will decrease and may not be true-to-type as the selected starting material. Therefore, it is important to have a system where the plants can be kept *in vitro* for a longer period of time per cycle particularly for selected or superior plants and to be in control of the production.

Materials and Methods

Shoot proliferation and elongation

Nodal segments of *L. pumila* var. *alata* (clones kf12, kf13, Kf14, kf22 and kf23) obtained from the laboratory at the Center for Biotechnology Bioenterpreneur, FRIM used as explants. These nodes were cultured in petri dish containing Murashige and Skoog, 1962 (MS) for macro and micro, modified MS vitamins supplemented with different concentrations of thidiazuron, TDZ (1-6 mg/L) for shoot induction. The observations of callus and shoot formation were recorded after 8 months in culture followed by transferring into MS hormone-free medium. After 8 months, cultures were transferred from Petri dishes into bottles containing MS hormone-free medium and kept for 7 months under control conditions *in vitro.*

Statistical analysis

Mean value of shoot elongation under the influence of TDZ were determined followed by ANOVA and Tukey's analyses for comparison of mean number of elongated shoots (i) among the six treatment and (ii) between six treatment groups for each clone (n=540).

Results and Discussion

The TDZ hormone was found to have positive effect on shoot induction in *L. pumila* var. *alata*. Further investigation found that the performance is clone-dependent whereby each clone responded differently towards TDZ. Observation showed the number of shoots primordial decreased as the concentration of TDZ increased in all clones. Treatment in 1 mg/L of TDZ was reported to be the best treatment which acted as a vigorous stimulant towards *L. pumila* var. *alata* nodes resulting in a maximum number of shoots primordial (per cm^2 area) (Figure 1-5). However, the treatments inhibit stem development which affected plant heights due to nodal segments developed closely together (Figure 6). The biggest callus was observed in clone Kf23 treated with MS medium containing 1 mg/L TDZ whereas clone Kf13 produced relatively smaller callus compared to the other clones. Kf23 produced a good quality of callus for shoot proliferation as it resulted in maximum number of shoot development (Table 1). Based on Table 2, the mean number of elongated shoots for Kf12 and Kf22 were not significantly different similar average number of elongated shoots per explants. On the other hand, different responses were observed in Kf13, Kf14 and Kf23 whereby the mean number of shoots were significantly different as different TDZ concentrations induced different numbers of shoots per explant for each clone (Table 2). These gave an indication that effect of varying concentration of TDZ on shoot proliferation and elongation dependent on the type of the clone. For example, clone Kf13 only produced shoots at 6 mg/L TDZ while Kf23 provided the highest shoot proliferation and elongation compared to other clones (Table 1).

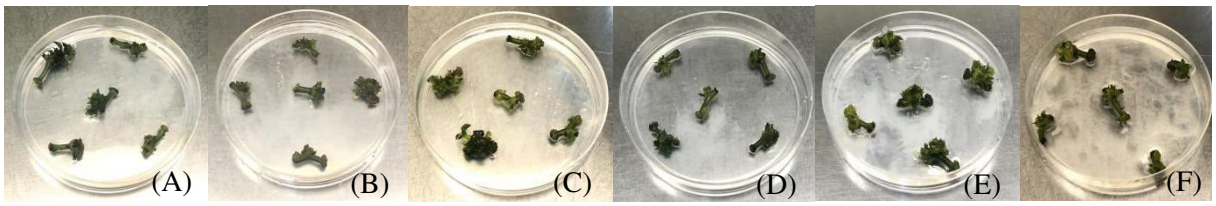

Figure 1: The shoot primordial development in *L. pumila* var. *alata* nodes (clone Kf12) in MS medium supplemented with TDZ: (A) 1 mg/L; (B) 2 mg/L; (C) 3 mg/L; (D) 4 mg/L; (E) 5 mg/L; (F) 6 mg/L.

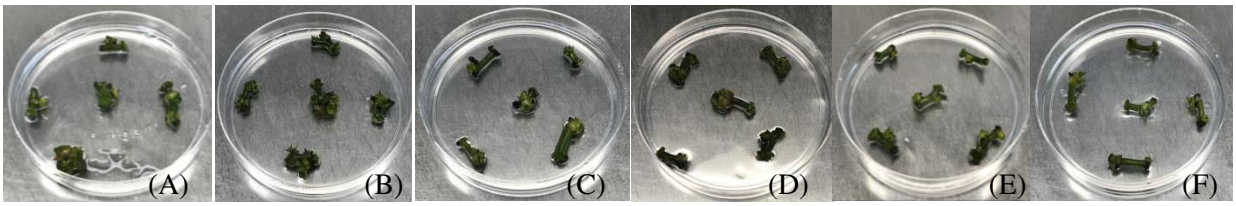

Figure 2: The shoot primordial development in *L. pumila* var. *alata* nodes (clone Kf13) in MS medium supplemented with TDZ: (A) 1 mg/L; (B) 2 mg/L; (C) 3 mg/L; (D) 4 mg/L; (E) 5 mg/L; (F) 6 mg/L.

After transfer into TDZ hormone-free medium, shoot elongation was observed with Kf23 gives the highest number of shoots (9.2 shoots/node) followed by Kf22 (6.5 shoots/node) that are treated previously in 5 mg/L TDZ and 3 mg/L TDZ, respectively. Further observation showed 6.0 mg/L TDZ produced biggest size of shoot primordial whilst 1.0 mg/L TDZ produced smaller shoot primordial, yielding in lower number of elongated shoot.

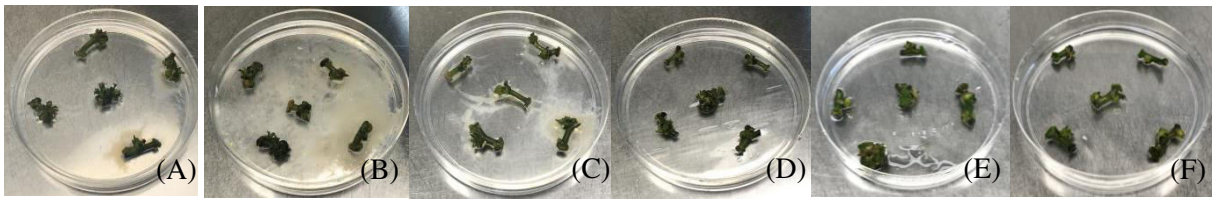

Figure 3: The shoot primordial development in *L. pumila* var. *alata* nodes (clone Kf14) in MS medium supplemented with TDZ: (A) 1 mg/L; (B) 2 mg/L; (C) 3 mg/L; (D) 4 mg/L; (E) 5 mg/L; (F) 6 mg/L.

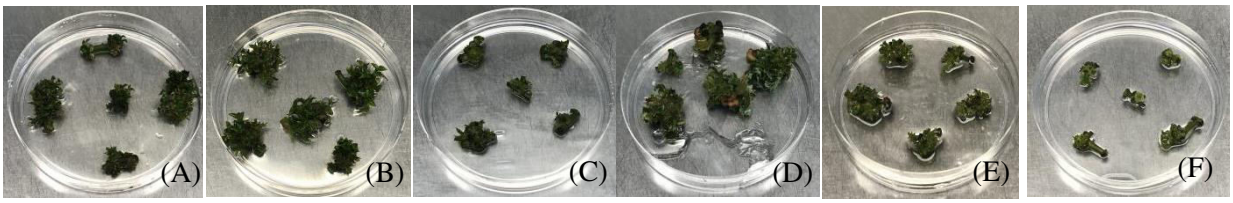

Figure 4: The shoot primordial development in *L. pumila* var. *alata* nodes (clone Kf22) in MS medium supplemented with TDZ: (A) 1 mg/L; (B) 2 mg/L; (C) 3 mg/L; (D) 4 mg/L; (E) 5 mg/L; (F) 6 mg/L.

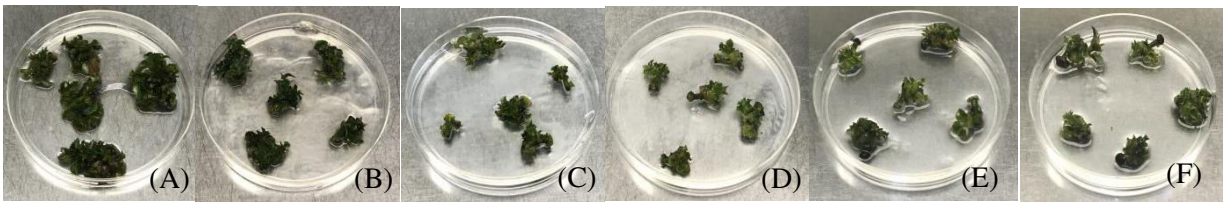

Figure 5: The shoot primordial development in *L. pumila* var. *alata* nodes (clone Kf23) in MS medium supplemented with TDZ: (A) 1 mg/L; (B) 2 mg/L; (C) 3 mg/L; (D) 4 mg/L; (E) 5 mg/L; (F) 6 mg/L.

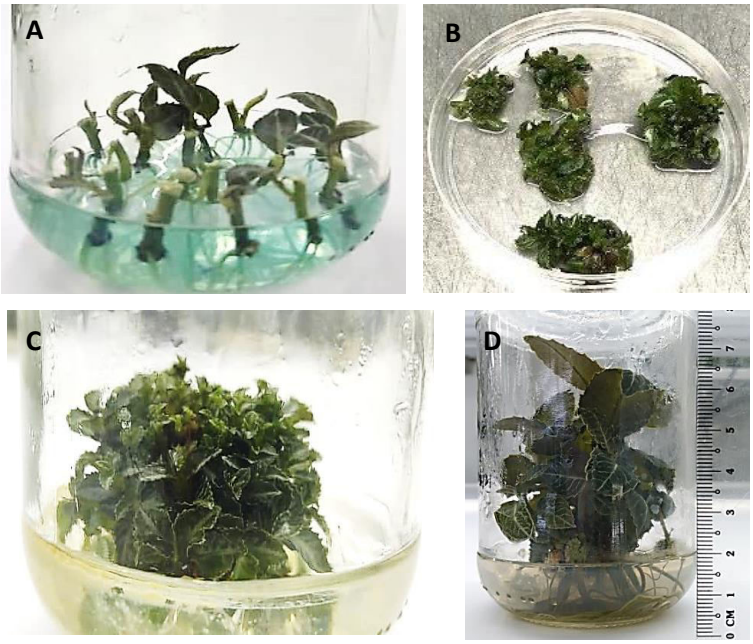

Figure 6: Eight months old culture in *L. pumila* var. *alata* culture in (A) MS medium without TDZ (single shoot per node), (B) with TDZ (high throughput shoot proliferation), (C) shoot elongation initiation occur at minimal rate after 2 months and (D) 7 months in hormone-free medium.

Table 1: Comparison of mean number of elongated shoots among six treatment groups for each clone (n=540).

Clone	TDZ concentration (mg/L)	Mean no of elongated shoots	F-statistic (df)	p-value
Kf12	1	2.47±2.99	1.63 (5)	0.183*
	2			
	3			
	4			
	5			
	6			
Kf13	1	1.39±2.25	2.80 (5)	0.034
	2			
	3			
	4			
	5			
	6			
Kf14	1	2.33±2.55	1.19 (5)	0.339*
	2			
	3			
	4			
	5			
	6			
Kf22	1	4.53±3.33	1.40 (5)	0.253*
	2			
	3			
	4			
	5			
	6			
Kf23	1	6.31±3.86	2.81 (5)	0.034
	2			
	3			
	4			
	5			
	6			

Tukey's multiple comparison tests were inapplicable as the means were not statistically significant.

Table 2: Comparison of mean number of elongated shoots between six treatment groups for each clone of the *L. pumila* var. *alata*.

Clone	Comparison of treatment	Mean difference (95% CI)	p<value
Kf13	TDZ 1 vs TDZ 2	-1.17 (-4.68, 2.35)	0.911
	TDZ 1 vs TDZ 3	0.00 (-3.52, 3.52)	1.000
	TDZ 1 vs TDZ 4	1.50 (-2.02, 5.02)	0.784
	TDZ 1 vs TDZ 5	2.17 (-1.35, 5.68)	0.437
	TDZ 1 vs TDZ 6	2.17 (-1.35, 5.68)	0.437
	TDZ 2 vs TDZ 3	1.17 (-2.35, 4.68)	0.911
	TDZ 2 vs TDZ 4	2.67 (-0.85, 6.18)	0.223
	TDZ 2 vs TDZ 5	3.33 (-0.18, 6.85)	0.071
	TDZ 2 vs TDZ 6	3.33 (-0.18, 6.85)	0.071
	TDZ 3 vs TDZ 4	1.50 (-2.02, 5.02)	0.784
	TDZ 3 vs TDZ 5	2.17 (-1.35, 5.68)	0.437
	TDZ 3 vs TDZ 6	2.17 (-1.35, 5.68)	0.437
	TDZ 4 vs TDZ 5	0.67 (-2.85, 4.18)	0.992
	TDZ 4 vs TSZ 6	0.67 (-2.85, 4.18)	0.992
	TDZ 5 vs TDZ 6	0.00 (-3.52, 3.52)	1.000
BKF 2/3	TDZ 1 vs TDZ 2	2.17 (-3.88, 8.21)	0.881
	TDZ 1 vs TDZ 3	-0.50 (-6.54, 5.54)	1.000
	TDZ 1 vs TDZ 4	4.33 (-1.71, 10.38)	0.276
	TDZ 1 vs TDZ 5	-2.33 (-8.38, 3.71)	0.845
	TDZ 1 vs TDZ 6	-0.50 (-6.54, 5.54)	1.000

TDZ 2 vs TDZ 3	-2.67 (-8.71, 3.38)	0.760
TDZ 2 vs TDZ 4	2.17 (-3.88, 8.21)	0.881
TDZ 2 vs TDZ 5	-4.50 (-10.54, 1.54)	0.239
TDZ 2 vs TDZ 6	-2.67 (-8.71-, 3.38)	0.760
TDZ 3 vs TDZ 4	4.83 (-1.21, 10.88)	0.177
TDZ 3 vs TDZ 5	-1.83 (-7.88, 4.21)	0.937
TDZ 3 vs TDZ 6	0.00 (-6.04, 6.04)	1.000
TDZ 4 vs TDZ 5	-6.67 (-12.71, -0.62)	0.024*
TDZ 4 vs TSZ 6	-4.83 (-10.88, 1.21)	0.177
TDZ 5 vs TDZ 6	1.83 (-4.21, 7.88)	0.937

ANOVA test was applied followed by Tukey's multiple comparison tests. Mean was significantly different at $p<0.05$ by Tukey's multiple comparison tests.

Conclusion

The application of TDZ may be used for the mass number of shoots/nodes in slow phase development which provides an alternative mode to fully utilized laboratory space and capacity which is suitable for plant stock purposes.

Acknowledgments

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In vitro Propagation of KLL 092, a Tectariaceae, from Spores

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Introduction

The traditional therapeutic applications of ferns have been passed down verbally from generation to generation for centuries or documented in old pharmacopeia books. Using ferns and allies as medicines have been around since ancient times, according to evidence gathered from many countries. Nowadays, many fern species grown throughout the world have been used to cure various ailments, especially in developing nations where herbal remedies still occupy a prominent part in primary healthcare for cultural and economic reasons (Ho et al., 2011). Tectariacea is one of the largest fern genera with the most confusing fern genus regarding its phylogeny and morphology. The fern genera are estimated to contain 210 species across Africa, the Americas, Asia, and the islands of the Indian and Pacific oceans (Zhang and Zhang, 2017). In Malaysia, the indigenous and the elderly still possess traditional knowledge. One species from the Tectariaceae family is known to have a high potential for medicinal use. KLL 092 is a code name given to the species since the species cannot be disclosed for specific reasons. The plant extract has been shown in scientific research to have the potential as an antidiabetic agent. An assessment of its safety level revealed that the standard extract KLL092 had no acute adverse effects (Firdaus et al., 2016; Firdaus et al., 2018). As the usefulness of this species is demonstrated, market interest in it will grow. Sustainable plantlet production must be carried out to avoid dependence on resources from the forest alone. Efforts to produce KLL092 plantlets through *in vitro* propagation have been initiated. For fern species, spores, and rhizomes can be used for initiation in a tissue culture medium. For this study, spores were used as a sample source.

Materials and Methods

Plant material

Mature leaves with clusters of sporangia at the back were received from the sample collection team, which consisted of a botanist and representatives from the Kensiu people. The spores were collected into test tubes using a spatula. Sterilized distilled water is used to clean the spores by a series of washes in the laminar flow. Due to its small size, the sterilization process requires Whatman paper for filtration to avoid samples being washed out. Sterilization solutions, such as 100% ethanol and Clorox® at 50% concentration, were used at 1- and 15 min exposure durations, respectively. Tween 20, a surfactant, was added into each sterilization process to increase the percentage of wet sample surface. After being rinsed with sterile distilled water, the samples were left to dry in laminar air flow for 2 hours before being cultured in Murashige and Skoog (MS) culture media without hormones.

Spore germination, prothalli propagation, and induction of young sporophyte production

After 3 months, germinated spores were transferred into a new fresh medium, MS-free hormone, and Woody Plant Medium (WPM) free hormone. Both media were supplemented with 3% (w/v) solidifying agent and 0.3% (w/v) sucrose as carbon sources. The media were autoclaved at 121 ^{o}C for 15 min. The prothalli were subcultured every 8 weeks into fresh medium. The cultures were put in slanting positions, and the positions frequently changed to facilitate the water vapor movement on the media surface.

Production of complete plantlets

KLL092 sporophytes were subcultured every 8 weeks into WPM hormone-free media to multiply and root. The rhizomes were excised into smaller clumps during subculture and cultured into new media. All cultures were kept on culture racks in light conditions (16 hours of light) at a temperature of 24±2 °C.

Acclimatization

KLL092 complete plantlets were removed from the culture media and washed under running tap water to remove the agar residual. The plantlets were soaked in 0.1% (w/v) fungicide for a few minutes. The Jiffy7® pellets were prepared by soaking in water for 15 minutes before planting the KLL092 complete plantlets. The KLL092 was kept in a weaning chamber for 1 month before being transplanted into a polybag with baked soil and peat moss (1:1).

Results and Discussion

Surface sterilization of KLL 092 sporangia using 100% (v/v) ethanol and 50% (v/v) Clorox has been successfully conducted. However, the initial trials without Whatman filter paper have led to almost all spores being washed out during a series of washings. Since the spores were small and there was lots of debris from the sorus and leaves, it was difficult to differentiate between the sporangia and the debris. Hence, all sterilized explants from the surface sterilization process were cultured in the media. Nevertheless, a 100% sterile culture has been successfully obtained. The viable spores germinated after 3 months of surface sterilization at around 50% of their germination rate, and these germinated spores were transferred to new fresh MS media to induce growth. The germinated spores grew into prothalli, which consist of female and male organs. This stage was also known as the gametophyte, where each female and male organ carried an n product. Fertilization of the gametophyte (egg and sperm) occurs spontaneously to produce embryos that will grow into young sporophytes, 2n. Water irrigation or sprinklers in the nursery has been reported to help fertilize. The water from condensation in the culture bottle is used as a mediator to facilitate sperm movement to the egg for embryo formation. In this study, the culture bottles were manually slanted twice a week to facilitate the water movement for the spores to fertilize the eggs. During the last few months, only mass propagation of prothalli was obtained without any sign of sporophyte growth. The process from surface sterilization of KLL 092 until the production of prothalli is shown in Figure 1.

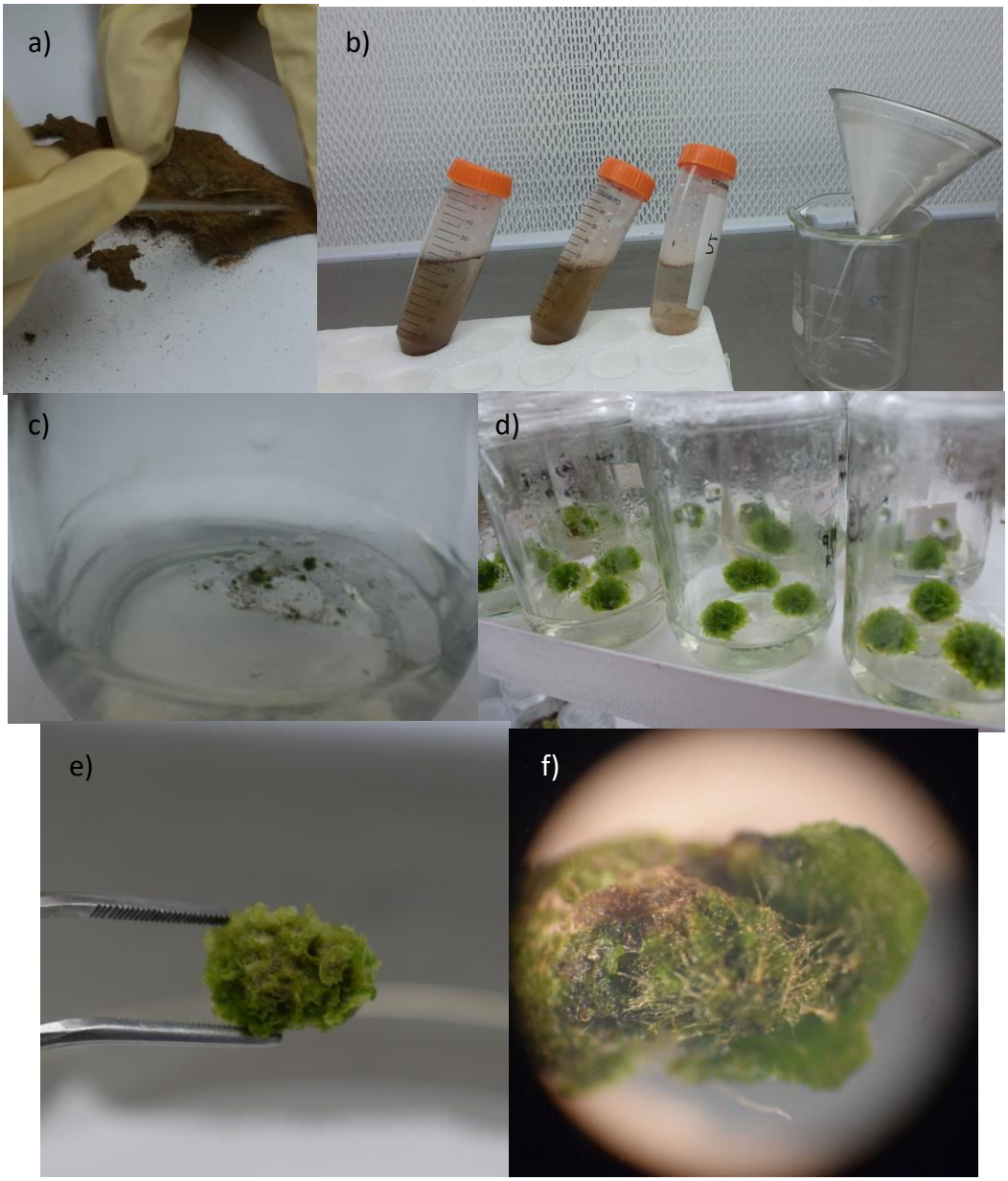

Figure 1: a) Spore extraction from sporangia located behind matured KLL 092 leaves; b) Spore surface sterilization process; c) Germinated KLL 092 spores; d) KLL 092 prothalli in WPM basal medium without hormone; e) KLL 092 prothalli under naked eye; f) KLL 092 prothallus under microscope Olympus, 10x magnification.

After 3 months, the formation of young sporophytes can be observed, indicating success in fertilization of the egg and sperm of the gametophytes. The young sporophytes were carefully subcultured into fresh media to facilitate further growth. The young sporophyte grew into more distinct features of actual leaves and roots. The WPM media basal without hormone proved suitable for the sporophytes to grow and multiply. At first, the young sporophyte turned brown, died, or showed no further growth even when cultured in the same medium. After a series of subcultures, the sporophytes become more stable and multiply without turning into prothallus or browning. In addition, a few trials on subcultures of KLL 092 sporophytes in media WPM or MS with a low concentration of 6-benzylaminopurine (BAP) have shown negative responses where the culture stunted, leaves turned brown and died. However, this study did not record these responses in a proper experimental design. In other fern cultures, the application of cytokines has shown an inhibitory effect on fern growth. BAP

and zeatin's presence significantly inhibits fern's growth, with the negative effect increasing with cytokinin concentration and exposure (Rolli et al., 2015). Even though cytokinins are well-known in many developmental processes in plants and are beneficial in promoting shoot growth and multiplication in other plants, their effects in fern culture were different.

Moreover, some reports that programmed cell death in plants was induced by high doses of BAP in several plant species, though cytokinin is generally considered an anti-sense hormone (Carimi et al., 2004; Carimi et al., 2005; Zottini et al., 2006; Rolli et al., 2015). Hence, the application of cytokinin in the culture media should be adequately studied because plant species may have different responses to the hormone. In this study, KLL 092 did not only grow and multiply well in hormone-free media but was also rooted. Complete plantlets of KLL092 were obtained using one type of culture media. Figure 2 shows the growth of an embryo into a young sporophyte in WPM basal-free media.

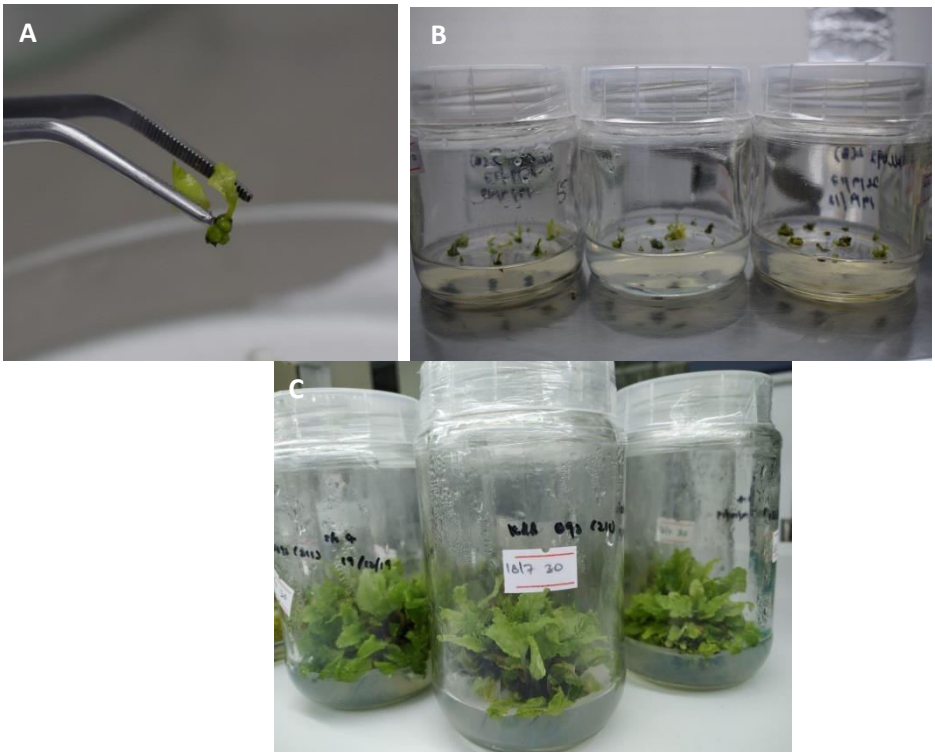

Figure 2: KLL 092-sporophytes in culture a) Embryo developing into a young sporophyte (2n), b) Young sporophyte subculture into new fresh media and c) Adult sporophyte in WPM basal culture media.

After complete plantlets were obtained, the sporophytes were acclimatized in the weaning chamber using Jiffy7® as media. Figure 3 shows the acclimatization process of KLL 092 complete plantlets. For 1 month, the KLL 092 plantlets were acclimatized with a 100% survival rate. The surviving plantlets were transferred into a polybag with a mixture of peat moss and baked soil (1:1). The surviving plantlets were kept on the bench with a watering routine twice daily. In this study, the acclimatization of KLL092 during the prothalli stage was also tested. After 1 month, the prothalli survived and were removed from the weaning chamber into the open air. However, after a few weeks, there was no sign of a young sporophyte growing from the prothalli. The prothalli were infested by small fly maggots or larvae and died a few weeks later. The KLL092 had high survivability during acclimatization but was prone to pest infestation, even after a few months in the nursery. Suitable pesticides should be considered as the KLL092 is used for oral consumption and is a safe end product.

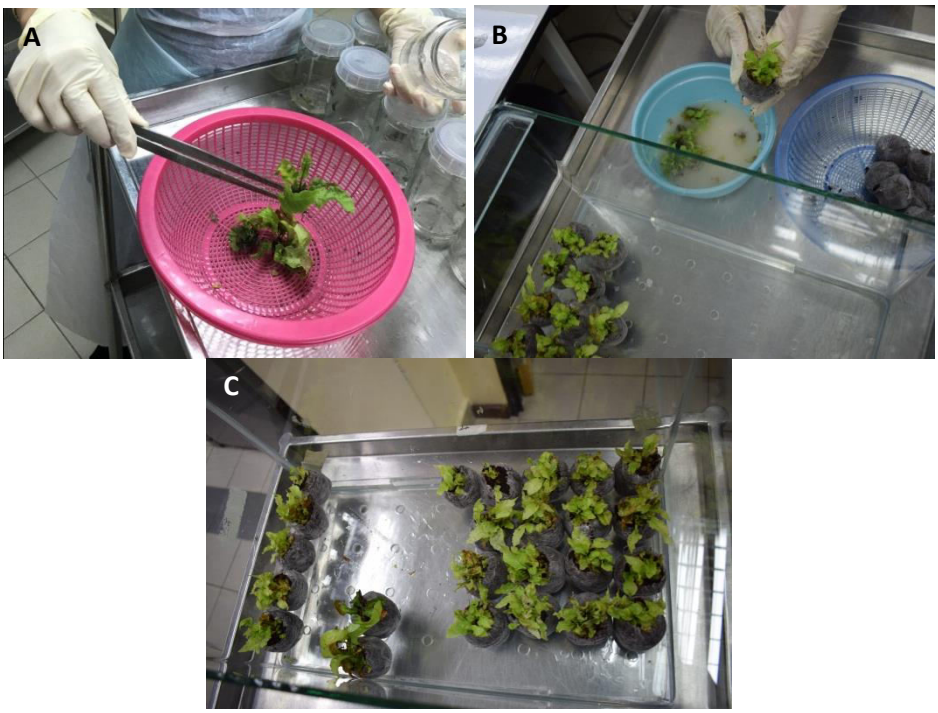

Figure 3: a) KLL 092 was removed from culture media, b) a 0.1% w/v fungicide was applied, and c) KLL 092 sporophytes were acclimatized in a weaning chamber using Jiffy7® medium.

Conclusion

The production of KLL092 tissue culture plantlets has been successfully carried out. The suitable medium for KLL092 is WPM basal media without hormone for growing prothalli and sporophytes into complete plantlets with roots. This media can be used for mass production of KLL092 and to assist in meeting commercial needs in the future.

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Development of Surface Sterilization Protocol for *Xylocarpus rumphii* (Nyireh Pasir) Culture Initiation

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Introduction

Xylocarpus is a small genus in the Meliaceae family, containing three mangrove species namely *Xylocarpus rumphii* (Nyireh pasir), *Xylocarpus granatum* (Nyireh bunga), and *Xylocarpus moluccensis* (Nyireh batu). *X. rumphii* can be found in China, East Africa up to Tonga, Indonesia, Peninsular Malaysia, Philippines, Vietnam, and Singapore. *X. rumphii* is medium sized tree and can reach up to 12 m height. The bark is finely fissured, greyish, and inner bark is bright pink to red colour. The wood is used for traditional carvings, boats making and the bark is used as a dye. This species has medicinal value, especially in Ayurvedic traditional medicine, where the seeds of *X. rumphii* are used to treat food poisoning and snake bites. The trees can be found near shores, rocky cliffs, and near sandy substrates above the high-water mark (Anon, 2022).

X. rumphii is an endangered species in Sri Lanka due to its limited distribution in coastal areas. In Sri Lanka, this plant is traditionally used to treat fish poisoning as well as alcohol poisoning (Kumara et al., 2009). Other than that, several compounds were identified from *X. rumphii* such as xylorumphiins A-D, mexicanolide limonoids and other limonoids from the seed kernels (Sarigaputi et al., 2010).

In Malaysia, this species is known to only be found in a few locations of coastal areas in Kedah, Pahang, and Perak. Although it is categorized as Least Concern in Malaysia, but not much information is known about this *X. rumphii* species compared to *X. granatum* and *X. moluccensis*, especially in terms of propagation. Therefore, it is important that propagation studies are carried out, so that this species can be continuously propagated and conserved.

Tissue culture techniques will be useful in hastening large-scale breeding, improvement, and conservation of this plant. The simplest method of tissue culture is to stimulate the development of axillary buds. Axillary buds are treated with hormones to break latency and produce new shoots. The shoots are then separated and rooting was induced to produce complete plantlets. Alternatively, the shoots are used as explant material for further propagation (Gregory and Hubstenberger, 1995). Many ornamental and woody species are commercially propagated by axillary bud propagation method (Chu, 1992). In this study, we adopted the similar technique to propagate *X. rumphii in vitro*.

Materials and Methods

Plant samples

Young shoots of *X. rumphii* were collected from matured plants in Pulau Pangkor and young seedlings grown in the laboratory (Figure 1). Nodal segments from these young shoots were used as explants in this study.

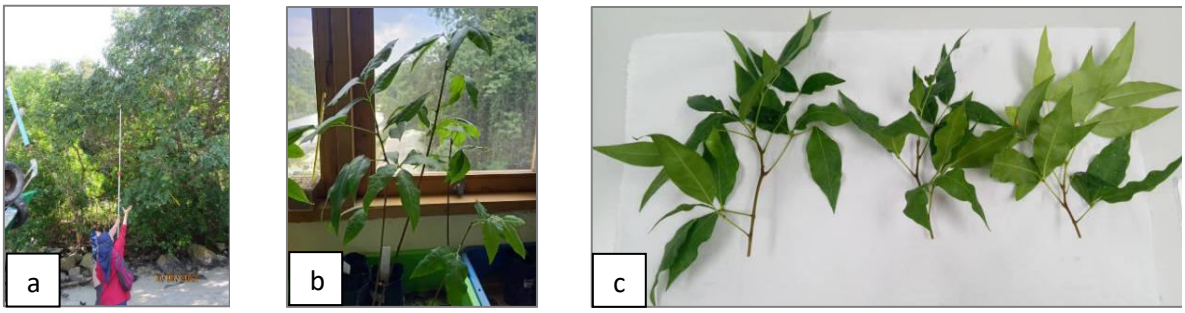


Figure 1: Plant samples of *X. rumphii* (a) *X. rumphii* mother tree, (b) Young shoots from Pulau Pangkor, and (c) Young seedlings.

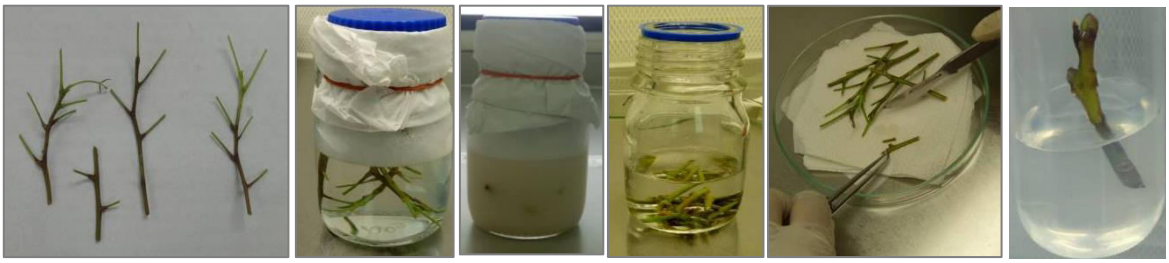
Culture media and conditions

MS basal medium (Murashige and Skoog, 1962) with 3% (w/v) sucrose solidified with 0.3% (w/v) gelrite agar was used in this study. The pH of the medium was adjusted to 5.8. The test tube containing the culture media were autoclaved at 121 ℃ for 15 min. All cultures were maintained in a culture room with a temperature of 22±2 ℃ under 16/8 h photoperiod.

Surface sterilization and culture initiation

Three surface sterilization methods; A, B, and C were applied for culture initiation using nodal segments (Figure 2) as explants collected from both matured trees and young seedlings. Explants were washed thoroughly with sterile distilled water and then soaked in fungicide for 45 min. After that, the explants were rinsed three times with sterile distilled water. Then, treated with 70% (v/v) Ethanol plus one drop of Tween 20 (as wetting agent) for 3 min, rinsed with sterile distilled water for three times and soaked in different concentration of Clorox® added with one drop of Tween 20. Method A comprise of 60% Clorox® - 20 min; Method B: 60% Clorox® - 25 min, and Method C: 50% Clorox® - 30 min.

After treatment with Clorox®, all explants were rinsed with sterile distilled water. Nodal segments were cut into small segments and dried in laminar flow. They were then cultured in shoot induction medium containing MS basal medium supplemented with 0.1 mg/L BAP (6-Benzylaminopurine*)*. The surface sterilization and transfer procedures were carried out in the laminar flow hood. All cultures are incubated in culture room at the temperature of 22±2 ℃ under 16/8 h photoperiod. Observation on percentage of clean/aseptic cultures and contamination were recorded after 4 weeks of culture.



(a) (b) (c) (d) (e) (f)

Figure 2: Surface sterilization process of *X. rumphii* explants. (a) Nodal segments of *X. rumphii* used as explants, (b) Explants soaked in 70% Ethanol, (c) Explants washed in Clorox® solution plus a drop of Tween 20, (d) Explants rinsed in sterile distilled water, (e) Explants cut into small pieces, and (f) Explants cultured in the test tube medium and incubated in the culture room.

Results and Discussion

Surface sterilization of plant materials especially from matured trees in open field is a very challenging step in establishing plant tissue culture protocol. Young shoots are an excellent starting/explant material for *in vitro* plant culture. However, the high level of microbial contamination they carry, particularly in axillary nodules, makes *in vitro* culture extremely difficult. Microbial contamination is typically addressed by effective surface sterilisation of explants. For the establishment of clean/aseptic culture, explants of all types, including seeds and leaves, are commonly surface sterilized with calcium hypochlorite, either alone or in combination with 70% (v/v) ethanol (Oluwakemi et al., 2018). However, in this study, only sodium hypochlorite or Clorox® (commercial bleach) and 70% (v/v) ethanol were used.

After 4 weeks in culture, it was observed that Method A gave the highest percentage (79%) of clean culture (Figure 3a) as compared to Method B (30%), and Method C (19%) from nodal segments explants (Table 1). It was observed that the percentage of contamination decreased with the increased duration of surface sterilization. One of the reasons for the low regeneration associated with long-term sterilization may be the effect of the active ingredient sodium hypochlorite (NaOCl) on delicate tissues, which can lead to the death of the actively growing parts of the explants (Teixeira et al., 2006). In the case of shoot induction, *X. rumphii* shoots started to induce (Figure 3b) only from nodal segment of young seedling after 6 weeks in culture, but the shoot growth in culture is very slow. Figure 3b showed the shoot germination after 9 weeks of culture from young seedlings. To date, there is no observation of shoot germination from matured explants.

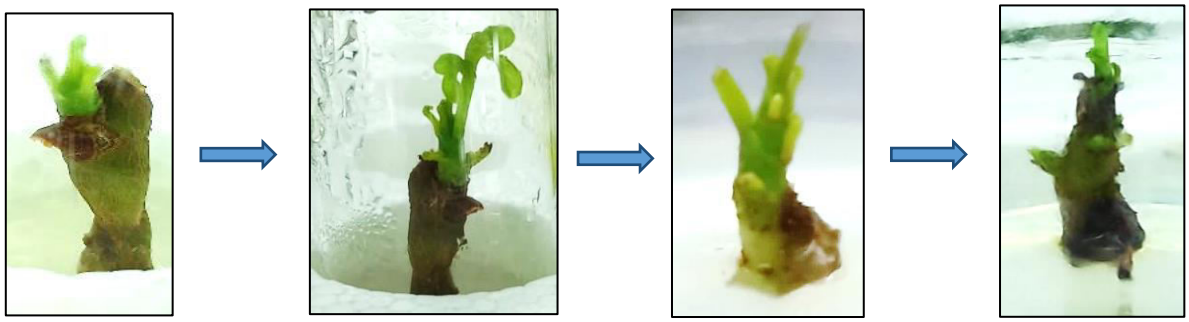
It was discovered from our previous study, that, despite the use of various surface sterilisation techniques, it is very challenging to obtain clean culture from mature trees. Thus far, the results of this investigation have demonstrated that the techniques used successfully produced clean and aseptic cultures of *X. rumphii*. It was also observed that young explants can induce shoot germination better compared to matured explants. Naghmouchi et al. (2008) also reported that actively growing shoots from juvenile plants are more responsive to shoot regeneration and proliferation than shoot explants from adult material. Figure 4 showed shoot development from young seedlings started from 6 weeks to 23 weeks of culture.

Table 1: The percentage of *X. rumphii* clean culture obtained after 4 weeks of culture.

Samples	Method	Percentage of clean culture	Percentage of contamination
Nodal segments from young seedling	A	78.6	21.4
Nodal segments from matured trees	B	30.0	70.0
Nodal segments from matured trees	C	19.4	80.6

(a) (b)

Figure 3: Clean culture and shoot germination of nodal segment (a) from matured trees and (b) from young seedling.



(a) (b) (c) (d)

Figure 4: Shoot development of *X. rumphii* from young seedlings explants; (a) Shoot induced after 6 weeks of culture, (b) shoot growth after 12 weeks of culture, (c) shoot growth after 18 weeks of culture, and (d) shoot growth after 23 weeks of culture.

The efficiency of the surface sterilisation process is critical to the success of tissue culture. Various chemicals were used by different researchers to sterilise the surface of the explants. Some of the researchers used the same chemical at the same concentration, but the duration of sterilisation varied, even when the plant material was very similar (Buah et al., 2015). Ethanol is an extremely powerful sterilizing agent, but also phytotoxic. It is usually used at the concentration of 70% (v/v) for only a few seconds or minutes, followed by treatment with other disinfectant(s). On the other hand, hypochlorite is a very powerful bactericide that drastically lowers bacterial populations during explant disinfection, even at concentrations as low as micro molar. Both sodium hypochlorite and calcium hypochlorite are commonly used hypochlorite compounds. However, it is recommended to use calcium hypochlorite because it is a mild steriliser rather than sodium hypochlorite (Abbasi et al., 2016).

Conclusions

In this study, it was observed that nodal segments from young seedlings surface sterilized with 60% Clorox® for 20 min gave higher percentage of clean culture as well as can induce shoot germination. Way forward, other disinfectants, and various explants, including seeds, may be employed to find the optimal surface sterilisation process and to encourage shoot germination for *X. rhumpii* in an effort to produce planting material.

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Effect of Cryoprotectants and Desiccation on Survival of (*Musa acuminata*) ssp. *malaccensis* Embryos in Cryopreservation

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Introduction

Musa acuminata is one of the wild banana species offering a possible source of resistance to diseases and would potentially be a good plant material for breeding and genetic strategies as they are fertile and produce a large number of seeds (Kayat et al., 2004). Since wild bananas produce seeds freely, the option of seed storage would be the most practical but this method is hindered by the slow and erratic seed germination due to dormancy. According to Chin (1994), some wild banana seeds germinated readily after harvesting and show no sign of seed dormancy, while others become dormant after drying.

Cryopreservation, or storage at ultra-low temperatures (-196 ^{o}C) appears to be an alternative for storage of germplasm of difficult materials. Accessions in a cryo-collection are not at risk from unfavourable weather, vandalism, pests or diseases and cryopreservation also requires much less space than a field genebank (Gotor and Cherfas, 2011). However due to relatively high moisture content in wild banana embryos, they must be pretreated using specific treatments before cryopreservation in order to avoid formation of ice crystals when exposed to liquid nitrogen. The most critical parameter in the cryopreservation procedure is the removal of the intracellular water fraction. Embryo moisture content is considered the most crucial factor for maintaining viability after exposure to liquid nitrogen (-196 °C) (Stanwood, 1985). It is capable of conversion to ice crystals during freezing or rewarming (Shalini, 2003). This can be achieved in a number of ways such as desiccation, usage of cryoprotectants and slow cooling. The use of cryoprotectants has been practised for better tolerance to freezing especially chemicals such as dimethyl sulfoxide, DMSO and glycerol. Cryoprotectants are some chemical compounds which prevent cells or tissues from being damaged due to freezing. Vitrification and thawing processes are mostly used in cryopreservation.

In view of the importance of the wild banana germplasm, zygotic embryos of wild banana, *M. acuminata* ssp. *malaccensis* was used to investigate the potential of cryopreservation for germplasm conservation as an alternative to the conventional method. Considering the above facts, this study was done to determine the effects of desiccation and freezing treatment on survival of banana embryos, without and with cryoprotectants.

Materials and Methods

Plant material

Mature wild banana fruits were collected from Serdang (ssp. *malaccensis*) at the low altitude (2°59'52.1"N 101°43'15.7"E). The fruit was allowed to ripen in the laboratory for 3 to 5 days. Upon ripening, the seeds were extracted by removing the pulp by rubbing it against a fine wire mesh with gentle pressure under running tap water. Those seeds that remained submerged in water were used and seeds that floated were discarded. Excessive water was removed by drying with paper towels. Figure 1 shows cleaned banana seeds that were ready to be used in this study.


Figure 1: Clean seeds after thoroughly washed under tap water.

Cryoprotection

A preliminary study was carried out using the embryo of banana with different exposure times to cryoprotectans but with a constant desiccation time and freezing treatments before the present study was conducted. Based on this preliminary study, an exposure time of 30 min was selected for the present study. Three cryoprotectants based on preliminary experiment were used. The zygotic embryos were excised aseptically and subjected to three cryoprotectants (5% (v/v) DMSO, 5% (w/v) sucrose and Murashige and Skoog (MS) basal media). A control without cryoprotectant was also included in the experiment. Embryo excision was done under the aseptic condition inside a laminar air-flow cabinet. Excised embryo of wild banana was immersed in 20 mL of cryoprotectant solutions in a sterile petri dish and sealed. The petri dish was placed in the laminar airflow cabinet for 30 min.

Embryo desiccation

After exposure to cryoprotectant, the solution was discarded and the embryos were carefully spread on sterile filter paper and desiccated in the laminar airflow cabinet (63±2% RH) for 1, 2 and 3 h. The moisture content of banana embryos was determined for each desiccation period, two replicates of 20 embryos were used for actual moisture content determination.

Moisture content was determined using 10 embyros placed in a weighed aluminium foil boat (replicated twice). The boat was weighed before drying in a ventilated oven at 130 °C for 1 h (Vineesh et al., 2015). After drying, the boats were cooled in an airtight desiccator and weighed using four decimal electronic balances. Moisture content (MC) was calculated as loss in weight and expressed as percentage (%) of fresh weight using the formula:

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

Where,
W1: weight of aluminium boat;
W2: weight of aluminium boat and samples before drying; and
W3: weight of aluminium boat and sample after drying

Freezing

Desiccated embryos were placed in sterilised cryovials secured to crycanes and plunged directly into liquid nitrogen (-196 °C) for at least 16 h. The cooling rate was approximate -200 °C per min. As a control, 10 desiccated embryos were also cultured directly onto MS media without any prefreezing treatment.

Thawing

Rapid thawing was used in this study. The frozen materials were plunged directly into a water bath at 38±2 °C for 10 min with constant agitation. After thawing, the embryo was cultured onto enriched MS medium.

Incubation of culture

The cultures were incubated in a culture room (maintained at 26-28 °C) with 24 h photoperiod and approximately 55 $\mu molm^{-2}s^{-1}$ intensity provided by fluorescent light. Twenty embryos were cultured for each replicate of each treatment to evaluate their survival. Survival was recorded after 6 weeks, when there were signs of shoot and root development or formation of calli.

Experimental design and data analysis

The treatments comprising three cryoprotectant treatments with control (no cryoprotectants) and three desiccation hours were arranged in a Randomized Complete Block Design (RCBD) with three replications. Data were analysed by Analysis of variance (ANOVA) carried out using Statistical Analysis Software (SAS) version 9.3, and differences between treatment means were compared using the Duncan's Multiple Range Test (DMRT) test at $P \leq 0.05$.

Results and Discussion

The percentage of moisture content (MC) and survival of *M. acuminata* ssp. *malaccensis* embryo in three cryoprotectants and three desiccation durations without and with freezing in liquid nitrogen (LN) was shown in Table 1. There was a highly significant effect on percentage of moisture content at 5% level of probability according to DMRT test. The initial moisture content of freshly excised banana embryos without any desiccation and cryoprotectant treatment, CL0 was 38.5% (Table 1). When the embryos were subjected to desiccation for the first hour, CL1 in the laminar air flow cabinet, the moisture content rapidly reduced from 38.5% to 13.3% for the control (Table 1). This result was similar to that obtained by Chua (2000). This indicated that banana seeds readily lose their free water. The rapid loss of moisture content could be due to its small size of the embryos (0.218 g per 100 embryos) with such a large surface area (Kaya et al., 2020). This clearly showed that banana embryos easily lose their water content when subjected to desiccation.

The initial moisture content after immersion in cryoprotectants for 30 min in 5% DMSO (DM0), 5% sucrose (SUC0) and MS Basal (MS0) showed increments of 45.84%, 43.38% and 48.88%, respectively, compared to control (38.5%). The increment of moisture content might be due to the permeation of water into the cells. After 3 h of desiccation (MS3), the wild banana embryos had reduced moisture up to 84% with MS basal achieving the lowest moisture content of 7.6%.

There was no significant difference on embryo survival percentages after desiccation before freezing in liquid nitrogen (LN). Without exposure to liquid nitrogen, the percentage of survival of freshly excised banana embryos of *M. acuminata* ssp. *malaccensis* was 86.7% (CL0) (Table 1). After being pretreated with cryoprotectants, the survival of embryo was up to 90% at DM0 and MS0. Desiccation of embryos up to 3 h resulted in a decline in the percentage of survival with and without cryoprotectants up to 66.7% (control, CL3), 76.67% (5% DMSO, DM3), 73.33% (5% sucrose, SUC3), and 70% (MS basal). It suggested that prolonged desiccation may cause desiccation injury.

There was significant difference for percentage of survival after exposure in liquid nitrogen of *M. acuminata* ssp. *malaccensis* embryos at $P < 0.05$. There was no survival of embryos with and without cryoprotectants and desiccation (CL0, DM0, SUC0 and MS0), when the embryos were frozen in liquid nitrogen. This was due to the high moisture content where ice formation can cause lethal injury to the tissues in the embryos during exposure in liquid nitrogen (Chua, 2000). In the first hour of desiccation,

the percentage of survival without freezing in liquid nitrogen was 56.67%, and the survival rate was reduced to 46.67% after 3 h of desiccation. On the whole, as the desiccation hour increased, the moisture content of the embryos and the percentage of survival subsequently decreased. This was similar to 5% DMSO, 5% sucrose and MS Basal. This trend has also been reported previously (Chua, 2000; Vineesh et al., 2015).

Table 1: Effect of cryoprotectants and desiccation duration on percentage of moisture content (MC), percentage of survival without and with freezing in liquid nitrogen (LN) of *Musa acuminata* ssp. *malaccensis* embryos.

Cryoprotectants (CP)	Desiccation duration (h)	Codes	Moisture content (% ± SE)	Survival (%± SE)	
				Before LN	After LN
No CP (control)	0	CL0	38.48±1.3^{c}	86.67±3.33^{a}	0.67±0.67^{h}
	1	CL1	13.30±0.7^{de}	66.67±14.53^{a}	56.67±3.33^{fg}
	2	CL2	11.31±0.8^{def}	73.33±12.02^{a}	53.33±6.67^{fg}
	3	CL3	11.08±2.2^{def}	66.67±8.82^{a}	46.67±3.33^{g}
5% DMSO	0	DM0	45.84±2.8^{ab}	96.67±3.33^{a}	0±0^{h}
	1	DM1	14.87±1.4^{d}	80.00±15.28^{a}	93.33±3.33^{a}
	2	DM2	13.40±0.5^{de}	73.33±12.02^{a}	73.33±8.82^{bcd}
	3	DM3	10.90±0.5^{def}	76.67±8.82^{a}	60.00±5.77^{ef}
5% sucrose	0	SUC0	43.38±1.4^{b}	83.33±3.33^{a}	0±0^{h}
	1	SUC1	14.22±1.4^{d}	70.00±15.28^{a}	80.00±5.77^{abc}
	2	SUC2	11.09±0.9^{def}	60.00±11.55^{a}	86.67±3.33^{ab}
	3	SUC3	8.93±0.9^{ef}	73.33±16.67^{a}	80.00±5.77^{abc}
MS basal	0	MS0	48.88±3.9^{a}	90.00±10^{a}	0±0^{h}
	1	MS1	16.00±1.5^{d}	80.00±11.55^{a}	83.33±3.33^{abc}
	2	MS2	11.46±0.3^{def}	76.67±8.82^{a}	70.00±5.77^{cde}
	3	MS3	7.59±0.8^{f}	70.00±10^{a}	63.33±3.33^{def}

Note: SE – Standard Error; DMRT – Duncan's Multiple Range Test.
Different letters indicate significant differences within each column based on DMRT test at 5% probability level. Results were expressed as mean ± standard error.

This study showed that fresh embryos of *M. acuminata* ssp. *malaccensis*, if frozen in liquid nitrogen, did not survive. However, with cryoprotectants and desiccation treatment, some survival could be achieved. Desiccation to a moisture content of approximately 13% allows survivability after freezing in LN. It can be deduced that for long-term cryopreservation of *Musa* ssp. seeds in liquid nitrogen, the moisture content should be less than 17% for optimal viability and germination (Kaya et al., 2020).

Conclusion

Desiccation is an important factor, which can determine the survival of banana embryos cryopreserved in liquid nitrogen (LN) for *M. acuminata* ssp. *malaccensis*. Without desiccation prior to freezing, no survival was obtained, irrespective of any pretreatment used. Pretreatment with 5% DMSO, 5% sucrose and MS basal could enhance desiccation tolerance and survival in LN up to 80%. Without any cryoprotectant, desiccation with 9 to 14% moisture content (beyond 1 h) showed an increase of survival from 0% to approximately 80% after liquid nitrogen freezing. Desiccation combined with cryoprotectant pretreatment improved survival of wild banana embryos after exposure in liquid nitrogen (LN). This study demonstrated that *M. acuminata* ssp. *malaccensis* zygotic embryos have the potential for long-term preservation by cryopreservation.

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Posterity of Genetic Resources via *Ex situ* Conservation: DNA Banking and Cryopreservation

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Introduction

Oil palm genetic resources are the main backbone of the Malaysia Palm Oil Board (MPOB), which has been named the host of the world's largest oil palm germplasm collection. For the past 50 years, MPOB has started oil palm germplasm exploration in Africa and South America to broaden the genetic base of the current planting materials (Rajanaidu et al., 2000; 2017). Currently, the MPOB exploration team has covered eleven countries for *Elaeis guineensis* and eight countries for *Elaeis oleifera*. The governance of oil palm germplasm collections (collection, evaluation, utilization, and conservation) is crucial in generating a guideline for effective utilization in breeding. These collections are actively being utilized in breeding programme for crop improvement. A conservation initiative for genetic resources has been taken to ensure the posterity and sustainability of the palm oil industry. Although field gene bank provides easy access to germplasm, they run the risk of losing these precious collections to diseases, stresses, or disasters, and they require large areas of land and are labor-intensive. The main role of the MPOB gene bank is to guarantee the preservation as well as consistent availability of genetic resources for oil palm improvement. With high variability in the available material in the gene bank, MPOB strives to improve the quality of high-quality breeding material for the oil palm industry. *Ex situ* living collection (field gene bank), deoxyribonucleic acid (DNA) banking and cryopreservation methods are the best alternatives for accomplishing the conservation of oil palm genetic resources.

DNA Bank was developed as a strategy to sustain the oil palm genetic resource and to support molecular-based research. Consequently, DNA banking is an ideal way to ensure the consistent accessibility of the oil palm genome even after the palms are no longer available in the field. It is intended to serve as a repository for oil palm genetic stock to cater to the surging demands for their use in molecular applications. Thus, DNA bank can serve as a conservation tool at the biotechnological level (Quazi et al., 2021). Aside from that, cryopreservation techniques were also developed as an alternative and complementary method for more efficient long-term conservation at ultra-low temperatures (-196 °C) for an unlimited period; this method is widely used to conserve plant genetic resources (Engelmann 2000). Thus, both the DNA Banking and cryopreservation programmes have benefited the palm oil industry as a whole through the sustainable management of an invaluable genetic resource and its accessibility to interested parties.

Materials and Methods

Sample collection

The experiments were carried out on oil palm from various MPOB germplasm collections; Nigeria, Cameroon, Zaire, Tanzania, Madagascar, Angola, Senegal, Gambia, Sierra Leone, Guinea, and Ghana for *Elaeis guineesis.* Meanwhile, for MPOB *Elaeis oleifera* germplasm, the collection covered eight countries; Honduras, Panama, Nicaragua, Colombia, Costa Rica, Suriname, Ecuador, Peru, and Colombia, which were planted at MPOB Research Station Kluang, Johor, Malaysia. In addition, several lineages of *dura* (Ulu Remis, Serdang Avenue, Elmina, Banting, Ebor, and Johor Labis) and *pisifera* (AVROS, Yangambi, La Me, and Ekona) from MPOB's in-house breeding programme were also collected.

DNA banking

Spears leaves from selected palms were collected at the field gene bank, processed, and packed into two replicates of 20 g each for leaf storage at -80 ^{o}C, together with 4 g for DNA extraction. The DNA was extracted using the modified (cetyl trimethyl ammonium bromide) CTAB method (four-day procedure) (Dellapota et al., 1983). The leaf sample was ground with sand in liquid nitrogen to form a powder before being homogenised with 20 mL of CTAB buffer and incubated for 30 min in a waterbath at 60 ^{o}C for 30 min. A 20 mL solution of chloroform/isoamyl alcohol (24:1 v/v) was added and centrifuged at 12,000 rpm for 20 min. The supernatant was transferred into a sterile polypropylene tube, and 0.6 volume of isopropanol were added to precipitate the DNA. The mixture was centrifuged at 12,000 rpm for 15 min to form a pellet. Then the pellet was washed with 70% (v/v) ethanol containing 10 mM ammonium acetate before being dissolved in Tris EDTA (TE) buffer. Further, 5 uL of RNAse (10 mg/mL) was added to treat the DNA, followed by one-tenth of a volume of 7.5 M ammonium acetate before centrifuging at 12,000 rpm for 20 min. The supernatant was transferred into a new sterile polypropylene tube, and 2.5 volumes of absolute ethanol were used to precipitate the DNA and also washed with 70% (v/v) ethanol. The pellet was dried at 37 ^{o}C for 20 min before being dissolved in TE buffer. The dissolved DNA was transferred into 1.5 mL Eppendorf tubes and kept at 4 °C. The quality of extracted DNA was determined by an absorbance measurement (optical density) and digestibility test. The optical density (OD) was determined by checking the ratio of absorbance at 260/280 and 260/230. A digestibility test was carried out using two restriction enzymes, EcoR1, and Hea III. The DNA samples were incubated overnight before being loaded into a well of horizontal gel electrophoresis. Finally, the DNA data is stored in Microsoft Excel.

Cryopreservation

Embryos were excised from oil palm fruits. The moisture contents of embryo samples were measured using oven method described by the International Seed Testing Association, ISTA (1985). Cryopreserved oil palm embryos have a higher survival rate when the moisture content is between 10% and 20%. Embryos were then sealed in propylene cryovials and directly immersed in liquid nitrogen (-196 ^{o}C) for cryopreservation. For regeneration, the embryos were thawed in a 40 ^{o}C waterbath for one minute. Embryos were then transferred onto MS basal medium to determine rate of 24-25 ^{o}C under 16/8 (light/dark) photoperiod.

Results and Discussion

Currently, DNA collection has been accomplished for oil palm *E. guineensis* germplasm. In addition, a few lineages of the *dura* breeding programme and *pisifera* populations were also preserved, as the materials played a major role in the improvement of oil palm planting materials in Malaysia (Rajanaidu et al., 2013). Meanwhile, for cryopreservation, the collection has been covered with oil palm *E. guineensis* and *E. oleifera* germplasm.

A total of 9,078 leaf samples were collected mainly at MPOB Research Station Kluang, Johor, and from that, 6,053 samples have been extracted for DNA and stored at 4 ^{o}C. Preserved leaf samples were placed in the Biological Resource Centre room as captured in Figures 1a and 1b. The quality of DNA was expressed through purity (A260/280) with a range of 1.8-2.0, concentration 0.2-2.0 (ug/uL) and A260/230 ratio values greater than 1.5 (Pachchigar et al., 2016). Before being stored, the quality of each sample was also tested using restriction enzyme digestibility tests (*EcoR1* and *HaeIII*). The DNA cleaved at this specific size indicated high quality of DNA. The DNA samples were arranged in boxes accordingly and stored at 4 ^{o}C (Figures 1c and 1d). Creating well-documented databases is a crucial step toward this goal. Presently, the DNA data is stored in Microsoft Excel. The important details recorded include type of material, trial number, palm number, location, OD result, leaf tissue, and DNA storage location.

Figure 1: DNA genebank of MPOB oil palm germplasm. (a) Leaf samples stored in ultralow temperature freezers (-80 ^{o}C) (b) Freezers at Biological Resource Centre, MPOB HQ (c) Storage boxes containing DNA in 1.5 mL Eppendorf tubes (d) DNAs stored in chiller (4 ^{o}C)

Meanwhile, oil palm zygotic embryos are being cryopreserved in the MPOB cryo tank in (Figures 2a, b, and c). More than 68,000 zygotic embryos of accessions from *E. guineensis* and *E. oleifera* germplasm are currently conserved. Oil palm seed demonstrated intermediate seed storage behaviour, with the ability to be desiccated to around 10%-12% moisture content and to withstand freezing temperatures (Ellis et al., 1991). Because of its small size in comparison to the seeds and kernels, the zygotic embryo is ideal for oil palm cryopreservation; larger tissues are more constrained by desiccation and freezing sensitivity. Many factors must be in place for successful cryopreservation, including source-plant status, materials, personnel, culture conditions, pre-treatment conditions, cryopreservation techniques, cryogenic facilities, organizations, and post-thawing (Reinhoud et al., 2000; Reed et al., 2004). To observe the efficacy, some five-year cryopreserved embryos have been germinated on MS basal media as shown in Figures 2(d) and (e), with a success rate of 70%. Similar results were obtained in citrus at the National Bureau of Plant Genetic Resources (NBPGR) in India, where 69%-81% of the accessions retained more than 70% of their viability after desiccation and after an average of 6.3-8.4 years of cryo-storage (Malik et al., 2012).

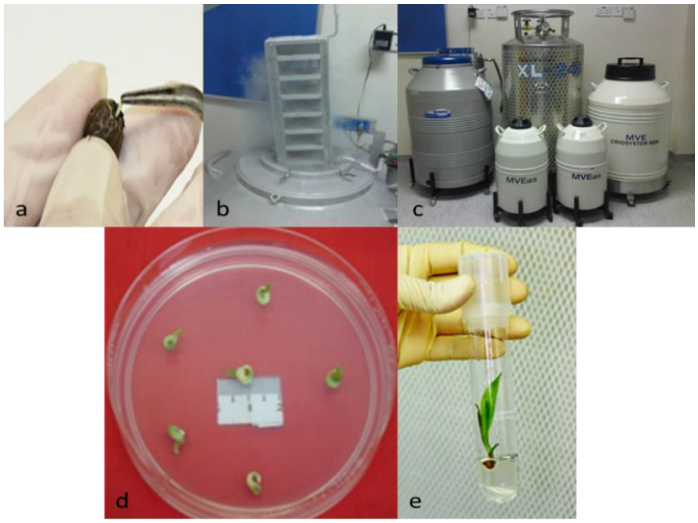

Figure 2: cryopreservation of zygotic embryos of oil palm germplasm. (a) Excising embryos from oil palm kernel, (b) rack with cryotubes filled with embryos are directly immersed in liquid nitrogen, (c) Cryopreservation facility at MPOB Research Station Kluang, Johor.

DNA banking is an efficient, simple, and long-term way to conserve genetic information. DNA banks can now be considered a form of complementary conservation. DNA storage is particularly useful for those species that cannot be conserved in traditional seed or field genebanks nor conserved *in situ* due to high risk in that area. Molecular techniques are becoming increasingly important in the study and management of genetic resources. DNA has been routinely extracted and stored to support various molecular-based research. The DNA can be stored at low cost in DNA banks as an insurance policy against the loss of oil palm diversity. In contrast with field genebank, the potential for DNA storage is promising due to the small sample size for storage of genetic information and the stable nature of DNA in cold storage. However, the use of DNA banks in conservation is limited, as whole plants cannot be directly reconstituted from DNA. Regardless of this fact, the field of molecular biology is advancing rapidly, and DNA banking is likely to become a more feasible option for the conservation of oil palm diversity in the future.

Cryopreservation techniques are also an essential tool for the long-term storage of germplasm materials, as they can be used as an alternate and complementary way for more efficient long-term conservation that is low maintenance and requires minimal space. It seems to be the most feasible method for storing recalcitrant seeds (such as those of the oil palm) and species that are vegetatively propagated. It is also effective since it requires low risk from environmental effects. The limitation of DNA preservation to produce the whole palm could potentially be covered via zygotic embryos preservation. Furthermore, the cryopreservation approach has the potential to be applied to other types of oil palm samples, such as pollen, kernels, somatic embryos, which would complement long-term conservation of oil palm germplasm as an alternative to the field genebank.

Conclusions

MPOB oil palm germplasm collections were successfully preserved in the form of DNA and cryopreservation. Both techniques have proven effective as an alternative for field conservation and have also managed to establish ex-situ oil palm genebank. DNA accessions could be used to accelerate upstream research using advanced molecular biology techniques. However, the advantage of establishing a DNA bank is to ensure the blueprint of these priceless germplasm collections can still be accessed for years to come. In addition, the long-term viability and stability of cryopreserved palms were also evaluated periodically. Thus, both the DNA banking and cryopreservation programmes have benefited the palm oil industry as a whole, through the sustainable management of an invaluable genetic resource and its accessibility to interested parties.

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Development of DNA Fingerprinting Technology for High-quality Banana Planting Materials Production in FGV

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Introduction

Banana (*Musa* spp.) is one of the most important crops worldwide and is widely consumed all over the globe. It has been estimated that more than 100 billion bananas are eaten globally every year (FAO, 2022). In terms of total global production of food crops, it ranks just behind wheat, rice and maize. The demand for bananas has also been increasing steadily at a compound annual growth rate of 4.5%. Cultivated banana originally comes from an inter-specific and intra-specific hybrid between two wild banana species, *Musa acuminata* (genome A) and *Musa balbisiana* (genome B) (Simmonds and Shepherd, 1955). The commercially consumed bananas are known to be either diploid with genome AA, AB, and BB, triploid with genome AAA, AAB, ABB, and BBB, or tetraploid with genome AAAA, AAAB, AABB, and ABBB. Triploid bananas are the most commonly observed commercial banana cultivar available (Valmayor et al., 2000). Due to its triploidy and male/female sterility issue, cultivated bananas are seedless and hence cannot reproduce sexually (Kang and Priyadarshan, 2008). Cultivated bananas propagate asexually using part of their underground rhizome to form daughter plantlets called suckers (Barker, 1959). For the mass production of commercial bananas, this characteristic has been exploited by commercial companies. Modern method using micropropagation of meristem tissue and other vegetative tissues as an explant is very efficient in generating a high number of planting materials (Suman, 2017).

This approach has been employed by FGV Agri Services Sdn. Bhd. to mass produce banana planting materials for commercial purposes. Before the planting of tissue culture clonal banana seedlings at the farm, several procedures must be undertaken. This includes suckers harvesting, the culture of meristem cells, the induction of roots and shoots growth, the transplanting of plantlets, and followed by the development of seedlings. As an additional quality control step, DNA fingerprinting technology can be utilised to confirm the legitimacy of banana planting materials.

DNA fingerprinting panel is typically developed using DNA markers. In bananas, various genetic studies have been conducted using different types of DNA markers such as random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), and amplified fragment length polymorphism (AFLP) (Howell et al., 1994; Wang et al., 2007; Hippolyte et al., 2010; Cenci et al., 2021). SSR in particular is a marker that is highly polymorphic, co-dominant, and relatively affordable for routine genotyping. Thus makes it suitable to be used for the development of DNA fingerprinting in bananas. In this study, we isolated SSRs from a few published scientific works, developed multiplex polymerase chain reaction (PCR) using primers labelled with fluorophores, and established unique SSR profiles for all commercially produced banana varieties in FGV.

Materials and Methods

Plant materials and DNA extraction

The materials used in this study were obtained from commercially produced banana varieties in FGV. Leaf tissues were collected from banana germplasm collection maintained in Pusat Penyelidikan Pertanian Tun Razak, Jerantut, Pahang, Malaysia. They are locally known as Pisang Saba, Pisang Nipah, Pisang Tanduk Lang, Pisang Rastali, Pisang Nangka, Pisang Raja, Pisang Emas, Pisang Lemak

Manis, Pisang Jari Buaya, Pisang Berangan, and Pisang Embun *(Cavendish).* DNA was extracted from freshly harvested banana leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Ying and Zaman, 2006). DNA concentration was quantified using NanoDrop Spectrophotometer ND-1000 (ThermoScientific, USA).

SSR markers and polymorphism screening

Thirty SSR markers were initially selected from four published literature (Ning et al., 2007; Hippolyte et al., 2010; Ravishankar et al., 2012; Rotchanapreeda et al., 2016). The retrieved forward primers of the SSR markers were added with M13 sequence (GGAAACAGCTATGACCAT) at their 5' end. Additionally, all reverse primers were added with PIG-tail sequence (GTTTCTT) at their 5' end. The polymorphism of each SSR marker was tested initially in a 10 µL singleplex PCR reaction. Each PCR amplification contains 2X MyTaq HS Mix (Bioline), M13-forward primer, PIG-tail-reverse primer, and 5' 6-FAM Fluorescein (FAM) labelled M13 primer (Integrated DNA Technologies). PCR reactions were carried out using Eppendorf thermal cycler with the following configuration; initial denaturation of 94 ℃ for 5 min, followed by PCR cycle of 94 ℃ for 30 sec, annealing at 55 ℃ for 90 sec, extension at 72 ℃ for 1 min, and final extension of 72 ℃ for 30 min. PCR products were run using fragment analysis setup on 3500xL Genetic Analyzer (Applied Biosystem, USA), and marker polymorphism analysis was conducted using Gene Mapper v 5.0 software (Applied Biosystem, USA).

Multiplex PCR and legitimacy panel establishment

Ten polymorphic SSR markers were selected from the initial screening process. For the final DNA fingerprinting panel, the amplification of the selected SSR markers was conducted in a multiplex PCR reaction. Instead of using M13 sequence, all forward primers for multiplex PCR were directly labelled at their 5' end using fluorophores of 5' 6-FAM Fluorescein (FAM), 5' HEX (HEX), and 5' ROX NHS Ester (ROX) from Integrated DNA Technologies, USA. The concentrations of forward and reverse primers were adjusted to get the best amplification profile readable using Gene Mapper v 5.0 software. A phylogenetic tree was constructed using DARwin software v 6.0. (CIRAD, France).

Results and Discussion

SSR markers acquired from four published literatures were screened using banana varieties in FGV to identify the most polymorphic markers. A multiplex PCR reaction was created using the ten SSR markers chosen. The size of the PCR products and the colour of their tagged fluorophores were significant factors in the multiplex PCR design. This was taken into consideration to ensure the PCR products of SSR markers labeled using the same fluorophore were not overlapping with one another. Scoring of the respective alleles for each SSR was done using Gene Mapper v 5.0 software. The usage of ten SSR markers in a multiplex PCR reaction allows high throughput DNA fingerprinting and at lower cost. Details of the DNA fingerprinting panel are shown in the Table 1.

Table 1: Ten polymorphic SSR markers used to create the DNA fingerprinting panel and their respective details.

SSR Markers	SSR source	Repeat motif	Allele sizes from multiplex PCR (bp)	Number of Alleles	SSR chromosome number in *Musa acuminata* subsp. *malaccensis* (reference genome)
Ba3	Ravishankar et al., 2012	(AG)	348-432	12	A06
Ba4	Ravishankar et al., 2012	(CT)	187-226	10	A11
Ba6	Ravishankar et al., 2012	(TC)	339-378	7	A03
Ba7	Ravishankar et al., 2012	(CT)	148-169	6	A08
Ba8	Ravishankar et al., 2012	(GA)	135-175	13	A02
Ba19	Rotchanapreeda et al., 2016	(GA), (CTC)	261-288	10	A09
Ba21	Ning et al., 2007	(GA)	168-243	12	A06
Ba25	Hippolyte et al., 2010	(TG)	304-307	6	A03
Ba26	Hippolyte et al., 2010	(CA)	252-276	7	A08
Ba30	Hippolyte et al., 2010	(TC)	446-490	12	A06

Overall, these ten SSR markers amplified 95 alleles when tested using the FGV collection of commercial banana varieties. The allele numbers demonstrate the polymorphism of this set of markers, hence justifying their suitability to be used in a DNA fingerprinting panel. The repeat motifs for all SSR markers are dinucleotide repeats, except for marker Ba19 which has both the dinucleotide and trinucleotide repeats present in the PCR amplicons. The SSR markers came from chromosomes A02, A03, A06, A08, A09, and A11 when referred to *M. acuminata* subsp. *malaccensis* strain Doubled-haploid Pahang (DH-Pahang) genome assembly. While not all 11 chromosomes in the banana genome are covered, the uniqueness of the DNA fingerprinting profile generated using the panel is deemed good enough to genetically differentiate all commercial banana varieties available.

A phylogenetic tree was built using the SSR genotype data from this fingerprinting panel. The tree showed distinct clustering of all banana varieties available (Figure 1). The phylogenetic tree built demonstrated that the selection of SSR markers in the DNA fingerprinting panel is sufficient to distinguish all banana varieties into different clades. The tree built here also confirms the accuracy of our genotype data, as the organization of clades in the tree is linked to the overall ploidy of the cultivated banana varieties (Valmayor et al., 2000).

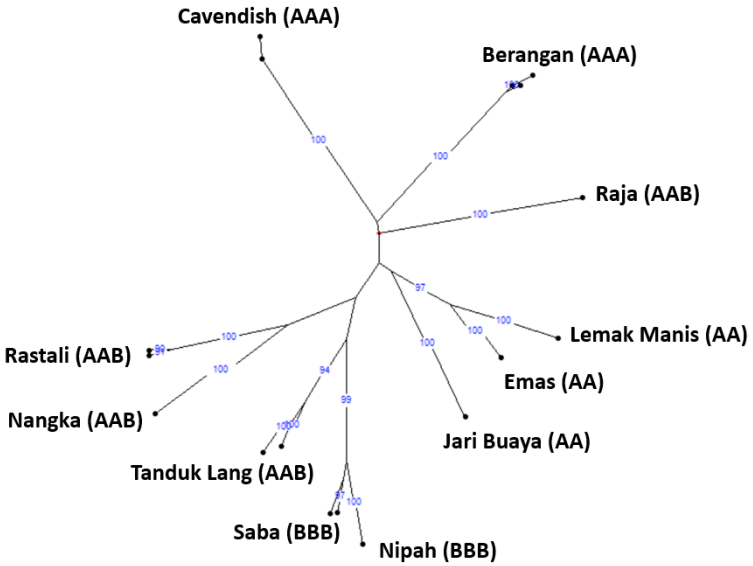

Figure 1: Phylogenetic tree of commercial banana varieties in FGV, constructed using SSR fingerprinting data.

This DNA fingerprinting panel is useful as a quality control step in the banana planting material tissue culture production. Banana suckers collected as the initial source for cloning can be tested using this panel to genetically confirm the variety of the mother plant. Only legitimate bananas will be allowed to be processed further down the line. Occasionally, plantlets within the production line can be tested to ensure that all banana varieties' legitimacy remains intact throughout the production process.

Conclusion

This study has successfully developed a DNA fingerprinting panel to be used in quality control protocol of banana tissue culture production. Thus, ensures the banana planting materials produced remain true-to-type until they reach the customers.

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SNP-based Assessment of Genetic Diversity in MPOB-Tanzania Oil Palm Germplasm

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Introduction

Oil palm *Elaeis guineensis* has been widely commercialised in South East Asia especially in Malaysia and Indonesia due to favourable climatic conditions, soil and availability of labour. Malaysia currently accounts for 35% of world palm oil production and 40% of world exports, ranked second after Indonesia (Farmimex, 2022). Oil palm breeding populations in Malaysia had been derived from few ancestral palms and seed producers exclusively used Deli dura as mother palms and resulted in narrow genetic base, thus restraint the enhancement of FFB yield, bunch number and bunch weight. Concerning the potential of this industry, Malaysian Palm Oil Board (MPOB) has collected *E. guineensis* from Africa and planted at MPOB Research Station Kluang, Johor. The objective of germplasm collection is to gather the maximum amount of genetic variability from the minimum number of samples (Marshall and Brown, 1975). One of the 11 African countries explored by MPOB was Tanzania which is located on the eastern part of Africa. The expedition was done in 1986 where MPOB collected one to seven open-pollinated palms from 13 sites located near Kigoma along the Lake of Tanganyika (Rajanaidu et al., 1992). A total of 42 *dura* and 18 *tenera* bunches were collected and further evaluation on this *dura* collection has shown the potentiality of high bunch index (BI: 0.6), high vitamin E (>1300 ppm), high carotene (>2000 ppm) low lipase (free fatty acids: 2-10%) and high protein kernel (>20%). Some dura materials also showed compact characteristics with height increment <0.30 m/year and rachis length <5.0 m (Marhalil, 2009). The most outstanding trait was extremely thin shell *tenera* with shell to fruit ratio was between 2.80 to 7.40%, compared to DxP commercial (>10%).

Zhou et al. (2015) and Okoye et al. (2016) previously studied molecular characterisation of oil palm using simple sequence repeats (SSRs). SSRs have also been the marker of choice for the assessment of several MPOB-oil palm germplasm studies of *E. guineensis* collected from Africa (Ting et al., 2010, Myint et al., 2021). However, these studies were based on a limited number of SSR markers (10 to 35 SSRs) and generated low genetic differentiation (Fst = 0.174 - 0.250) which indicated a high level of genetic divergence among the germplasms. Nowadays, a more advanced type of marker, single nucleotide polymorphisms (SNPs) is commonly used in oil palm genetics and molecular breeding. The present study aimed to provide an overview of SNP-based assessment on the genetic diversity and population structure among individual and populations in Tanzania germplasm collection. Finding from this study may help breeders establish the core collection and facilitate studies of variability and correlation with morphological traits of agronomic interest to the breeding program developed at MPOB.

Materials and Methods

Experimental plot and palm selection

Tanzania germplasm was planted at MPOB Research Station Kluang, Johor in 1990. Palms were laid out as an equilateral triangle design with 8.8 m planting distance between palms using randomized complete block design (RCBD) with 16 palms/plot, giving 148 palms/ha. This experiment was labelled as Trial 0.256 and each population was indicated as TZA01-13 according to the original collection sites in Tanzania, with the range of one to six families per population (Figure 1). Yield data collection (YR) and bunch analysis (BA) on individual palms were carried out from 1994 until 2000.

Bunch quality characters were evaluated using the protocol developed by Blaak et al. (1963). All data were systematically uploaded into MPOB-Breeding Information System (MPOB-BIS).

Figure 1: Collection sites of *E. guineensis* in Tanzania.

Palm selection for SNP genotyping was done by estimating the variance components for each population and analysis of variance (ANOVA) was calculated to estimate the variance explained by populations, by family within population and the error (palms within each family). Due to low variance components obtained, population that has only one family and/or the number of palms within a family of less than five were removed and its details is shown in Table 1. The phenotypic data of 322 palms above then was standardised (mean = 0, standard deviation =1) and subjected to cluster analyses utilising complete linkage method based on Eucladian distance. Populations that contributed to most of the variation based on ANOVA and cluster analysis were prioritised and 117 palms from TZA02, TZA03, TZA05 and TZA13 were selected for SNP genotyping. DNA stocks were obtained from MPOB DNA Banking program that were previously extracted from spear leaf samples using modified CTAB method (Rahimah et al., 2006). DNA quantification of each sample was measured using Multiskan Spectrophotometer (Thermo Scientific, USA).

Table 1: Details on the selected individuals palm from Tanzania.

Characteristics	No.
Number of trials	1 (0.256)
Number of palms	322
Number of populations	11
Number of families within population	2-5
Number of individuals within families (average)	5-10 (8.3)
Number of bunches (for phenotyping)	1-4
Number of samples for SNP genotyping	117
Population selected for SNP genotyping	TZA02, TZA03, TZA05 and TZA13

SNP genotyping and filtering

117 DNA samples were genotyped using the Illumina Infinium® oil palm customised SNP array containing 92,459 SNP markers on the HD iSelect Bead Chips platform following the manufacturer's protocol (Illumina Inc., Orion Genomics, USA). Data visualisation, inspection and scoring were carried out using the Genome Studio software (Illumina Inc., USA). Visual inspection was done for each palm to screen for any abnormalities in the allelic intensity ratio of the two alleles (A and B), namely theta (θ) distribution plots using all SNP markers. One palm with a shifted or abnormal θ distribution was removed from the analysis (Table 2). The ASSIsT (Automatic SNP Scoring Tool) software (Di Guardo et al., 2015) was then used as a tool for efficient filtering and calling of SNPs from Illumina Infinium arrays, specifically devised for custom genotyping arrays. After SNP filtering, 67,180 informative SNPs were loaded into R Studio and markers with >80% missing data with minor allele frequency <0.01 were removed from the dataset. This resulted in 66,940 SNPs were used to study the genetic diversity and population structure in 116 accessions in Tanzania oil palm germplasm.

Table 2: Number of samples representing each population in Tanzania germplasm.

Population	No. of palms within population	Total number of accessions	
		Genotyped	Normal θ distribution
TZA 02	41	117	116
TZA 03	39		
TZA 05	18		
TZA 13	19		

Diversity analysis

Diversity and population structure were all analysed using R vs 4.2 via different diversity analysis packages. SNP variation in whole dataset was plotted using "GenVisR" package to determine the frequency of transitions and transversions. Markers distribution and the density in each chromosome were determined and plotted. Observed (H_o) and expected heterozygosity (H_e), minimum allele frequency (MAF), fixation index (Fi) and polymorphism information content (PIC) values among germplasm populations were calculated using the "snpReady" package. Analysis of molecular variance (AMOVA) was analysed using levels of variation within and among populations. Pairwise Fst between populations (Weir and Cockerham, 1984) was calculated using "hierfstat" package Fst heatmap which was plotted using "ggplot" function. Principle component analysis (PCA) was computed by "snpRelate" package and neighbor joining (NJ) dendrogram was constructed using "poppr" and ape packages based on Nei genetic distances.

Results and Discussion

Characterisation and distribution of SNPs

A data set containing 49,961 high-quality SNPs out of 67,180 SNPs were physically mapped across 16 chromosomes with an average marker density of 40,395.13 kb per chromosome. The additional 17,219 SNPs were unmapped and included in the analysis as extra SNPs for diversity analysis. A genome-

wide SNP density plot (Figure 2) revealed that the highest number of SNPs were physically mapped to chromosome 4 (12.6%, 6,313 SNPs). The highest and lowest marker densities were observed on chromosome 1 (67,845 kb) and chromosome 16 (720,744 kb), respectively. Generally, transition SNPs (61.49%, 46,952 SNPs) were more frequent than transversions (38.51%, 20,228 SNPs), with a ratio of 2.32. The A/G transitions (31.23%) accounted for the highest frequency, while G/C transversions (7.92%) occurred at the lowest frequency among all the six SNP scenarios (Table 3).

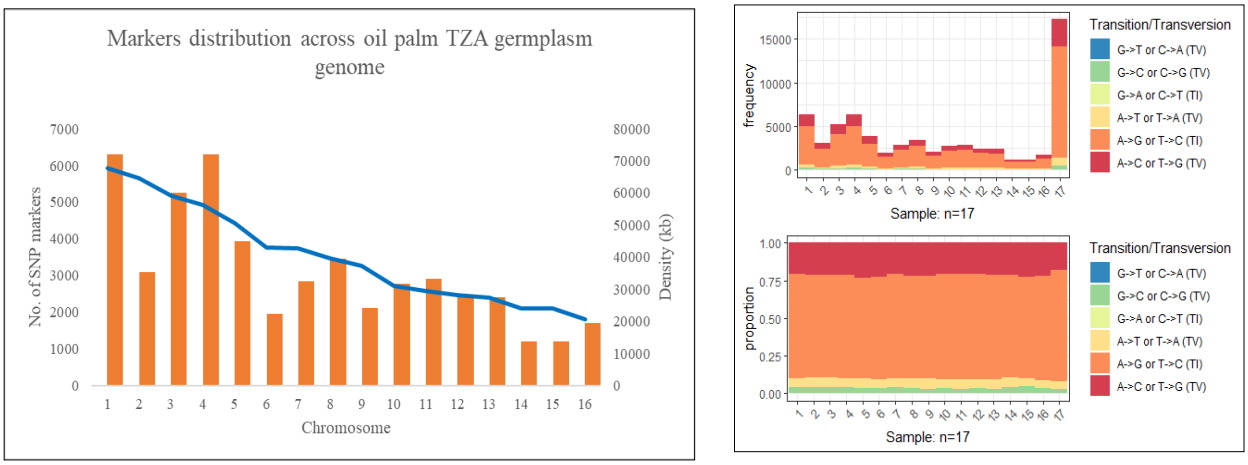

Figure 2: Markers distribution, the density and SNP variation in each chromosome.

Table 3: Percentage of transition and transversion SNPs across the *E. guineensis* genome.

SNP type	Transitions		Transversions			
	A/G	T/C	A/T	A/C	T/G	G/C
No. of allelic site	23525	23427	4008	6916	6903	2401
Frequencies (%)	31.23	30.26	11.23	9.91	9.45	7.92
Total percentage	46,952 (61.49%)		20,228 (38.51%)			

Diversity analysis

All populations showed comparable diversity patterns with almost similar values for all genetics parameters used (Table 4). The observed (H_o) and expected heterozygosity (H_e) were very similar among the population and all values were low signifying the presence of higher admixture that could arise due to the existence of historical exchanges of seeds through informal seed system within nearby provinces in Tanzania. A total of 22,707 (33.9%) SNPs had a MAF less than 0.2. TZA02 obtained the highest polymorphism content (PIC) among the populations. As expected, H_e value was slightly greater than the PIC value since PIC values will always be smaller than H_e and will become closer to H_e with more alleles and with increasing evenness of allele frequencies (Shete et al., 2000). Botstein et al. (1980) reported that markers with a PIC value of 0.25 to 0.5 were moderately informative as observed in this study. H_e and PIC values are both measures of genetic diversity among genotypes in breeding populations, which reflect the evolutionary pressure on the alleles and the mutation rate per generation per locus (Shete et al., 2000).

Table 4: Genetic parameters used to determine TZA *E. guineensis* diversity.

Populations	H_o	H_e	MAF	Fi	PIC
TZA02	0.36	0.36	0.27	-0.006	0.29
TZA03	0.35	0.35	0.27	0.015	0.28
TZA05	0.37	0.36	0.27	-0.045	0.28
TZA 13	0.37	0.35	0.27	-0.042	0.28

Ho = Observed Heterozygosity; He = Expected Heterozygosity; MAF = Minimum Allele Frequency; F = Fixation Index; PIC% = Percentage of Polymorphic Information Content.

Analysis of molecular variance (AMOVA)

Low pairwise F_{ST} values (0.026-0.032) were obtained according to Nei's genetic distance analysis indicating low differentiation and high gene flow between populations (Table 5). An $F_{ST} = 0$ indicates no differentiation among populations, $F_{ST} > 0.15$ indicates significant in differentiating populations while $F_{ST} = 1$ indicates complete differentiation (Bird et al., 2017; Frankham et al., 2002). Low F_{ST} value coincided with the AMOVA results where the vast majority of total variation (93%) was accounted for within-population variations while only 7% of the total variation was accounted for by among-population variations (Table 6). This finding was predictable because the collection sites between populations in Tanzania were not more than 20 km apart and might contribute to the low variability.

Table 5: Pairwise F_{ST} values among four populations in Tanzania.

Populations	TZA02	TZA03	TZA05	TZA13
TZA02	0.000			
TZA03	0.028			
TZA05	0.031	0.028		
TZA13	0.026	0.035	0.032	0.000

Table 6: Analysis of molecular variance (AMOVA) of the genetic variation among and within Tanzania populations.

Source	d.f.	Sum of squares	Mean squares	Est. Var.	%
Among populations	3	388,041.2	129,347.08	2,924.93	7%
Within populations	112	5,402,784.2	48,239.14	48,239.14	93%
Total	115	5,790,825.4		51,164.07	100%
PhiPT: 0.057		P(rand >= data): 0.001		N_m : 4.14	

Probability, P(rand >= data), for PhiPT is based on standard permutation across the full data set.
PhiPT = AP / (WP + AP) = AP / TOT; Nm = [(1 / PhiPT) - 1] / 4; AP = Est. Var. Among Pops, WP = Est. Var. Within Pops; d.f. is the degrees of freedom.

Population structure analysis

The principal component analysis (PCA) indicated that the 116 *E. guineensis* accessions could be clustered into three subgroups (Figure 3), and these results coincided with the dendrogram analysis (NJ tree) (Figure 4). Group 1 clustered half of the total accessions from the four populations clustering, irrespective of their geographical origin. This might be due to genetic exchange among geographical regions, which were located close to or overlapping each other in Tanzania. The remaining accessions from population TZA02 and TZA13 were clustered in Group 2 without any single accessions from TZA03 and TZA05. On the other hand, a small number of accessions from TZA03 deviated and formed three small subgroups (Group 3) while the majority of them can be somewhat clustered into both Group 1 and Group 2. This subgroup can be clearly seen on the NJ dendrogram which showed that 18 accessions from TZA03 formed a separate group at a different branch from the rest of TZA03. This finding suggested that certain specific traits intentionally or unintentionally selected by germplasm collectors might also lead to population structure. However, it was suggested that the admixture of accessions between two Groups would exist if a larger sample size was included in the analysis.

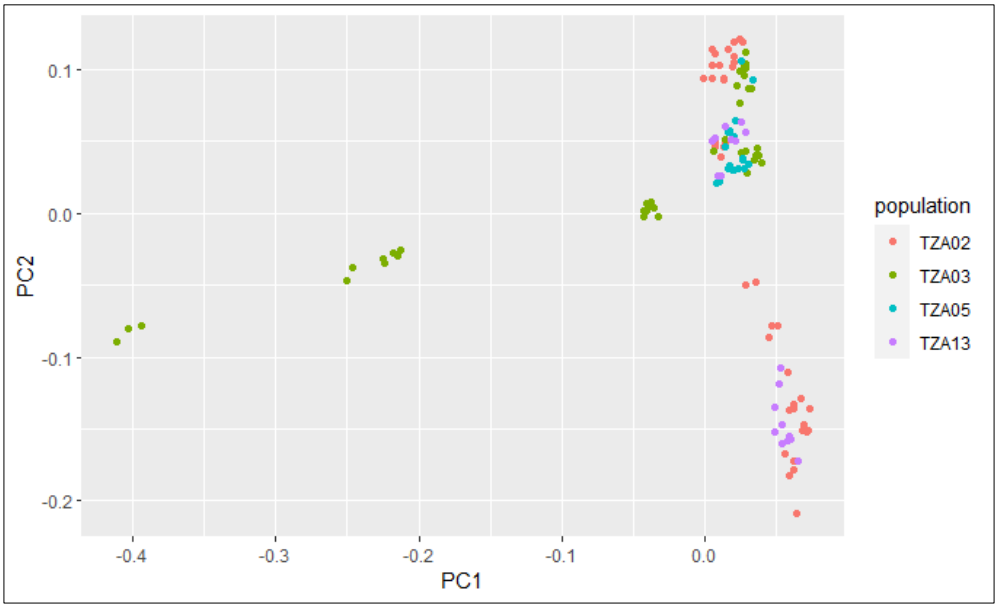

Figure 3: PCA based on genetic distance showing three subpopulations within studied *E. guineensis* accessions.

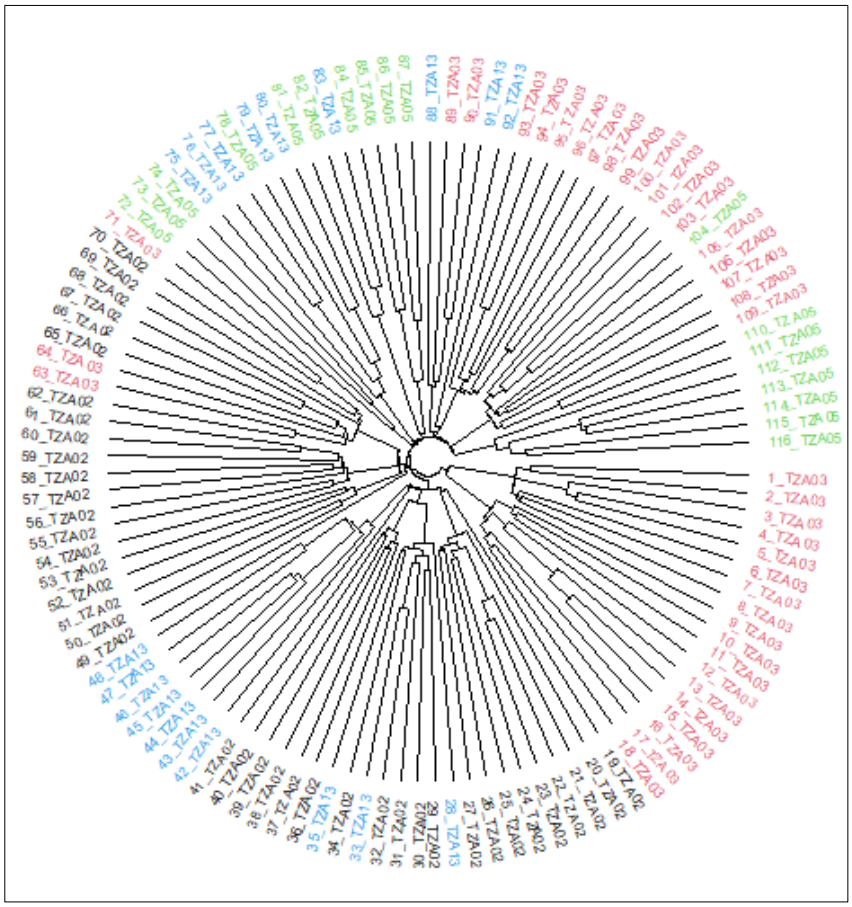

Figure 4: The NJ phylogenetic tree based on genetic distance matrix of *E. guineensis* accessions.

Conclusions

In this study, high-throughput SNPs was used to explore genetic diversity and population structure among MPOB-Tanzania oil palm accessions and the possibility of using 92K SNP array in genomic analysis. This level of genetic diversity could be the basis to understand the variability of MPOB-Tanzania *E. guineensis* germplasm and important for the genetic enhancement of desirable traits, as well as establishing the core collection without losing too much genetic source. Our study identified three subpopulations, which could be explained by their close proximity of geographical location and natural selection. This knowledge of population structure and genetic diversity of MPOB-Tanzania accessions should be extended to all Tanzania populations and other germplasm populations using at least 30-50% of SNPs used in this study as a foundation for future marker-assisted selection (MAS), genomic selection (GS) and genome-wide assosication study (GWAS) in *E. guineensis* breeding programs at MPOB.

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The Effect of Different Latex Stimulants on *Hevea brasiliensis* Transcriptome and Sucrose Metabolism

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Introduction

Latex, a rubber-containing cytoplasmic component, is produced in laticifers, which are highly differentiated cells that synthesise and store latex in the inner bark of rubber tree (*Hevea brasiliensis*). In natural rubber production, latex is harvested by periodical tapping of the trunk bark. Wounding of the bark caused by tapping induces endogenous ethylene production. Ethylene is a gaseous plant hormone involved in the regulation of diverse biochemical, physiological and developmental processes in plants. The role of ethylene in defense responses to wounding, herbivory and pathogen infection has been widely studied in plants (Dubois et al., 2018). Ethephon, an ethylene releaser, effectively increases the latex yield in rubber trees and ethylene stimulation is commonly practiced in rubber tree plantations worldwide (Zhu and Zhang, 2009). Ethephon application to the bark enhances and prolongs latex flow and latex regeneration, usually resulting in a 1.5- to 2-fold increase in latex yield (Zhu and Zhang, 2009). However, intense mechanical damage to bark tissues by excessive tapping and/or overstimulation with ethephon induces severe oxidative stress in laticifer cells, which often causes the tapping panel dryness (TPD) syndrome in rubber tree.

Several types of approaches, including transcriptome analyses, have been conducted previously to explore the molecular mechanisms involved in the response of rubber tree to ethylene (Duan et al., 2010; Chow et al., 2011; Wang et al., 2013; An et al., 2015; Putranto et al., 2015; Dai et al., 2016; Montoro et al., 2018; Nazri, 2020). Transcriptome analysis is a powerful approach to understand gene regulatory mechanisms. However, previous studies have provided only limited information on ethylene-specific events in rubber trees because ethephon was applied to tapping panels of mature trees in a plantation before or after tapping and, therefore, the effects of wounding were not excluded (Nie et al., 2016). Thus, an experimental system suitable for analysis of the ethylene-specific response needs to be established.

In the present study, we used mature rubber tree for latex stimulant application of RRIM HYDROBEST™ andethephon as well as untapped rubber trees to obtain gene expression profile. The availability of *H. brasiliensis* transcriptomic and genomic resources made it possible to identify all the different genes acting in the ethylene and jasmonate signalling pathway and characterise their implication during development, and in response to TPD. Dynamic and specific changes in gene expression profiles will be revealed in response to various latex stimulant treatments. The study provides valuable information on the biochemical and metabolic responses to ethylene in rubber trees, in addition to the establishment of a model experimental system useful for studying latex biology and rubber biosynthesis.

Materials and Methods

Plant materials and RNA extraction

Mature rubber trees from RRIM 3001 *Hevea* genotype and tapped on first virgin panel (BI-1) with S/2 d3 (half spiral, tapped once in three days) tapping system at the experimental Field 109, RRI Experimental Station (RRIES) Pelepah, Kota Tinggi, Johor was selected for the study. The latex sample was collected from four groups of trees namely healthy trees without latex stimulation (Control), untapped trees, RRIM HYDROBEST™-treated trees and Ethephon-treated trees. Each

group of trees had three biological replicates with one tree per replicate. For RNA extraction, an extraction buffer (300 mM LiCl, 10 mM disodium salt EDTA, 10% SDS and 100 mM Tris) was added with the same amount of latex and was then kept on ice. Once arrived in the lab, the same amount of TRIzol was added to the mixture and total RNA was extracted according to the manufacturer's instructions (Invitrogen Life Technologies RNA, UK).

RNA-seq preparation

Total RNA of each sample was quantified and the quality determined using Agilent 2100 Bioanalyzer (Agilent Technologies) and NanoDrop (Thermo Fisher Scientific Inc.). Total RNA (1 µg) with RNA Integrity Number (RIN) value above 7 was used for library preparation. Next generation sequencing library preparations were constructed according to the manufacturer's protocol (NEBNext® Ultra™ RNA Library Prep Kit for Illumina®). Paired-end sequencing was performed using Illumina HiSeqX platform (Illumina, San Diego, CA, USA) for three independent biological replicates. Quality of raw sequencing reads were assessed by FastQC v0.11.8 (Andrews, 2010). Pre-processing of the data were performed using Trimmomatic v0.38 (Bolger et al., 2014) with the parameters comprising ILLUMINACLIP:TruSeq3-PE.fa:2:30:10 LEADING:20 TRAILING:20 SLIDINGWIND OW:8:20 MINLEN:50. Clean reads of each sample were mapped to the reference genome (*H. brasiliensis*, RefSeq assembly accession: GCF_001654055.1) using HISAT2 v2.1.0 (Kim et al., 2015) with the following parameters comprising minimum intron length = 50, maximum intron length = 15,000, and reports alignments tailored specifically for cufflinks.

Expression and differential expression

Analyses of reference gene expression and differential expression were carried out using Cufflinks v2.2.1 (Trapnell et al., 2010; Roberts et al., 2011) and Cuffdiff v2.2.1 (Trapnell et al., 2010, 2013) with the following parameters namely maximum fragments allowed in a bundle before skipping = 5,000,000, use 'rescue method' for multi-reads (more accurate), and use bias correction - reference fasta required. The expression level of each gene was expressed as Fragments Per Kilobase of gene per Million fragments mapped (FPKM). Differentially expressed genes were defined as genes that are measured to be at least 4-fold different in expression level (log2 fold-change $\leq$ -2 or log2 fold-change $\geq$ 2) with false discovery rate less than 5% ($q \leq 0.05$). In addition, genes of which the expression detected only in one condition with $q \leq 0.05$ were also considered differentially expressed.

Determination of sucrose content

The objective of this experiment was to evaluate the sucrose content in different tapping system using Cary 60 UV-Vis Spectrophotometer and to relate the findings to the transcriptomics analysis results. Three different tapping systems were tested namely trees without latex stimulant, trees with Ethephon 5.0% 9/y, and trees with RRIM HYDROBEST™. Test samples were prepared by diluting 0.1 mL of latex serum with 0.4 mL of trichloroacetic acid. Sucrose content was determined using the anthrone method described by Scott and Melvin (1953). Absorbance of the samples were measured at 620 nm using a UV-VIS Spectrophotometer (SHIMADZU UV-1800). Sucrose content of the samples were determined using a calibration curve prepared with different sucrose concentrations ranging from 40-200 $\mu g ml^{-1}$.

Statistical analysis of data

Data were analysed using SAS software. ANOVA was done for the variables followed by mean separation using Least Significant Difference (LSD) at the significance level of 0.05.

Results and Discussion

Latex transcriptomes sequencing and characterization

A total of twelve samples were sequenced using Illumina HiSeqX system resulting in 12 transcriptome datasets. The throughput for all 12 samples was at least 6.4 Gb data per sample. Sequencing reads were pre-processed to obtain high-quality data for further analysis. Quality assessment of sequencing reads were carried out on each dataset before and after data pre-processing. In brief, more than 92% of sequencing data were retained after quality filtering process, indicating sequencing was performed well. Paired clean reads were aligned to reference genome (cultivar Reyan7-33-97) (Tang et al., 2016). Approximately 95% of reads from each sample were found mappable to the reference genome, with unique mapping rate achieving about 90%.

Clean reads mapped to genes annotated in reference genome were quantified and normalized to gene expression unit termed as FPKM (Fragments Per Kilobase of gene per Million fragments mapped). Genes with FPKM = 0 were not expressed, while genes with FPKM > 0 were considered to be expressed genes. In brief, an average of around 59% of the 42,489 reference genes tested were found to be expressed in each sample.

Identification of differentially expressed genes (DEGs)

Differential expression analyses among sample groups were conducted in pairwise, i.e. in "Test vs. Control (HT)" design. Genes were regarded as significantly differentially expressed (DEG) if the expression is ≥4 folds different in a comparison with $q \leq 0.05$. Genes of which the expression detected only in one condition with $q \leq 0.05$ were also considered differentially expressed. The differential expression analysis results are summarised in Table 1. Genes related to treatments using Ethephon (ET), RRIM HYDROBEST™ (RHB) and untapped trees (UT) recorded 7586, 5462, and 6782 DEGs, respectively.

Table 1: Number of statistically significant differentially expressed genes (DEGs) in each sample group comparison.

Comparison	Total DEG	Upregulation	Downregulation
ET vs. HT	7586	3855	3731
RHB vs. HT	5462	2831	2631
UT vs. HT	6782	3477	3305

HT= control, ET= ethephon; RHB= RRIM HYDROBEST™; UT = untapped trees

Functional classification and characterisation of DEGs

Based on sequence identity, GO terms associated with DEGs were tested using enrichment analysis. Numbers of GO terms achieving $p \leq 0.1$ are summarised in Table 2. All related genes were mostly associated with biological process, followed by molecular function and cellular component.

Table 2: Number of enriched GO terms in DEGs tested.

Comparison	Total GO terms enriched	GO category (Percentage)		
		Biological process	Cellular component	Molecular function
ET vs. HT	487	229 (47%)	103 (21%)	155 (32%)
RHB vs. HT	369	180 (49%)	69 (19%)	120 (32%)
UT vs. HT	440	219 (50%)	87 (20%)	134 (30%)

To further understand the functions of DEGs, we mapped all DEGs to KEGG pathways and compared them with the whole-transcriptome background, with the goal of searching for genes involved in metabolic or signal transduction pathways that were significantly enriched. Numbers of Pathways achieving $p \leq 0.1$ are summarised in Table 3. Interestingly, pathways related to RHB was the highest

with 12 and only 3 pathways were involved with ET simulation. Both RHB and UT but not ET have pathways related to natural rubber biosynthesis such as terpenoid backbone biosynthesis.

Table 3: List of pathways in each sample group comparison.

Comparison	Total significant pathways enriched	List of identified pathways
ET vs. HT	3	(1) Betalain biosynthesis, (2) Synthesis and degradation of ketone bodies, (3) Ribosome
RHB vs. HT	12	(1) Carbon metabolism, (2) Terpenoid backbone biosynthesis, (3) Fatty acid degradation, (4) Fatty acid metabolism, (5) Ribosome, (6) Synthesis and degradation of ketone bodies, (7) Pyruvate metabolism, (8) Valine, leucine and isoleucine degradation, (9) beta-Alanine metabolism, (10) Propanoate metabolism, (11) Butanoate metabolism, (12) Taurine and hypotaurine metabolism
UT vs. HT	7	(1) Glycosphingolipid biosynthesis - globo and isoglobo series, (2) Protein processing in endoplasmic reticulum, (3) Various types of N-glycan biosynthesis, (4) Arachidonic acid metabolism, (5) Betalain biosynthesis, (6) Terpenoid backbone biosynthesis, (7) N-Glycan biosynthesis

Sucrose metabolism analysis

The transcriptomes of rubber tree latex revealed that the sucrose metabolism was heavily affected by both types of latex stimulants and tapping practice. Sucrose is subject of interest as it is a precursor for rubber biosynthesis. The effect of latex stimulants and tapping on sucrose could be beneficial for the rubber tree productivity. The key genes related to sucrose metabolisms have similar expression profile in both RHB and ET treatments except for the *HbSPS3* gene with its expression was downregulated when treated with ET. The *HbSPS3* gene involved in the synthesis of sucrose in the cell and its downregulation suggested that ET could not enhance the synthesis of sucrose. The expression of *HbSPS3* gene was not upregulated nor downregulated. For the untapped rubber tree, the expression profile contrasted with the two latex stimulants as the group of sucrose catabolism genes were downregulated, however the group of sucrose synthesis genes were upregulated. This suggested that by not tapping rubber tree, there is no need to break down sucrose for natural rubber biosynthesis.

Sucrose content in the latex under RRIM HYDROBEST™ and other types of stimulation

Figure 1 summarised the results for the comparison of sucrose content in latex obtained from different stimulation practices obtained during medium-yielding period. The sucrose contents in latex treated with ET was the only markedly lower than control suggesting the application of ET could increase sucrose utilization in rubber trees. However, the application of RHB stimulation had similar effect on sucrose content compared to the control.

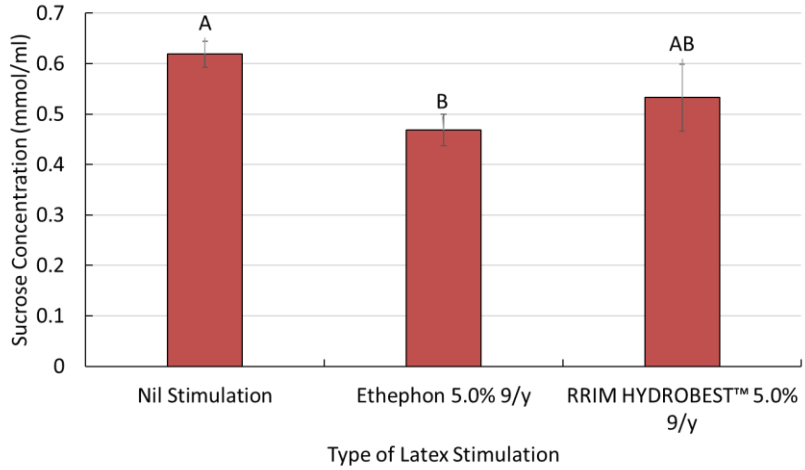

Figure 1: The sucrose content in latex with different tapping system. The ANOVA test and the Newman–Keuls test were used in the statistical analyses ($p < 0.05$).

Conclusion

Previous study on RHB revealed that it produced better rubber tree productivity than ET but with less TPD incidence. Our transcriptomics study concluded that the added benefits of using RHB could be due to the ability of RHB in enhancing sucrose metabolism, which subsequently enhances the long process of rubber biosynthesis starting from Calvin cycle to isopentenyl diphosphate (IPP) metabolism. In addition, RHB induced more pathways compared to ET, which could contribute further to its beneficial ability. Further work can be conducted to characterise the DEGs and the roles of pathways other than rubber biosynthesis. The knowledge and understanding from this study can be utilised to improve rubber tree production in the future.

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Real-time PCR SYBR Green Specific Detection Method of *Bt* Brinjal Event EE-1

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Introduction

Brinjal or eggplant (*Solanum melongena* L.), a member of the Solanaceae family, is an important crop that is widely cultivated and consumed in Asia's South-Eastern and Southern regions (Kuwardadra et al., 2020). This crop is widely grown in India, Bangladesh, Pakistan, China, and the Philippines. However, it is vulnerable to many insect and pest attacks, particularly the Eggplant Fruit and Shoot Borer (EFSB), which has caused significant damage in brinjal cultivation, with yield losses of up to 80% (Prodhan et al., 2018). EFSB is caused by the *Leucinodes orbanalis* Guenee family of Lepidoptera, which causes damage to brinjal crops during both the vegetative and reproductive stages (Raina and Yadav, 2018).

In view of the pest and diseases serious problems, genetically modified insect-resistant brinjal commonly known as *Bt* brinjal has been developed by Maharashtra Hybrid Seeds Company (Mahyco), India. *Bt* brinjal designated as EE-1 contains insect resistance gene (*Cry1Ac*) under the control of cauliflower mosaic virus 35S (CaMV 35S) promoter, with neomycin phosphotransferase II (*nptII)* gene as the selectable marker and terminator of *nopaline synthase* (T-NOS). The gene construct was incorporated into brinjal genome using *Agrobacterium*-mediated genetic transformation to control feeding damage caused by EFSB (Mainali, 2014). *Bt* brinjal has been approved for cultivation in Bangladesh and the Philippines in 2014 and 2021, respectively (Shelton et al., 2017).

In Malaysia, brinjal is mainly imported from Thailand and Bangladesh in order to meet market demand. Therefore, a reliable, accurate, and sensitive analytical method for detection of living modified organisms (LMOs) is required in order to meet the regulatory obligations and legislative requirements of Malaysia Biosafety Act 2007. PCR assay is the most widely used method for LMO detection because of their simplicity, sensitivity, and reliable protocols. Among PCR based assay, Real-time PCR (qPCR) method is considered to be easy, useful, and accurate. *TaqMan* chemistry based on the simultaneous addition of two primers and a specific probe is one of the most commonly used assays in LMO detection. While SYBR Green which exhibits fluorescence enhancement upon binding to the double-stranded amplification product offers an inexpensive alternative (Hernandez et al., 2004).

LMO detection strategies commonly used in LMO detection are element-specific, construct-specific, and event specific. Among all, event specific detection is the best, most accurate and most important method in LMO detection. These methods identify the presence of the specific DNA sequences that span the junction region between the plant host DNA and the inserted foreign DNA. Since the position of integration of the gene construct in the plant genome is unique to the particular event, the use of an event specific method will specifically identify the event of the LMO in question (Xu et al., 2009). Hence, in this paper we report the development of event-specific detection method using qPCR-SYBR Green for detection of *Bt* brinjal event EE-1 which would be useful to meet the regulatory obligations.

Materials and Methods

Plant materials

Certified reference materials (CRM) MON531 (AOCS 0804-C) contains gene construct similar to EE-1 brinjal, which consist of *Cry1Ac* and *nptII* gene as well as 35S promoter and NOS terminator. The CRM purchased as dry powders from Monsanto Company via Team Medical and Scientific Sdn. Bhd. was used in PCR efficiency determination. Seed of *Bt* brinjal strictly for research purposes was provided by Department of Biosafety Malaysia.

DNA extraction

In each reaction, 0.1 g of either CRM 531 powder or *Bt* brinjal seeds were lysed using tissue lyser (Qiagen, Germany) before the genomic DNA was extracted. DNeasy Plant Mini Kit (Qiagen, Germany) was used according to the manufacturer's protocol to extract the genomic DNA. The extracted DNA was then quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific, US). The DNA concentration was estimated at A_{260} value where 1 absorbance unit is equal to 50 ng/µL, while the purity was assessed via absorbance ratio of $A_{260/230}$, with a value of ≈ 1.8 being considered as pure.

Primer synthesis, optimize and verification

DNA sequence of primers specific for plant, element, construct, and event were mined from sequence available in the public domain. Primers specific to *Bt* brinjal EE-1 were retrieved from Ballary et al., 2012. All primers were synthesised by Integrated DNA Technologies (IDT, Singapore). The primers were then optimized and verified. Selected primers sequences and expected amplicon size are listed in Table 1.

Table 1: Primer sequence used in this study.

Primer	Sequence	Amplicon size (bp)
rbcL-146 (F)	5'-CTTGGCAGCCTTCCGAGTAA-3'	146
rbcL-146 (R)	5'-AGCATCGCCCTTTGTAACGA-3'	
35S-147 (F)	5'-GACAGTGGTCCCAAAGATGG-3'	147
35S-147 (R)	5'-GTCTTGCGAAGGATAGTGGG-3'	
Nos-69 (F)	5'-GATTAGAGTCCCGCAATTATACATTTAA-3'	69
Nos-69 (R)	5'-TTATCCTAGKTTGCGCGCTATATTT-3'	
Cry1Ac-74 (F)	5'-GAGGAAATGCGTATTCAATTCAAC-3'	74
Cry1Ac-74 (R)	5'-TTCTGGACTGCGAACAATGG-3'	
NptII-114 (F)	5'-TGCCTGCTTGCCGAATATCA-3'	114
NptII-114 (R)	5'-ATATCACGGGTAGCCAACGC-3'	
EE1 (F)	5'-CGTTTCCCGCCTTCAGTTTA-3'	151
EE1 (R)	5'-GCGGTGATAATTGAATGCAT-3'	
BtB-nptII (F)	5'-ACGCCGGCTGGATGATC-3'	126
BtB-NOS (R)	5'-AAGACCGGCAACAGGATTCA-3'	

qPCR SYBR Green

qPCR-SYBR Green assay were performed in 7500 Fast Real-time PCR (Applied Biosystems, USA). All qPCR reactions were carried out in 96-well micro-titer plate with a total volume of 20 µL reaction containing 10 µL of SYBR Select Master Mix (Applied Biosystem, USA), 10 pM of forward and reverse primers, DNA template, and RNase free water. The thermal cycling program comprise a single cycle of DNA polymerase activation for 10 min at 95 °C followed by 40 amplification cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 1 min. All reactions were performed in triplicate. Negative and positive control were analyzed in parallel, in addition ribulose-bisphosphate carboxylase (rbcL) was used as an internal positive control (IPC) to evaluate the absence of PCR

inhibition. Data were collected and processed using ABI 7500 fast Real-time PCR System v.2.3 software (Applied Biosystems, USA). C_T value below 38 was considered as a positive amplification.

PCR amplification efficiency of each primer was determined by standard curve method. Genomic DNA of CRM MON 351 or *Bt* Brinjal at serial dilution of 100 000, 10 000, 1000, and 100 were used as template. Standard curve was established from the amplification plot (Rxn vs. cycle). The slope of the calibration curve was used to calculate the amplification efficiency using the formula of Efficiency = $10^{(-1/\text{slope})}$ -1 (European Network of GMO Laboratories).

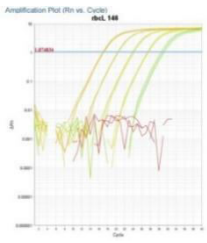
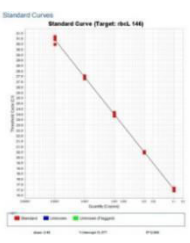
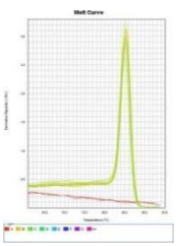
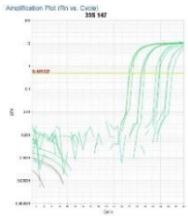
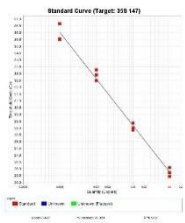
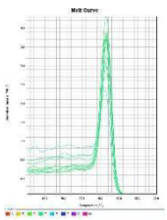
Melting curve analysis was used to determine the specificity of the qPCR assay. A melting curve was performed after the amplification cycles have been completed. The temperature is incrementally increased around 0.5 °C per cycle starting at 60 to 95 °C. As the temperature is increased, the fluorescence will gradually decrease evenly as the dye is pulled off the double stranded DNA. The melting temperature (T_m) curve of the amplicons was generated by plotting the negative derivative reporter versus temperature (-Rn vs T). A single distinct peak in the plot indicates amplification of double-stranded DNA product is specific.

Results and Discussion

Primer selection

SYBR Green-based detection is the least expensive and easiest method available for qPCR. However, to obtain consistent and accurate results, good controls are crucial. A high percentage of PCR efficiency of primers used is one of these key controls. Many researchers use the standard curve method to estimate PCR efficiency. In this study only primers that amplified diluted sample of CRM MON351 with more than 90% PCR efficiency were selected.

Specificity of the primer is another key control in SYBR Green-based qPCR. SYBR Green which detection is based on binding into double stranded DNA may detect any double-stranded DNA including non-specific amplicons or primer dimers. Therefore, optimal design of primers is essential to ensure that no unexpected double-stranded DNA is created during amplification. Hence only primer pairs that has one specific pick on melting curve were chosen for qPCR SYBR Green analysis. Analysis of PCR efficiency and specificity on each selected primer was shown in Figure 1. The percentage of PCR efficiency, slope, and correlation coefficient value were listed in Table 2.

Primer	A	B	C
rbcL			
35s promoter			

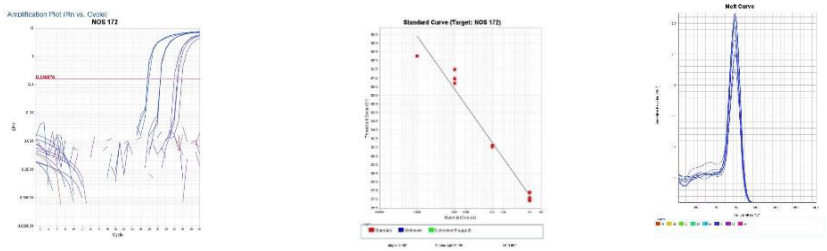
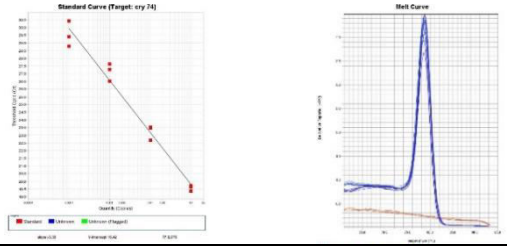
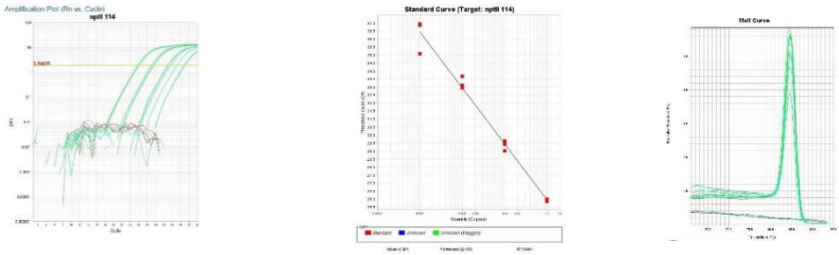
NOS terminator	
Cry1Ac	
nptII	

Figure 1: PCR efficiency and specificity analysis of primers selected. Column (A) shows amplification curves generated by serial dilution of CRM MON531 genomic DNA at 100 000, 10 000, 1000, and 100 dilution. Column (B) shows standard curve showing diluted template vs. threshold cycle (C_t) generated a slope value as listed in Table 2. Column (C) shows melting curve analysis of each primer shows single specific peak with T_m value listed in Table 2.

Table 2: List of selected primer for universal plant (rbcL), element specific (35S promoter, NOS terminator, and Cry1Ac) with percentage of PCR efficiency, slope, and correlation coefficient value.

Sequence Name	Percentage PCR Efficiency	Slope	Correlation Coefficient value (R^2)
rbcL	95%	-3.46	0.999
35s promoter	96%	-3.43	0.989
NOS terminator	101%	-3.29	0.966
Cry1Ac	98%	-3.38	0.976
nptII	93%	-3.50	0.984

Bt brinjal EE-1 detection

The *Bt* brinjal EE-1 gene construct (Figure 2) consists of *Cry1Ac* gene, which encodes for CrylAc protein (δ-endotoxins) of 130 kDa that is highly specific to *Lepidpoteran* larvae. The gene is flanked by CaMV 35S promoter and 7S-3' terminator. Two marker genes are included in the construct to identify transformed cells containing the *Cry1Ac* gene. The first marker is *nptII* gene, which encodes for enzyme neomycin phosphotransferase II. The gene is flanked by CAMV 35S promoter and the *Agrobacterium tumerficiens* napoline synthase terminator (T-NOS). Another marker is *aad* gene, which encodes for the bacterial selectable marker enzyme 3" (9)-O-aminoglycoside adenyl transferase (AAD) and allows for the selection of transformed bacteria on media containing spectinomycin or streptomycin.

Routine LMO detection are usually based on the promoter, terminator, and genes elements exist in the construct. Hence, primer for element specific of *Bt* brinjal (*35S*, *nptII*, *T-NOS,* and *Cry1Ac*) were optimized. qPCR SYBR Green assay carried out using the primers showed the amplification of respective amplicon in *Bt* brinjal but absence in native brinjal (Figure 3). Element specific detection has the lowest specificity and is mainly used for rapid first-level screening at relatively lower cost.

Figure 2: Schematic diagram of recombinant construct of *Bt* brinjal event EE-1 (Ballari et al., 2012). Location of primer and amplicon size used in this study are shown on top of the diagram corresponding to element specific [p-35S (147 bp), *nptII (144 bp)*, T-NOS (69 bp), and *Cry1Ac* (74 bp)], construct specific (*BtB-nptII* (F) and *BtB*-NOS (R), 126 bp) and event specific (EE-1, 151 bp) of *Bt* brinjal EE-1 detection.

The primer pairs *BtB-nptII* (F)/*BtB-NOS* were used to detect construct-specific of *Bt* brinjal EE-1. The primer targeted the integration of *nptII* gene and NOS terminator. The 126 bp fragment of the junction region between the *nptII* and NOS terminator was amplified only in *Bt* brinjal but absence in native brinjal (Figure 3). However, gene construct detection was not able to distinguish between two different events transformed with the same plasmid (Holst-Jensen et al., 2009). Event-specific detection can precisely distinguish legitimate transgenic events from related illegal varieties transformed with similar or identical transgenic constructs, thus it is often used to evaluate the legality of a GMO sample (Wu et al., 2014). The primer pair EE-1 (F)/(R) is unique to *Bt* brinjal EE-1, and detects the junction region between the right border of T-DNA and the 3' transgene brinjal integration site (Figure 2). Amplification was detected only in *Bt* brinjal EE-1 and was absent in native brinjal (Figure 3). The result of conventional PCR from a previous study carried out on other GM plants also indicated that the amplification of EE-1 is specific to *Bt* brinjal EE-1 (unpublished data).

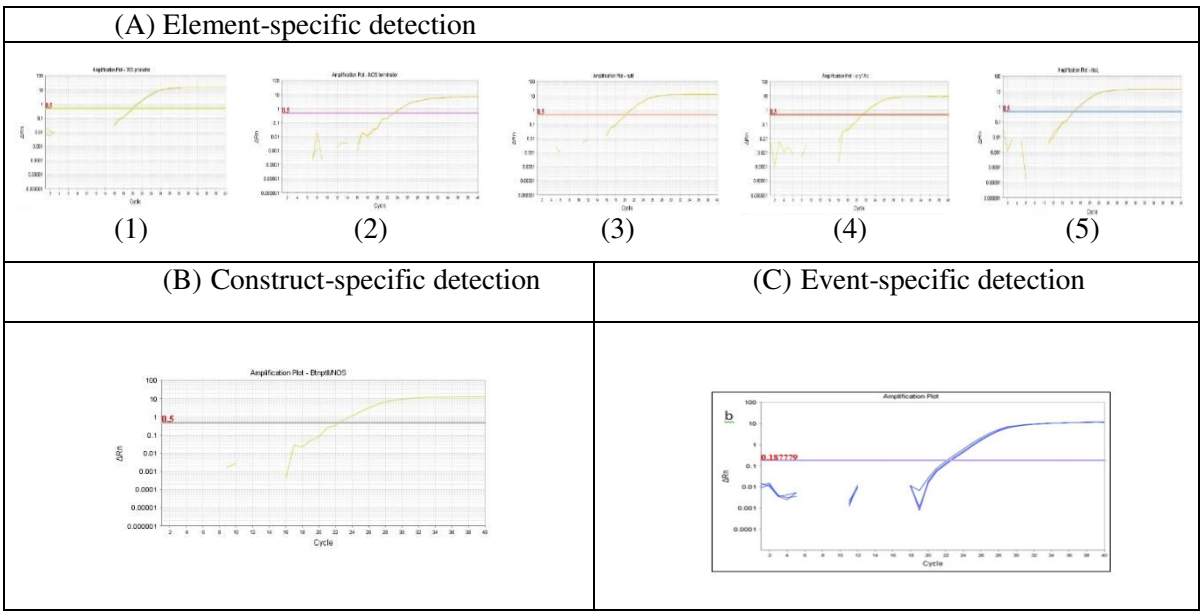


Figure 3: Amplification plot of *Bt* brinjal EE-1 with; (A) Element specific detection of 35S promoter (1), NOS terminator (2), *nptII* (3), *Cry1Ac* (4), and rbcL (5) at threshold value of 0.500. (B) Construct-specific and (C) event-specific detection shows the amplification of *BtB-nptII*/NOS at threshold value of 0.500 and EE-1 at threshold value of 0.187, respectively. All the Ct values of amplification are less than 30.

Conclusion

In the present study, detection method for *Bt* brinjal EE-1 using qPCR-SYBR Green method has been optimized based on previous finding reported by Ballari et al. (2012) with slight modification. Primers for element-specific, construct-specific, and event-specific have been verified and could be used in LMO detection. EE-1 primer has been verified to exclusively amplify DNA of event EE-1. The methods developed are ready for *Bt* brinjal detection in Malaysia and it is hoped that they will help the Department of Biosafety regulate LMO activities in Malaysia more effectively.

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Antibacterial Screening of Selected Nanoparticles and Essential Oils Against *Bukholderia glumae*

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Introduction

Burkholderia glumae, a gram-negative, non-fluorescent, rod-shaped bacterium with a polar flagellum, is the primary cause of the rice disease known as Bacterial Panicle Blight (BPB). It is a seed-borne rice pathogen, and BPB can induce 75% yield loss in severely infested fields (Zhou-qi et al., 2016). BPB is present in 18 countries and it is considered to be one of the most important emerging diseases in rice-growing regions around the world. It has distinctive characteristics that differ from other rice diseases, which include changes of panicle color, discoloration of emerging grains, grain rot and spikelet sterility (Zhou, 2019). The disease first hit Malaysia in 2017 in Sungai Ache, Penang and has now affected nearly 445 hectares of paddy fields nationwide (Ramachandran et al., 2021). Currently, there no known complete resistance variety for this disease and only a few partially resistant varieties are commercially available. As for chemical control agent, oxolinic acid is the only known chemical that is able to limit the spread of the disease. However, this chemical is not registered for agricultural purposes in some countries including Malaysia and the natural occurrence of oxolinic acid-resistant strains limits the usage of this chemical. Given the challenges currently faced in controlling this economic importance disease, alternative strategies to cope and overcome the disease efficiently are being developed.

The antimicrobial effect of nanoparticles (NPs) has opened a new avenue for the development of nano-technology-based formulations for targeting plant pathogens. One of the most known NPs' toxicity mechanisms is the interaction between the bacterial cell membrane and NPs, which leads to the disruption of the bacterial membrane integrity and finally results in the death of the microorganism (Wang et al., 2017; Sharmin et al., 2021). Nanoparticles suitability as anti-phytopathogenic agents is further supported by research results indicating that nanoparticles can have a positive effect on the growth and development of crop plants and has the ability to induce tolerance to abiotic stress conditions in plants (Khan et al., 2019). In this study, as the first step in developing a formulation against *B. glumae* that incorporates nanoparticles, three nanoparticles, which are silver nanoparticles, zinc oxide nanoparticles and copper nanoparticles, were tested against *B. glumae.* These nanoparticles were selected as they have received much attention in targeting plant pathogens (Elmer et al., 2018). Seven essential oils, which are also known to exhibit antimicrobial properties, were also screened against *B. glumae.*

Materials and Methods

Materials and B. glumae bacterial strain

Three different nanoparticles, which are, copper nanoparticles at two different sizes 40-50 nm and 60-80 nm, zinc oxide nanoparticles <100 nm and silver nanoparticles <100 nm were purchased from Sigma-Aldrich Co. (St Louis MO). Copper sulphate, $CuSO_4$, in powder form, was purchased from a local seller. Distilled water was added to the nanoparticles and $CuSO_4$ powder to achieve the tested concentrations. All seven essential oils (EOs), which are tee tree *(Melaleuca alternifolia),* lemon myrtle (*Backhousia citriodora*), nilam (*Pogostemon cablin*), gelam (*Melaleuca cajuputi)*, cinnamon (*Cinnamomum verum*), citronella (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*), were supplied by MARDI Station Kuala Linggi. The *B. glumae* local isolate, coded B35, was obtained from MARDI Station Seberang Prai.

Antibacterial activity of selected nanomaterials and essential oils against Burkholderia glumae

The antibacterial activity of the nanoparticles, $CuSO_4$ and essential oils were evaluated against local *B. glumae* on Luria-Bertani (LB) agar plate using well diffusion assay. Briefly, 100 µL of *B. glumae* overnight culture (10^7 CFU/mL) was spread evenly on LB agar plate with a sterile cotton swab. A 9-mm-diameter well was then punched in the middle of the plate using a sterile cork borer. Next, 40 µL of each nanomaterial and $CuSO_4$ samples at different concentration (2, 4, 6, 8, 10, 20, 30, 40 and 50 mg/mL) was added into the well. For EOs, the initial stock solution was added into the well. The plates were incubated at 30 °C overnight and on the following day, the diameter of the inhibition zone was calculated edge to edge across the centre of the well.

Results and Discussion

It has been reported that metal and metal oxide nanoparticles, which include Ag, Cu and ZnO, exhibit a wide spectrum of antimicrobial activity against gram-positive and gram-negative bacteria as well as fungi (Usman, 2013). Out of the three different types of nanoparticles tested, only copper nanoparticles, Cu-NPs, showed antibacterial activity against *B. glumae*. It was also found that the size of copper nanoparticles did not affect the ability of the nanoparticle to inhibit *B. glumae* growth. Cu-NPs have been reported to have antibacterial effect on the bacterial cell functions in multiple ways, including adhesion to gram negative bacterial cell wall due to electrostatic interaction, oxidation of proteins and cleavage of DNA and RNA molecules (Longano et al., 2012). Previously, Mondal and Mani (2012) has reported the effectiveness of copper nanoparticles in controlling the bacterial blight of pomogranate caused by *Xanthomonas axonopodis* pv. *punicae*, which is also a gram-negative bacterium. The effect of copper nanoparticles on *B. glumae* was also compared with soluble copper compounds - copper sulphate, $CuSO_4$, which has been used as traditional inorganic antibacterial agents. It was found that Cu-NPs were able to inhibit the growth of *B. glumae* at a lower concentration than $CuSO_4$ (Table 1).

Table 1: Diameter of inhibition zone for $CuSO_4$, Cu-NP (60-80 nm) and Cu-NP (40-50 nm).

Sample solution concentration mg mL^{-1}	Diameter of inhibition zone (mm)		
	$CuSO_4$	Cu-NP 60-80 nm	Cu-NP 40-50 nm
2	0	0	0
4	0	0	0
6	0	0	0
8	0	0	0
10	0.00	14.7±0.3	14.8±0.2
20	12.3±1.5	16.0±0.6	15.0±0.3
30	14.3±0.7	15.0±0.6	18.0±0.4
40	15.0±2.0	20.0±0.0	18.0±0.2
50	18.0±0.8	21.7±0.9	18.5±0.6

For zinc oxide nanoparticles, Zn-NPs, it was observed that the nanoparticles were able to suppressed the growth of *B. glumae* (the halo zone is present yet not clear). On the other hand, biogenic ZnO-NPs synthesized by Ahmed et al. (2021) were reported to show significant antibacterial activity against *B. glumae* and *B. gladioli.* The green synthesis of the ZnO-NP could have modified the surface or intrinsic physiochemical properties of ZnO making it able to inhibit the growth of *B. glumae* better in comparison to commercially available ZnO-NP (da Silva et al., 2019). No inhibition zone was observed on all silver nanoparticles, Ag-NPs plates. Our observation match that of Ruparelia et al., 2008, where they observed Cu-NPs were more efficient in inhibiting and killing the bacteria *B. subtilis* compare to Ag-NPS, suggesting it may be due to more affinity of the Cu-NPs to surface amines and carboxyl groups of *B. subtilis*. The comparison of each nanomaterial and $CuSO_4$ inhibition zone at 40 mg mL^{-1} is presented in Figure 1. The presence of a halo zone for Cu-NPs and $CuSO_4$ samples indicates both were able to inhibit the growth of *B. glumae*. A hazy halozone was observed for Zn-NPs

suggesting that the nanoparticle may able to suppress but not inhibit the growth of *B. glumae* on LB agar plate. No halo zone was seen on all tested Ag-NPs agar plates.

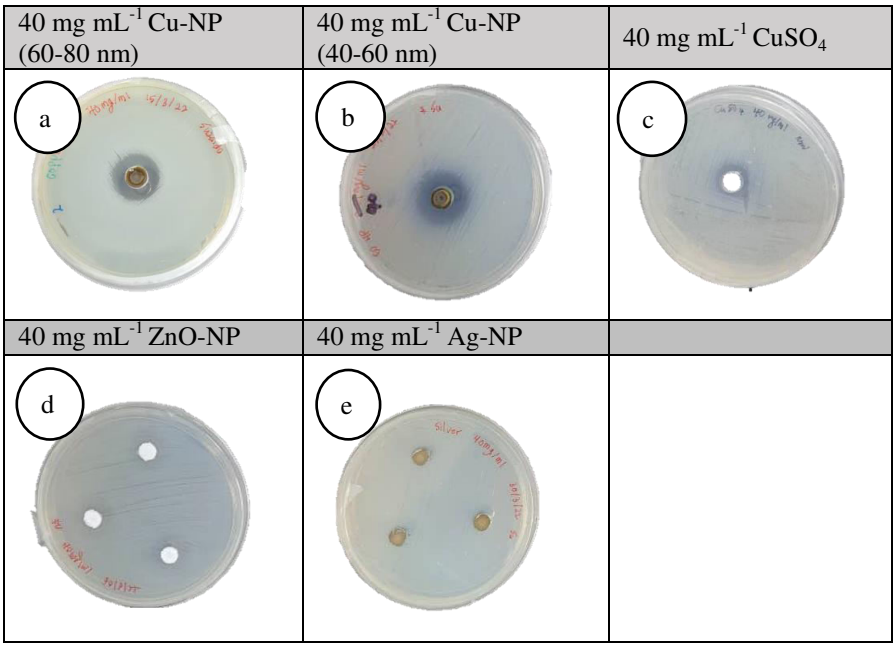

Figure 1: Halozone indicating presence or lack of antibacterial activities of various nanoparticles and samples at 40 mg mL^{-1} (a) 60-80 nm Cu-NP (b) 40-60 nm Cu-NP (c) $CuSO_4$ (d) ZnO-NP and (e) Ag-NP.

The minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC) of Cu-NPs were then determined. The MIC and MBC value of Cu-NPs against *B. glumae* was determined at 10 mg mL^{-1} and 20 mg mL^{-1} respectively.

As for the essential oils, 6 out of the 7 tested EOs showed antibacterial activity against *B. glumae*, which are tea tree, cinnamon, gelam, lemongrass, citronella and lemon myrtle. Tea tree has the biggest inhibition zone, 25 mm in diameter (Table 2). Nilam did not show any inhibition.

Table 2: Diameter of inhibition zone for tested essential oils.

Essential oil	Diameter of inhibition zone (mm)
Tea Tree	25
Cinnamon	20
Gelam,	15
Lemongrass	15
Citronella	15
Lemon Myrtle	12
Nilam	0

Conclusions

For the nanomaterials, it was observed that Cu-NPs inhibit while Zn-NPs suppressed the growth of *B. glumae* on LB agar plate. Silver nanoparticles were not able to inhibit or suppress the growth of *B. glumae* at all tested concentrations. We have also determined the minimum inhibition concentration (MIC) value and minimum bactericidal concentration MBC value of Cu-NPs against *B. glumae,* which are 10 mg mL^{-1} and 20 mg mL^{-1}, respectively. Essential oils were also tested against *B. glumae* and 6 essential oils were able to inhibit the growth of *B. glumae*. Moving forward, bacterial isolates that has antagonistic and anti-QS activity will be sequence to determine their species and a consortium microbe against *B. glumae* will be formulated. The suitability to incorporate nanomaterials or other active compounds in the formulation will also be studied.

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A Practical Approach Towards Genomics Lab Operation Utilising the Lab Inventory Management System

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Introduction

Oil palm is a valuable crop, producing 40% of the world vegetable oil while occupying only 6% of the land used for oil crops (Soh et al., 2017). Through molecular breeding, the oil yields from wild palm groves that were previously less than one tonne per hectare (t/ha) have increased up to 9 t/ha, with a potential output of up to 18 t/ha (Soh, 2018).

Many ongoing research projects are still being done on how to improve the properties and biomarkers of oil palm. Therefore, it is important to preserve the original source of materials for future use and references, especially the ortets and highly valuable palms with specific characteristics that are beneficial to the company. Therefore, a large number of samples need to be processed in the genomics laboratory to ensure that the best source of planting materials is secure for future use for the breeding unit and other departments in FGV. Manual inventory recording can be time-consuming and error-prone, therefore jeopardising the data accuracy. Thus, data digitalisation is the preferable method to manage huge number of samples for inventory purposes.

Over the past 10 years, new technologies have emerged, driving research labs to change how they organise and computerise information. Laboratory Inventory Management Systems (LIMs) was developed to meet these demands (Avery et al., 2000). It is essential to have LIMs capable of storing all sample-related information and preventing data loss during procedures. Through LIMs, research lab productivity will increase while expenses decrease (Aghimien et al., 2018).

Current practise for inventory, data management and sample retrieval is laborious, time-consuming and prone to error. This is because research laboratory generates an enormous amount of varied and complex data, requiring a more systematic format for data storage. Hence, LabCollector LIMs were applied in the genomics lab to completely migrate into data digitalisation. This is because LIMs was also reported to facilitate data tracking and manage the information resources, particularly for valuable and important data (Fiori et al., 2018; Paszko and Turner, 2018).

Materials and Methods

Components of LabCollector LIMs software

Active modules of the LabCollector LIMs software for lab inventory management comes with powerful modules such as Samples Module, Strains and Cells Module, Plasmids Module, Sequences Module, Reagents and Supplies, Documents Module, Equipment Module and Address Book Module. Graphical flow chart for any lab workflow templates for the stated modules can be created. All those workloads and inventory can be tracked easily from the dashboard view.

Samples modules for database inventory purposes

Samples Module enables laboratories to keep track of the samples from fields up until the storage freezer. All materials such as the leaves, pollen, inflorescence, embryo and cabbage are tracked in real-time basis. The barcode system supports the tracking in a way that it enables custom fields for data entry. At first, all the information from the sample request form will be transferred into the

LabCollector systems for record. It includes the samples name, types of samples received, date of sampling, date of samples delivery to the laboratories, types of tests required, location of samples in the freezer and also the person in charge of selected samples. All of this information will be stored in one barcode system generated by the LabCollector software. Hence, once the samples are received in the lab, staffs will scan the materials to be tallied with the barcode generated by the system. Data will then be imported in the Samples Module for easy tracking. JPEG, PNG, GIF and other file formats for the images are intergrated into the gallery and will be uploaded into the systems if special traits or characteristics of the palms need to be documented, by utilising the import functions through media files and albums.

Samples ID tracking and data customisation

One must sign in to LabCollector LIMs in order to retract information about any individual samples. Picklist the samples ID information based on sample information available such as samples name, samples delivery or project lists. Users can quicky check and update any information for the samples by simply scanning or uploading predefined scan files throughout the search functions during samples tracking. The LabCollector LIMs allows us to record all types of content through extensive customisation such as the experiment workflows, standard operating procedure used for selected samples and spreadsheet templates required for samples information and updates.

LabCollector data backup

LabCollector LIMs can be hosted either locally or in cloud. In FGV, a local host server is chosen allowing end users to set up a daily data backup system in the FGV Innovation Centre (FGVIC) server. The daily data backup is scheduled to run and backup on a daily basis.

Results and Discussion

First developed for the purpose of lab inventory, LabCollector LIMs is now capable of extensive and easy customisation to manage any R&D lab activity in Genomics Unit. By utilising the LabCollector LIMs, Genomics Unit can easily track all inventory data under a centralised system supported by an automated data entry barcode system that allows quick information search and retrieval, thereby enhancing data entry speed and precision. Additionally, LabCollector LIMs can also be linked to the associated person in charge in Genomics, so the team can easily and accurately track all the inventory efficiently. The internal database server will centrally catalogue all data, and the programme will grant authenticated users access to it. A web-based system with an intuitive interface will encourage users to actively participate in the digitalisation and utilisation of information (Kapenieks, 2013). Previous conventional management and data tracking have some drawbacks as it requires the use of papers, lab workbooks and excel spreadsheets. This type of tracking system is extremely time-consuming to maintain, elusive, difficult to locate and susceptible to data loss or error (Hashim and Arifin, 2013). In addition, conventional inventory data is restricted to specific individuals or projects.

Manual recording techniques employed by the Genomics Unit for more than 15 years are now less effective due to the large number of samples received for various types of molecular services. The technique described above required not only commitment, time and energy, but most importantly the space required for inventory purposes.

For the inventory purposes to develop the database in Genomics unit, the Samples Module used stores information of any type of samples that the genomics lab works with. It serves as the foundation of samples data inventory, allowing staffs to perform various functions with the samples and they may link to other records in the LabCollector. Hence, with the Samples Module used, samples types can be customised, derived samples can be created for both parents and progenies, the workflow or process related to the samples can be linked and connected, the analysis record can be kept with additional storage location registered. Furthermore, the samples image from the field can also be uploaded in the

LabCollector systems and last but not least, it allows Batch samples processes to ease samples inventory in bulk. Figures 1, 2 and 3 below provide an example on how data were retrieved for sample ID: UA0012 using LabCollector LIMs systems. Graphical User Interface (GUI) was used for the data search.

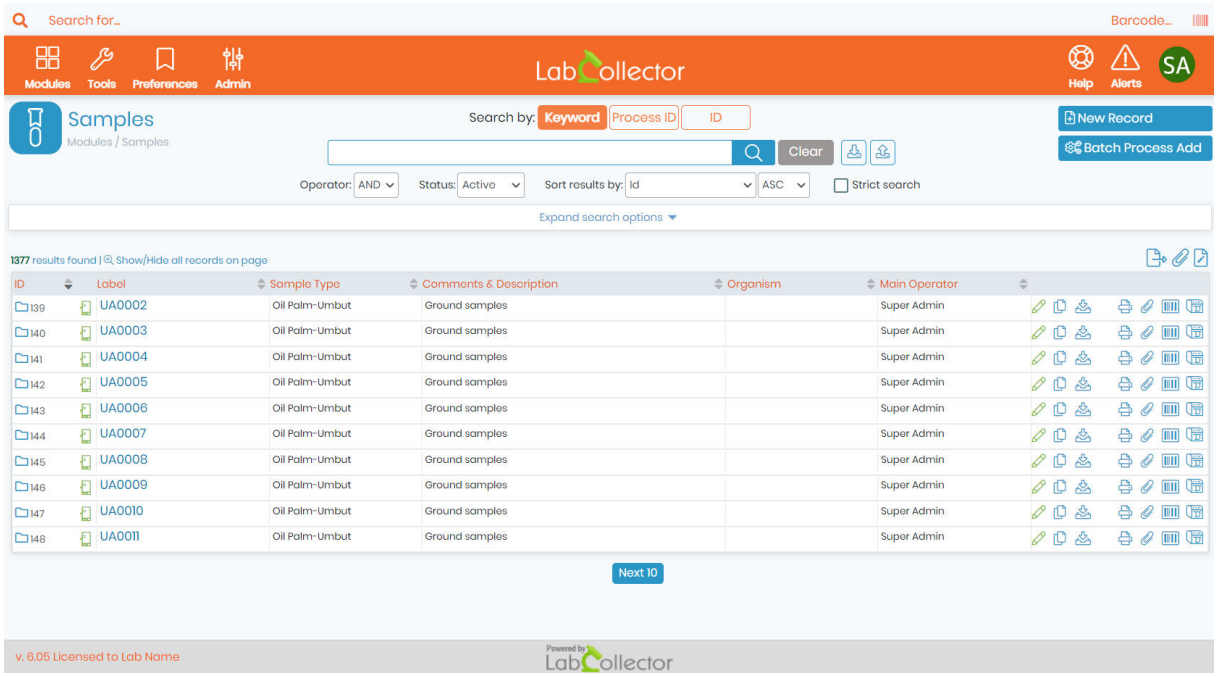

Figure 1: LabCollector LIMs Graphical User Interface (GUI) on Samples Module for tracking purposes. UA0012 samples name needs to be keyed in on the text box (search column) provided.

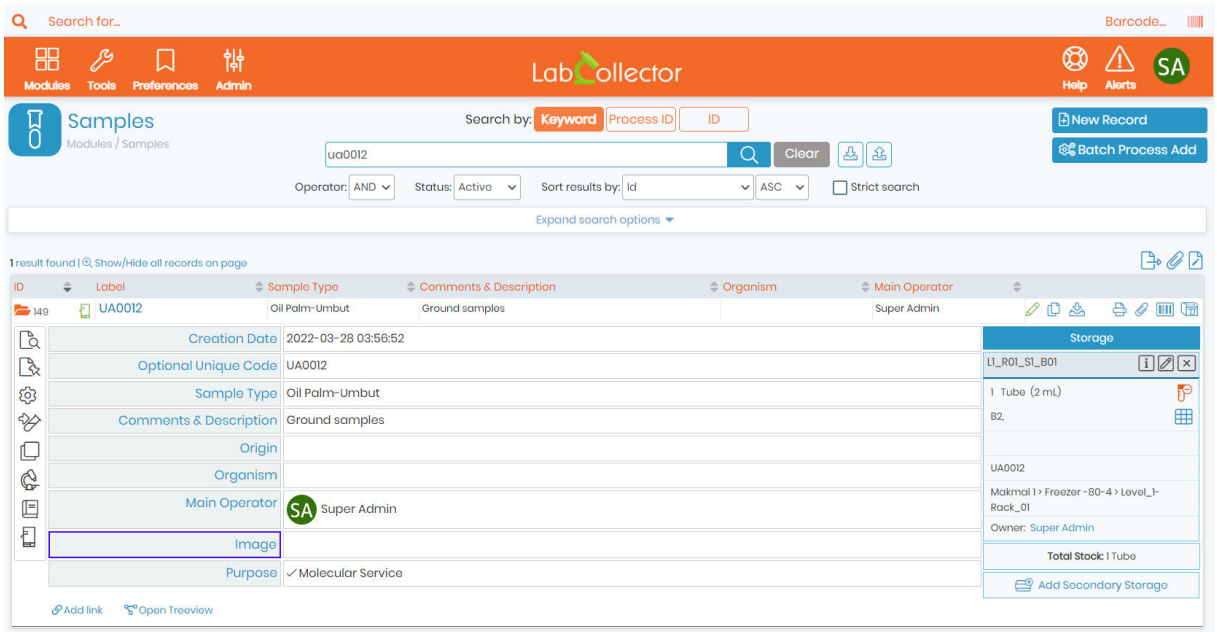

Figure 2: LabCollector LIMs Graphical User Interface (GUI) shows the retrieved information for UA0012 ground samples. The samples type, samples description and also the project purpose will be displayed.

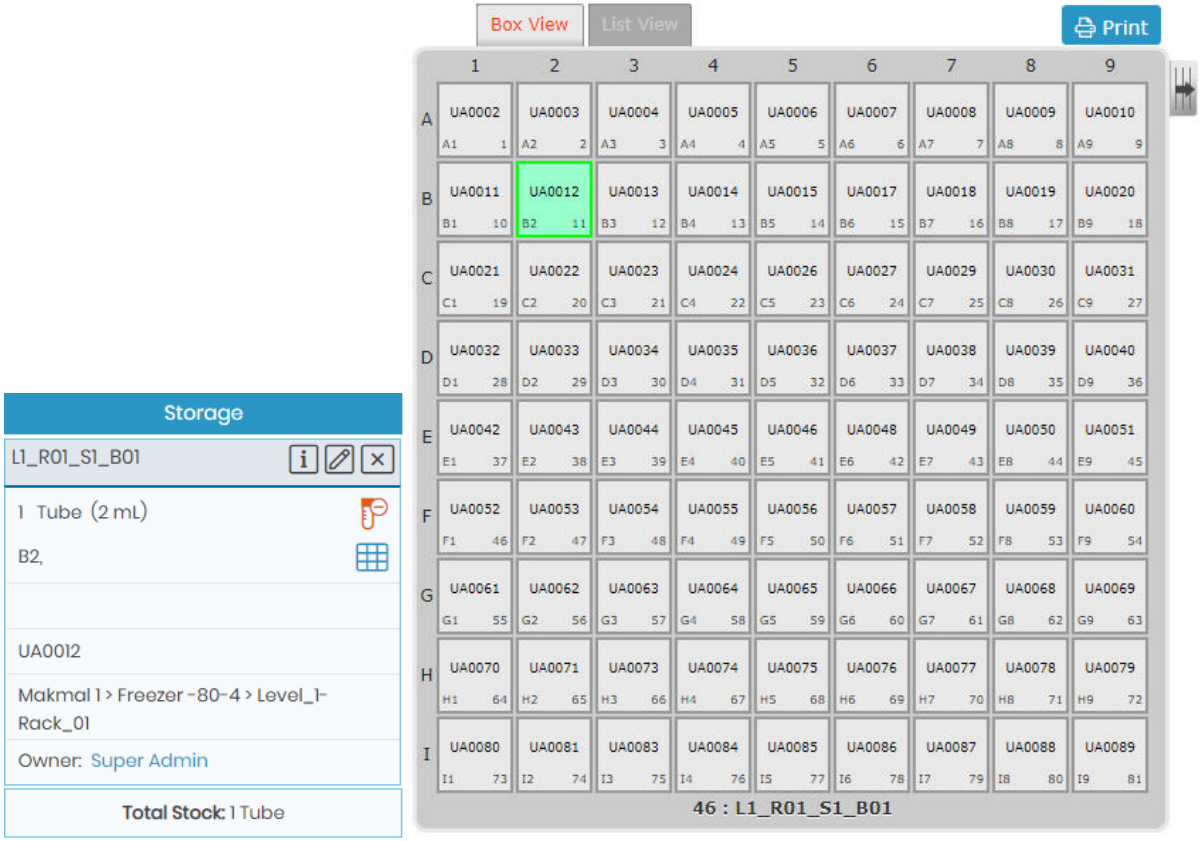

Figure 3: LabCollector LIMs Graphical User Interface (GUI) shows the storage location for UA0012 ground samples. The UA0012 location will be highlighted and displayed based on the box view interface.

A total of five users can access the LabCollector LIMs at one particular time. The LabCollector databases audit trails and data versioning are tracked through history logs that are kept on file. LabCollector LIMs allows samples that were previously housed in plastic bags, open racks and containers of varying sizes to be stored on a shelf and tray system, saving up to 80% of the space required. For real-time tracking, the LabCollector LIMs saves up to 83% time by utilising the excel spreadsheet in the LabCollector systems.

Conclusions

Implementing LabCollector LIMs improves the overall performance and operational efficiency especially when dealing with a very large sample numbers for inventory purposes. The LabCollector LIMs saves the overall time that would have been spent logging all the data manually, thus making it a practical approach for lab inventory management systems. Storage space is being used to its maximum potential, hence preserving samples of oil palm materials efficiently.

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